

## 9. Highly pathogenic avian influenza infections in foxes: can the 2021 H5N1 strain adapt to mammals?

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During the 2020-2022 epizootic of highly pathogenic avian influenza virus (HPAI) several infections of mammalian species were reported in Europe. In the Netherlands, HPAI H5N1 virus infections were detected in three wild red foxes (*Vulpes vulpes*) that were submitted with neurological symptoms between December 2021 and February 2022. Histopathological analysis demonstrated the virus was mainly present in the brain, with limited or no detection in the respiratory tract and other organs. Phylogenetic analysis showed the three fox viruses were not closely related, but were related to HPAI H5N1 clade 2.3.4.4b viruses found in wild birds. In addition, limited virus shedding was detected suggesting the virus was not transmitted between the foxes. Genetic analysis demonstrated the presence of mammalian adaptation E627K in the polymerase basic two (PB2) protein of the two fox viruses. In both foxes the avian (PB2-627E) and the mammalian (PB2-627K) variant were present as a mixture in the virus population, which suggests the mutation emerged in these specific animals. The two variant viruses were isolated and virus replication and passaging experiments were performed. These experiments showed mutation PB2-627K increases replication of the virus in mammalian cell lines compared to the chicken cell line, and at the lower temperatures of the mammalian upper respiratory tract. This study showed the HPAI H5N1 virus is capable of adaptation to mammals, however more adaptive mutations are required to allow efficient transmission between mammals. Therefore, surveillance in mammals should be expanded to closely monitor the emergence of zoonotic mutations for pandemic preparedness.