ZKBS
The Central Committee on Biological Safety

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Recommendation of the ZKBS on the risk assessment of

influenza A viruses of subtype H5N8

as donor or recipient organisms according to Article 5 paragraph 1 GenTSV

General

Influenza A viruses have a single-stranded, negative-oriented, segmented RNA genome. They belong to the *Orthomyxoviridae* family and are subdivided into subtypes based on the antigenic properties of their glycoproteins hemagglutinin (HA) and neuraminidase (NA). To date, 18 different HA (H1-18) and 11 different NA (N1-11) subtypes are known, which can occur in different combinations (termed HxNy) [1; 2].

The subtype H5N8 is a group of avian influenza viruses, which has been increasingly observed as a cause of fowl plague in commercial poultry flocks and wild and zoo animals since 2010. Starting in Asia, the subtype spread in 2014 and 2015, presumably due to the migration of migratory birds, first to Europe and Africa and finally to North America. A key role in this spread is attributed to various duck species, which are described as asymptomatic carriers and favour the spread and further reassortment between different H5 subtypes [3 - 5]. Viruses of the subtypes H5N8 and H5N5 are currently triggering the most severe and long-lasting outbreak of avian influenza in Europe, which has been ongoing since autumn 2016 and now extends to 29 European countries. In Germany, so far approximately 1100 confirmed H5N8 cases have been reported in wild birds and 92 outbreaks of this subtype in poultry farming and 15 in zoological gardens or zoos (as of May 2017) [5].

Studies of two H5N8 strains isolated in Korea in 2014 and in India in 2016 showed an intravenous pathogenicity index of 3 [6; 7] in six-week-old chickens. Furthermore, the hemagglutinin of this subtype has a cleavage site with multiple basic amino acids [7; 8]. Accordingly, this subtype meets the criteria for a highly pathogenic avian influenza virus (HPAIV) as defined in Council Directive 2005/94/EC of 20 December 2005.

Compared to the likewise highly pathogenic subtype H5N1, H5N8 may have a lower transmissibility between chickens [7]. This subtype is also described as less pathogenic for mice than other H5 subtypes [8 - 10]. Ferrets could be infected, but showed no or only mild symptoms. Moreover, no transmission of the viruses to sentinel animals was observed [8; 9; 11; 12]. Furthermore, in contrast to the situation in chickens, no systemic replication of the virus could be detected in ferrets and mice [7 - 9; 11; 12]. Besides mice and ferrets, dogs and cats can also be experimentally infected [10]. Human infections have not yet been described [3].

The first animal experiments suggest that the strains studied have not yet adapted to mammals. This assessment is additionally supported by *in vitro* experiments and genome analyses. On the one hand, HA preferentially binds to α2,3-linked sialic acid found in birds, whereas only

a minimal binding to α2,6-linked sialic acid typical for humans was measured [8; 13]. Furthermore, the virus replicates poorly in cultures of human lung cells and does not exhibit the high acid stability typical of human-adapted viruses [8; 9]. Finally, no mutations were found in a genome analysis which are regarded as signs of adaptation to mammals [8]. According to these data, the WHO currently estimates the likelihood of human infection as being low [3]. In mice, however, an adaptation was achieved after only five passages, which was accompanied by a significantly increased morbidity. This mouse-adapted virus also showed a significantly increased replication efficiency in cultures of human lung cells [14].

H5N8 viruses often show no cross-reactivity with antibodies directed against H5 vaccine strains. As part of the global pandemic preparedness program, WHO has therefore recommended a reassortant with the HA and NA genome segments of the strain A/gyrfalcon/Washington/41088-6/2014 (H5N8) and the other segments of the laboratory strain A/Puerto Rico/8/1934 (H1N1) as a vaccine candidate [4]. A first Phase I study with the monovalent inactivated vaccine has been ongoing in the US since January 2017 [15]. In addition, it has already been shown that individual strains are sensitive to the approved neuraminidase inhibitors oseltamivir and zanamivir as well as the experimental drug peramivir [8; 13]. In contrast, the presence of a known resistance mutation in the M2 protein suggests resistance to adamantanes [8].

In Technical Rules for Biological Agents (TRBA) 462 “Classification of viruses in risk groups” influenza A viruses of subtype H5N8 are assigned to risk group 2 with the addition “t3”[1] [16].

Recommendation

According to Article 5 paragraph 1 GenTSV in conjunction with the criteria in Annex I GenTSV, influenza A viruses of the subtype H5N8 are assigned to **risk group 3** as donor and recipient organisms for genetic engineering operations.

Reasoning

Influenza A viruses of subtype H5N8 meet the criteria for a highly pathogenic avian influenza virus (HPAIV) as defined in the Council Directive 2005/94/EC in Annex I, point 2 of 20 December 2005. According to the “Recommendation of the ZKBS on the risk assessment of highly pathogenic avian influenza virus A strains of subtypes H5 and H7 and derived laboratory strains” (file number 6790-05-02-34, updated 2015), isolates and strains meeting the above definition are assigned to risk group 3.

References

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[1] t3: due to its pathogenicity for vertebrates animal health regulations may require safety measures comparable to measures of class 3, which prevent the release of the virus into the environment or adjacent workspaces.

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