ZKBS
Zentrale Kommission
für die **Biologische Sicherheit**

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Recommendation of the ZKBS on the risk assessment of Influenza A virus A/bat/Egypt/381 OP/2017 (H9N2) as donor and recipient organism according to § 5 (1) GenTSV

General information

Alphainfluenzavirus influenzae (Influenza A viruses, FLUAV) of the subtype H9N2 were first isolated in 1966 in North America in poultry farms from turkeys [1] and are distributed worldwide in poultry farms and wild birds [2, 3]. The viruses have a broad host range and infect various mammalian species in addition to poultry [4–7]. The H9N2 subtype comprises a large number of strains that have emerged through reassortment with other circulating avian FLUAVs [8]. The viruses can be transmitted through air, dust, feed or water [4].

In birds the infection is usually asymptomatic. Rarely, infected farm-reared animals exhibit altered plumage care or increased salivation [4]. However, infection increases the likelihood of a secondary bacterial infection, which is associated with a mortality rate of 10% [4].

Transmission to pigs and mink kept on farms was observed for individual strains of the H9N2 subtype, which displayed varying symptoms. While infection of pigs with the strain A/duck/Hong Kong/Y280/1997 was associated with a respiratory syndrome [5], infection of mink with the strains Dk/HK/Y280/97 and A/chicken/Hebei/4/2008 was associated with mild symptoms such as weight loss and lethargy [6]. Infection of mice, e.g. with the strain A/chicken/Shandong/244/02 or A/chicken/Beijing/243/05, is generally asymptomatic [9].

Individual strains of the H9N2 subtype or their derivatives (e.g. A/duck/Hong Kong/Y280/97 and A/quail/Hong Kong/G1/97) are also capable of infecting humans [10, 11]. This resulted in the viruses adapting to α2,6-linked sialic acid receptors in the upper respiratory tract [10, 11]. Infection occurs particularly frequent in people who have close contact with poultry [4]. Studies from China have thus shown that 2.3 - 4.6% of poultry farm workers have antibodies against H9 [4]. Human-to-human transmission cannot be ruled out [11]. However, infection in humans is usually accompanied by mild flu-like symptoms [4].

In accordance with the recommendation of the ZKBS on the "Risk assessment of influenza viruses as donor or recipient organisms for genetic engineering operations according to § 5 (1) GenTSV" (Ref.: 6790-05-02-29, updated November 2015), FLUAV of the subtype H9N2 are assigned to **risk group 2**.

The strain A/bat/Egypt/381 OP/2017 was isolated in 2017 from oral and rectal swabs of Egyptian fruit bats (*Rousettus aegyptiacus*) [12]. The animals showed no symptoms of disease. A phylogenetic analysis of the eight genome segments showed that the strain is most closely related to avian FLUAV of the H9N2 subtype [12]. Seroprevalence studies indicate that avian influenza A viruses of the H9N2 subtype are in principle capable of infecting other African fruit bat species [13]. However, A/bat/Egypt/381 OP/2017 appears to be adapted to the Egyptian fruit bat, as it has not yet been possible to be experimentally infected with other H9N2 strains

[13]. The isolated virus has an affinity for α2,3-linked sialic acid receptors and replicates in embryonated chicken eggs, but not in experimentally infected chickens [12]. In contrast, replication lasting up to eleven days was detected in freshly hatched turkeys that were infected oronasally [14]. In further experiments, it was also demonstrated that, unlike other H9N2 strains, A/bat/Egypt/381 OP/2017 is capable of effective and sustained replication in ferrets [14, 15]. For this purpose, animals were initially infected intranasally with $10^{4,8}$ tissue culture infectious dose 50 ($TCID_{50}$). The viral genomes were detected by quantitative RT-PCR from samples of rectal swabs and nasal lavages. Viral genomes were detected up to ten days after infection. Maximum virus replication with 10^8 mL^{-1} viral genomes was achieved two days post-infection. Furthermore, the strain showed a high transmission rate among ferrets [14]. Cohabitation in the same cage system of infected and non-infected ferrets, led to detection of viral genomes in all previous non-infected ferrets within one day. Previous infection attempts in ferrets with other H9N2 strains caused a mild course of disease [16]. In contrast, 13% of the animals infected with A/bat/Egypt/381 OP/2017 lost weight and 93% of the animals developed high fever [14]. However, none of the infected animals died from the infection [14]. The virus replicated predominantly in the nasal conchae, but viral genomes were also detected in the trachea, pulmonary lobes and colon.

In addition, human *ex vivo* lung cultures could be infected with the strain A/bat/Egypt/381 OP/2017 [14]. The strain replicated more efficiently than the seasonal human FLUAV strain A/Panama/2007/1999 (subtype H3N2) [14]. Also, antibodies isolated from human serum samples that are directed against the seasonal H1N1 and H3N2 strains showed no cross-reactivity with the N2 of A/bat/Egypt/381 OP/2017 [14].

Avian influenza A viruses are capable of adapting to replication in humans and enabling human-to-human transmission through several adaptations [17]. One of the key steps in the successful adaptation of FLUAV to humans is the evasion of the human antiviral myxovirus resistance protein 1 (MxA), a major mediator of interferon-mediated immune defence. This adaptation is made possible by specific mutations in the nucleoprotein [17]. Unlike previously known H9N2 strains, A/bat/Egypt/381 OP/2017 is able to evade the antiviral activity of MxA in mice expressing human MxA via a still unknown mechanism [14].

Recommendation

According to § 5 (1) GenTSV in conjunction with the criteria set out in Annex 1 of the GenTSV, the Influenza virus strain A/bat/Egypt/381 OP/2017 is assigned to **risk group 3** as a donor and recipient organism for genetic engineering operations as a precautionary measure.

Rationale

The FLUAV strain A/bat/Egypt/381 OP/2017 differs significantly in several aspects from other H9N2 strains that have occurred in mammals. Firstly, the strain isolated from a fruit bat shows high transmissibility (100%) among ferrets. Secondly, this strain replicates more efficiently in explanted human lung tissue than human-adapted influenza A viruses do. Therefore, even though the strain has an affinity for α2,3-linked sialic acid receptor, transmission to humans and replication in human tissues cannot be ruled out. Furthermore, infection experiments in mice expressing human MxA suggest that the strain is capable of evading the human interferon-mediated immune response. Furthermore, no pre-existing immunity is to be expected in humans, so that, according to the ZKBS statement on the "Risk assessment of influenza viruses as donor and recipient organisms for genetic engineering operations according to § 5 (1) GenTSV" (Ref.: 6790-05-02-29, updated November 2015), an increased pandemic risk cannot be ruled out at this stage.

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