ZKBS
The Central Committee on Biological Safety

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Recommendation by the ZKBS on the risk assessment for *Mammalian 1 orthobornavirus* as a donor and recipient organism according to § 5 para. 1 GenTSV

General Information

The Mammalian 1 orthobornavirus (previously *Borna disease virus*, BoDV) is an enveloped virus of the *Bornaviridae* family (genus *Orthobornavirus*) that has a non-segmented RNA genome of negative polarity. Two subtypes are distinguished based on the similarity of the genome sequences, whereby field isolates until now are assigned almost exclusively to subtype 1 (BoDV-1) [1].

In 2010 it was shown that numerous cDNA copies of genes of BoDV-like viruses are integrated in the genome of humans and a number of other mammals, and in that of reptiles, fish, spiders, and insects [2; 3]. In humans, seven copies of the nucleoprotein (N) gene and one copy of the glycoprotein (G) gene are present as independent or fusion genes. At least two of these so-called *endogenous borna-like elements* (EBL) are expressed in humans as functional proteins (hsEBLN-1 and hsEBLN-2) [4]. Both proteins feature a 41% sequence identity to BoDV N [2]. Their function is currently not clear. It has been verified that they integrate directly with various cellular proteins and influence the expression of other proteins. Based on this, it is assumed that hsEBLN-1 has a pro-tumour effect and hsEBLN-2 an anti-tumour effect [5]. Moreover, regulatory, non-coding RNA molecules (piRNA or lncRNA) are transcribed of human EBL [4].

In infection trials in humans and canine cell cultures and in the mouse, it was shown that genes of BoDV can also integrate nondirectionally in the genome of host cell. This is made possible by the persistent, non-lytic replication of the virus in the nucleus of the host cell and, in humans, is probably mediated by the reverse transcriptase of the endogenous retrotransposon *long interspersed nuclear element 1* (LINE-1) [2; 3]. Although the integration efficiency is less than with retroviruses [3], an insertion mutagenesis accompanying infections by BoDV can therefore not, in principle, be ruled out.

BoDV is the pathogen of the so-called Borna disease in horses and sheep. This disease, which was already described in the 17th century, has until now occurred exclusively in parts of Germany, primarily in the southern and eastern federal states, parts of Austria and Switzerland and Lichtenstein [6]. In addition, animal and humans specimens from other regions have also demonstrated viral RNA and antibodies that are reactive with BoDV. Various work groups postulate a global distribution [1]. In addition to horses and sheep, among others donkeys, cows, goats, dogs, cats, rabbits and humans can also become infected with BoDV. Aside from horses and sheep, however, the incidence of infections is very low. Experimentally, monkeys, chickens, and various rodents can additionally be infected intranasally, intracranially, intramuscularly, intradermally, subcutaneously and, with low efficiency, intravenously [7]. The virus usually exhibits neurotropism; it first replicates within the neurons at the entry site and

then spreads along the nerve tract to the brain. Natural BoDV infection therefore presumably takes place via the olfactory route [6]. The cellular receptor is unknown to date [3]. Direct transmission of the virus between livestock or livestock and humans has not been described to date. It is also not clear whether the indicated animals shed infectious particles. Viral particles have thus been verified only rarely in the bodily fluids of symptomatic horses [7]. In contrast, it is assumed that the bicoloured shrew (*Crocidura leucodon*) can shed the virus via urine, saliva and faeces and thus these can serve as a source of infection. In addition, a role played by the bank vole (*Myodes glareolus*) as a reservoir has also been discussed [6]. Experimentally infected mice and rats can also transmit the virus. In rats, this takes place horizontally via the urine, in mice, however, vertically to the offspring [7].

In investigations on the prevalence of antibodies, 12% of tested horses were seropositive in endemic areas of Germany. Outside of these regions, however, the seroprevalence was also 12% [8]. Based on possible cross-reactivity of the antibodies, however, no statement can be made with regard to the incidence of infections. Moreover, in another study antibodies were verified in only 30 - 40% of verifiably infected animals. Symptomatic disease arose in 20% of seropositive horses and in fewer than 40% of seropositive sheep [7]. In total, these are respectively fewer than 100 annual cases [1; 7].

In horses the incubation time for the virus is four weeks to three months. Thereafter, non-specific symptoms such as fever, loss of appetite, and depression first occur, leading to disorders of movement, paralysis, and acute encephalitis. Eighty percent of ill horses finally die 1 – 4 weeks after symptoms arise. Moreover, in non-deadly infections, persistent behavioural disorders and/or a blindness often result [6; 7]. The symptoms thereby are probably not attributable to the virus itself, but rather to the reaction of the immune system and the brain damage associated therewith [9]. Aside from acute progressions, in 10% of cases chronic infection develops with recurring symptoms. Sheep show similar symptoms at a lethality rate of 50% after symptomatic infection [6; 7].

In humans a BoDV infection is initially associated with various psychiatric illnesses, such as schizophrenia or depression. A confirmation of this association, however, is absent due to investigational results that vary [10; 11]. In October 2018 a number of BoDV-1 cases with a deadly progression were reported. On the one hand, this concerned two recipients of donor kidneys from the same donor from Bavaria. Both developed encephalitis and died approx. six months after the transplant. Brain specimens from both patients allowed verification of almost the entire genomic RNA of BoDV-1. The sequence showed the greatest similarity to wild isolates of the donor's home region. A third patient who had the liver of the donor transplanted also developed an encephalopathy, but survived. He is, however, blind. The organ donor showed no neurological symptoms or signs of a viral infection [12]. Another publication reported on a previously healthy 25-year old from Bavaria who also developed neurological symptoms and died of diffuse encephalopathy after 23 days. Also in this case, the entire genome of BoDV-1 was verified be means of sequencing [13]. There is also a further case report of deadly encephalitis in a previously healthy 17-year old man from Bavaria with subsequent verification of BoDV-1 [14]. In a retrospective study of specimens of patients from Bavaria who died due to unexplained encephalitis, the BoDV-1 genome was verified in five of eight cases [15]. There is no information on the immune status or possible previous illnesses for these patients. There is also no information on the mode of transmission of the virus, except for the case of the transplant recipient. German research and diagnostic labs have been handling BoDV-1 for decades. To date, there have been no lab associated infections.

Presently there is neither an approved vaccine nor an antiviral substance against BoDV. In the 1920s, however, an inactivated vaccine was developed in Germany for horses and sheep, which was replaced as of 1947 by various attenuated, live vaccines. Attenuated live vaccines were used in West Germany until 1978 and in East Germany until 1992. The efficacy of this vaccine was never proven, however. The approval for the last vaccine ran out in 1992. Since then there has been no increased incidence of the Borna disease [1].

Previously, BoDV was assigned **risk group 2** as a donor and recipient organism for genetic engineering operations.

In the "Technical Regulation for Biological Substances 462: Classification of Viruses" BoDV is also assigned **risk group 2.**

Recommendation

According to § 5 para. 1 GenTSV in conjunction with the criteria in annex I GenTSV, *Mammalian 1 orthobornavirus* is further assigned to **risk group 2** as a donor and recipient organism for genetic engineering operations.

Reasoning

According to the current state of knowledge the *Mammalian 1 orthobornavirus* can also cause severe encephalitis in humans that can also lead to death in immunocompetent individuals. Regarding the lethality rate in humans, no verified statement can be made presently. Additionally, the path of transmission to humans is unknown to date. An infection via the olfactory route is assumed in animals. Many years of experience in handling BoDV in the lab, however, show that the currently applied safety measures of level 2 adequately counteract the risk of a BoDV infection.

Notes

Mice and rats infected with BoDV are always to be kept in *individually ventilated cages* (IVCs) to avoid airborne transmission of the virus. Additionally, cage transfer stations are to be used to transfer the animals.

Literature

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