

**Recommendation of the ZKBS on the risk assessment of the  
*Reston ebolavirus* (RESTV) as donor or recipient organism according to Art. 5  
Paragraph 1 of the German Genetic Engineering Safety Regulation (GenTSV)**

**General considerations**

The *Reston ebolavirus* (RESTV) belongs to the family *Filoviridae* in the order *Mononegavirales*. Next to the *Zaire ebolavirus* (EBOV), the *Tai forest ebolavirus* (TAFV), *Bundibugyo ebolavirus* (BDBV) and the *Sudan ebolavirus* (SUDV) it represents a species of its own within the genus *Ebolavirus* [1]. RESTV are filamentous, enveloped viruses possessing a linear single-stranded RNA genome of negative polarity. The closest relative of RESTV is SUDV [2]. The amino acid sequence of proteins from EBOV and RESTV display an identity of 58 – 81 %, with the sequence of the gene encoding the glycoprotein being minimally, and that of the matrix protein gene *vp24* being maximally conserved [3; 4].

RESTV was first diagnosed in 1989, when cynomolgus monkeys perished from a disease in a quarantine unit in the city of Reston, Virginia, USA. The macaques had been previously imported into the USA from a breeding facility in the Philippines [5; 6]. An investigation revealed that the monkeys succumbed to infections with the *Simian hemorrhagic fever virus* and the then unknown filovirus. In the years 1989, 1990, 1992 and 1996, further RESTV breakouts occurred in the USA and in Italy among cynomolgus monkeys that had been imported from the Philippines [7 - 11], the incidences could be retraced to the same breeding facility in the Philippines. The lethality of the monkeys ranges between 50 and 84 % [9; 12]. In comparison, all seven experimentally infected Ethiopian vervet monkeys survived. In cynomolgus monkeys, the course of RESTV infections was milder and the lethality was lower than that of EBOV infections in which the viremic phase started earlier and was of shorter duration [9]. RESTV is not endemic outside Asia.

In 2008, RESTV was identified at an epizootic in pigs of two geographically separated pig breeding facilities in the Philippines. The pigs were additionally co-infected with the *porcine reproductive and respiratory syndrome virus* (PRRSV) and the *porcine circovirus type-2* [13]. In subsequent studies, the pigs which were experimentally infected exclusively with RESTV

display no clinical symptoms of disease, however, they excreted the virus [14]. Furthermore, in 2011, RESTV was detected by RT-PCR in PRRSV-infected piglets in China [15].

Experimentally, guinea pigs, rabbits and mice could be successfully infected with RESTV. RESTV does not replicate in mosquitoes and ticks [16]. However, antibodies directed against RESTV antigens and, to some extent, against the nucleic acid segments of the RESTV genome were also found in wild-living microbats (Chiroptera) and megabats (Megachiroptera) from the Philippines, Bangladesh and China [17 - 20].

A study with humans who had contact with the infected monkeys or pigs revealed that a total of 15 were seropositive for antibodies against RESTV, however, none displayed any clinical symptoms of the disease [5; 21 - 24]. Among them was an animal caretaker who infected himself with the virus by a cut injury during the necropsy of an infected monkey. Tests confirmed that he was viremic on Days 9, 10 and 11 after inoculation, but displayed no further symptoms [6; 23]. All examined contact persons of seropositive persons were shown to be seronegative [21], for which reason a human-to-human transmission of RESTV is not to be expected.

As is the case with other viruses of the same genus, the transmission of RESTV proceeds via the feces, blood, urine and other body fluids. Ebola antigens were identified in alveoli of the lungs and in intraalveolar infiltrations of infected pigs and cynomolgus monkeys, so that for the macaques and pigs a transmission via aerosols cannot be ruled out [12; 13].

Due to the poor proofreading function of its polymerase, RESTV has a high mutation rate just like other RNA viruses. The pathogenicity of ebolaviruses can change distinctly within only few passages. For example, EBOV could be adapted by serial passaging of only few passages to guinea pigs which were not susceptible to EBOV before passaging [25; 26]. After the fifth passage the lethality of an infectious dose of  $10^3$  TCID<sub>50</sub> was 100 %.

In addition, the WHO recommends handling RESTV as well as other filoviruses only under conditions of biosafety level 4, because pathogenicity for humans cannot be ruled out. Pathogenicity for humans is considered as possible, since seropositive persons who had contact with infected monkeys or pigs were exclusively healthy male adults, of which only one (the animal caretaker who infected himself with RESTV during a necropsy and was viremic) had diabetes as an underlying disease. According to the WHO, it therefore cannot be ruled out that an infection of pregnant women, children or immunosuppressed patients could take a more serious progression [21].

On the other hand, EBOV whose glycoprotein gene was replaced by the glycoprotein gene from RESTV, and RESTV carrying the glycoprotein gene from EBOV were both attenuated. This suggests that, other than in EBOV, numerous factors would contribute to the attenuation of RESTV, whereas single mutations could not directly elevate the pathogenic potential for humans to that of an organism assigned to risk group 4 [27].

The "Technical Rules for Biological Agents 462: Classification of Viruses in Risk Groups" assigns the virus to risk group 2, with the notice that safety measures of level 4 must be maintained because of the pathogenic potential for vertebrates. Internationally (e.g. Switzerland, Belgium, USA, Canada), RESTV is exclusively handled by observing the conditions of biosafety level 4.

### **Recommendation**

Pursuant to Art. 5 Paragraph 1 of the German Genetic Engineering Safety Regulation (GenTSV) in conjunction with the criteria in Annex I of the GenTSV the *Reston ebolavirus* as a donor and recipient organism for genetic engineering operations is assigned to **risk group 4**.

### **Reasons**

The ZKBS recommends assigning RESTV to risk group 4 because (i) it has a high risk potential for non-human primates, (ii) is closely related to other highly pathogenic ebolaviruses, (iii) the cause of the attenuation of RESTV, as compared to other ebolaviruses, is not completely understood and (iv) it is an RNA virus whose genome is subject to a high mutation rate.