ZKBS
Zentrale Kommission
für die **Biologische Sicherheit**

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Recommendation of the ZKBS on the risk assessment of *Orthobornavirus bornaense* as donor and recipient organism according to § 5 (1) GenTSV

General

Orthobornavirus bornaense (formerly Mammalian 1 orthobornavirus, Borna disease virus, BoDV) is an enveloped virus of the family *Bornaviridae* (genus *Orthobornavirus*) that has a non-segmented RNA genome of negative polarity. Based on the similarity of the genome sequences, two subtypes are distinguished, with field isolates almost exclusively assigned to subtype 1 (BoDV-1) so far [1].

In 2010, it was shown that several cDNA copies of genes from BoDV-like viruses are integrated into the genome of humans and a variety of other mammals, as well as into 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 stand-alone or fusion genes. At least two of these so-called endogenous borna-like elements (EBL) are expressed as functional proteins in humans (hsEBLN-1 and hsEBLN-2) [4]. Both proteins have a 41% sequence identity with BoDV N [2]. Their function is currently unclear. It has been shown that they interact directly with various cellular proteins and influence the expression of other proteins. Based on this finding, a pro-tumour effect of hsEBLN-1 and an anti-tumour effect of hsEBLN-2 are assumed [5]. In addition, regulatory, non-coding RNA molecules (piRNA or lncRNA) are transcribed by human EBLs [4].

Infection experiments in human and canine cell cultures and mice have shown that BoDV genes can also integrate into the genome of the host cell in an undirected manner. This is made possible by the persistent, non-lytic replication of the virus in the host cell nucleus and is probably mediated in humans by the reverse transcriptase of the endogenous retrotransposon long interspersed nuclear element 1 (LINE-1) [2, 3]. Although the integration efficiency is lower than that of retroviruses [3], insertion mutagenesis cannot be ruled out in principle upon infection with BoDV.

BoDV is the pathogen that causes Borna disease in horses and sheep. This disease, which was first described in the 17th century, has so far only occurred in parts of Germany, mainly in the southern and eastern federal states, parts of Austria and Switzerland, and in Liechtenstein [6]. In addition, viral RNA and antibodies reactive to BoDV have also been detected in animal and human samples from other regions. Various working groups postulate a global distribution [1]. In addition to horses and sheep, donkeys, cattle, llamas, alpacas, goats, dogs, cats, rabbits, bicoloured shrews, bank voles and humans can also become infected with BoDV. However, except in horses and sheep, the incidence of infection is very low. Experimentally, monkeys, chickens and various rodents can also be infected intranasally, intracranially, intramuscularly, intradermally, subcutaneously and, with low efficiency, intravenously [7]. The only reservoir host confirmed to date is the bicoloured shrew (*Crocidura leucodon)*, which can become asymptomatically and persistently infected [8, 9]. In addition, the bank vole (*Myodes glareolus)* is also being discussed as a reservoir [6, 7]. In the bicoloured shrew, the virus can

infect many different tissue types [10]. In other hosts, it is usually neurotropic, initially replicating within the neurons at the site of entry and then spreading along the nerve pathways to the brain. A natural BoDV infection therefore presumably occurs via the olfactory route [6]. The cellular receptor is still unknown [3]. Direct transmission of the virus between farm animals or between farm animals and humans has not yet been described. It is also unclear whether the animals mentioned shed infectious virus particles. Virus particles have only rarely been detected in body fluids of symptomatic horses [7]. In contrast, it is suspected that the bicoloured shrew can shed the virus via urine, saliva and faeces, which thus serve as sources of infection. Experimentally infected house mice and rats can also transmit the virus to other laboratory animals of the same species. In rats, this occurs horizontally via urine, but in house mice it is transmitted vertically to offspring [7].

In studies on the prevalence of antibodies, 12% of horses tested in endemic areas in Germany (southern and eastern federal states) were seropositive. Outside these areas, however, the seroprevalence was also 12% [11]. Due to possible cross-reactivity of the antibodies, no statement can be made regarding the incidence of infections. Furthermore, in another study, antibodies were detected in only 30 to 40% of animals that were proven to be infected. Symptomatic disease occurs in 20% of seropositive horses and in less than 40% of seropositive sheep [7]. Overall, there are fewer than 100 cases per year in the endemic region [1, 7]. Unlike other hosts, bicoloured shrews are frequently seropositive (10 to 67% of bicoloured shrews examined at the respective locations in the BoDV endemic region) [8, 9, 12–20].

In horses, the incubation period of the virus is four weeks to three months. This is followed by initial non-specific symptoms such as fever, loss of appetite and depression, and finally movement disorders, paralysis and acute encephalitis. 80% of infected horses eventually die 1 to 4 weeks after the onset of symptoms. In non-fatal infections, permanent behavioural disorders and/or blindness often occur [6, 7]. The symptoms are probably not caused by the virus itself, but by the immune system's response and associated brain damage [21]. In addition to acute cases, 10% of cases develop into a chronic infection with recurring symptoms. Sheep show similar symptoms with a mortality rate of 50% after symptomatic infection [6, 7].

In humans, BoDV infection was initially associated with various psychiatric disorders, such as schizophrenia and depression. However, this link has not been confirmed due to differing study results [22, 23]. Since 2018, a total of almost 50 (as of the end of 2023) cases of meningoencephalitis caused by BoDV-1 have been reported in Germany over the last 30 years, most of which were fatal [12, 15–20]. The three surviving patients described have lost their sight, require in-patient care or suffer from severe cognitive and psychological impairments [15, 18, 24]. The infections occurred after organ transplantation (three patients who had received a kidney or liver from the same donor) or without any known external cause. Some of the infections were discovered during retrospective examination of samples from fatal cases of encephalitis of unknown cause. No underlying immunosuppression is known for the majority of patients [25].

Symptoms of human infection include fever, headache, cognitive impairment and worsening neurological symptoms such as unsteady gait, convulsions, memory loss and coma, which usually lead to death [17]. Symptoms of Guillain-Barré syndrome may also occur [26].

Extensive serological tests were conducted to investigate the human pathogenic potential of BoDV-1. An analysis of 1,109 serum samples from veterinarians and blood donors revealed one positive sample from a veterinarian. The veterinarian had indicated in the questionnaire that he had experienced joint pain [27], but did not report any other symptoms of disease. Further follow-up was not possible because the questionnaire had been completed anonymously. In another study, 216 serum samples from blood donors and 280 serum samples from transplant recipients from the endemic area tested negative for antibodies against BoDV-1. In the same study with 288 serum and plasma samples from encephalitis patients from the endemic area with suspected infection with *Orthoflavivirus encephalitidis* (Tick-borne encephalitis virus),

one sample was positive. BoDV-1 RNA was subsequently detected in the patient's cerebrospinal fluid [28]. All serum samples from residents of a village where two independent cases of BoDV-1 had previously occurred were negative (43% of the village residents had been tested) [12]. However, tests for antibodies against BoDV-1 in serum samples and cerebrospinal fluid samples often turn positive only late in the clinical course of the disease [17]. Bornavirus RNA was detected only in meningoencephalitis patients and only in brain tissue, peripheral nerve tissue and cerebrospinal fluid. Serum samples were negative [17].

Except in isolated cases, antibodies against BoDV-1 or BoDV-1 RNA are therefore only detectable in patients with meningoencephalitis. One possible explanation is that mild BoDV-1 infections that are asymptomatic or subclinical are extremely rare in humans. Another explanation is that mild cases do occur but cannot be detected by seroprevalence studies because the available methods detect antibodies against BoDV-1 only late in the clinical course (median 32 days after hospitalisation [29]).

The route of transmission of the virus is unknown except in cases of transplant recipients. Household members of patients with BoDV-1 infections or persons in contact with BoDV-1-infected farm animals were always asymptomatic and seronegative, with the exception of the positive sample from the veterinarian, in whom the source of infection is unclear, so that human-to-human transmission or transmission from farm animals to humans is not to be expected [20, 29–31]. Risk factors for BoDV-1 infection include living in rural areas or on the outskirts of towns [17, 32]. It is assumed that infection occurs through contact with excretions from infected bicoloured shrews, whereby the virus is initially taken up via the respiratory tract, infects the olfactory nerve and then spreads to the brain [30, 33]. Although BoDV-1 has been handled in German research and diagnostic laboratories for decades, no laboratory-associated infections have been reported to date. Even documented laboratory accidents such as needle stick or cut injuries while handling BoDV-1-contaminated sample material or exposure of the skin to cerebrospinal fluid from a BoDV-1-infected person did not lead to seroconversion or disease in laboratory personnel [34].

There is currently no approved vaccine or proven treatment with antiviral agents against BoDV. Ribavirin and favipiravir have been shown to be effective against bornaviruses, including BoDV-1, in cell culture and animal experiments [35–37] and have also been used in the treatment of BoDV-1 infections, but they were unable to prevent the death of patients or the onset of serious long-term effects [24, 30]. In the 1920s, an inactivated vaccine for horses and sheep was developed in Germany, which was replaced by various attenuated live vaccines from 1947 onwards. Attenuated live vaccines were used in West Germany until 1978 and in East Germany until 1992. However, the efficacy of these vaccines has never been proven. In 1992, the licence for the last vaccine expired. Since then, there has been no increase in the incidence of Borna disease [1].

The notification requirement for Bornavirus infections in animals was abolished in 2011. However, since 2020, Bornavirus infections in animals and humans are once again notifiable (Ordinance on Notifiable Animal Diseases and § 7 of the German Infection Protection Act).

To date, BoDV has been classified as a donor and recipient organism for genetic engineering operations in **risk group 2**.

In the "Technical Rules for Biological Agents 462: Classification of Viruses", BoDV is also assigned to **risk group 2**.

Recommendation

According to § 5 (1) GenTSV in conjunction with the criteria in Annex 1 GenTSV, *Orthobornavirus bornaense* continues to be assigned to **risk group 2** as a donor and recipient organism for genetic engineering operations.

Small mammals infected with BoDV must always be kept in individually ventilated cages (IVCs) to prevent aerogenic transmission of the virus. In addition, cage changing stations must be used for cage swapping of experimental animals.

Reasoning

According to current knowledge, BoDV can in rare cases also cause severe encephalitis in humans, which can lead to death even in immunocompetent individuals. No reliable statement can be made about the mortality rate in humans at present, as it cannot be ruled out that there are undetected asymptomatic or subclinical infections. In addition, the exact route of transmission to humans is still unknown, although there are indications that the infection is transmitted via the olfactory route. According to current knowledge, there is no human-to-human transmission. Many years of experience in handling BoDV in laboratories show that the current level 2 safety measures are sufficient to counteract the risk of BoDV infection.

Literature

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