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Replication of an H5N1 clade 2.2 virus in a mammalian host forces reversion to PB2 627K in presence of its nucleoprotein

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H5N1 HPAIV of clade 2.2 spread from Southeast Asia to Europe. Intriguingly, they share the PB2-627K mutation in contrast to the majority of all other avian strains possessing 627E. To investigate the relevance of this mutation for the broad host range of clade 2.2 strains, we changed K to E in A/Swan/Germany/R65/06 (R65) resulting in the mutant virus R65-PB2_K627E. Compared to R65, multicycle growth of R65-PB2_K627E was considerably impaired in mammalian, but not in avian cells. Correspondingly, polymerase activities were reduced to 5% in mammalian cells and to 50% in avian cells. Whereas virulence in chickens was unaffected as both viruses caused death in all animals within 3 days, the LD50 of R65-PB2_K627E in mice was increased by three magnitudes. Strikingly, R65-PB2_K627E reverted to PB2-627K after one passage in mice, however not in chickens. To reveal whether additional genes of R65 are required for reversion, we passaged reassortants of R65-PB2_K627E containing genes from A/Hong Kong/156/97 (H5N1) (not carrying PB2-627K) in avian and mammalian cells. Reversion to PB2-627K occurred only in mammalian cells provided that the R65 nucleoprotein (NP) is present. Thus, this observation corresponds to in-vitro results of others that PB2-627K restores an impaired association of PB2 and NP of avian strains in mammalian cells. Since this mutation appears not necessary for virus prevalence in avian hosts, it has not been eliminated. Overall, fast reversion to PB2-627K in MDCK cells and mice suggests that the clade 2.2 H5N1 HPAIV could have a history of several, perhaps mammalian hosts.