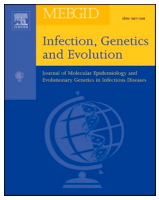
Infection, Genetics and Evolution 134 (2025) 105806

Contents lists available at ScienceDirect

Infection, Genetics and Evolution

journal homepage: www.elsevier.com/locate/meegid



Short communication

Screening for Borna disease virus 1 (BoDV-1) in Austria: Absence of human cases in a retrospective case-finding study

Markus Bauswein [a,*], Lisa Arnold [b], David N. Springer [c], Monika Redlberger-Fritz [c]

[a] Institute of Clinical Microbiology and Hygiene, University Hospital Regensburg, 93053 Regensburg, Germany
[b] Institute of Medical Microbiology and Hygiene, University of Regensburg, 93053 Regensburg, Germany
[c] Center for Virology, Medical University Vienna, 1090 Vienna, Austria

ARTICLE INFO

Keywords:
Borna disease virus 1 (BoDV-1)
Epidemiology
Austria
RT-qPCR
Serology

ABSTRACT

Background: Borna disease virus 1 (BoDV-1) is a zoonotic virus with a recently confirmed potential to cause rare but severe cases of encephalitis in humans. While the bicolored white-toothed shrew (*Crocidura leucodon*), which represents the reservoir, is widely distributed over eastern, central, and southern Europe as well as south-west Asia, human infections have so far only been reported from Germany. As infections in sentinels such as horses indicate the endemic circulation of the virus also in circumscribed regions of neighboring countries (Austria, Liechtenstein, Switzerland), we initiated a retrospective case-finding study to investigate whether there were so far undetected human infections in Austria.

Methods: For this purpose, biobank samples from the Center for Virology in Vienna were selected based on available clinical characteristics consistent with possible neurological symptoms of human BoDV-1 infections to be screened for BoDV-1 RNA (859 cerebrospinal fluid samples) and anti-BoDV-1 IgG antibodies (366 corresponding serum samples).

Results: BoDV-1 RNA or confirmed anti-BoDV-1 IgG antibodies were not detected in any of the cerebrospinal fluid or serum samples, respectively.

Conclusion: Our result demonstrates that if human BoDV-1 infections occur in Austria, they must be very rare even in patients with neurological symptoms. Further research using samples with a more distinct geographical link to the circumscribed endemic rural region in Upper Austria, however, will be necessary to complement the preliminary finding of this study.

1. Introduction

The zoonotic potential of Borna disease virus 1 (BoDV-1) has recently been confirmed, at first in a cluster of solid-organ transplantation (Schlottau et al., 2018). In the meanwhile, approximately 50 human cases of severe BoDV-1 infection have been notified to German health authorities (Pörtner et al., 2023). Patients showed symptoms of a severe encephalitis such as altered mental status, memory impairment, paresis, ataxia, visual impairment, and in most cases eventually deep coma (Pörtner et al., 2023).

While the bicolored white-toothed shrew (*Crocidura leucodon*) as potential reservoir is widely distributed over eastern, central, and southern Europe as well as south-west Asia (Dubey et al., 2007; Haring et al., 2024; Bourg et al., 2013; Dürrwald et al., 2014; Nobach et al., 2015), BoDV-1 positive reservoir animals and dead-end sentinel animals have only been reported from eastern and southern Germany, Switzerland, Liechtenstein, and Upper Austria (Kolodziejek et al., 2005; Weissenböck et al., 2017). Additionally, the closely related BoDV-2 has been described in one diseased horse in Upper Austria (Nowotny et al., 2000).

Sequences of viral isolates of both reservoir/sentinel animals and diseased humans cluster regionally, indicating rare spill-over events as most plausible causes of infections (Niller et al., 2020). Despite endemic infections in horses from Upper Austria, however, no human cases of BoDV-1 encephalitis have been reported from Austria. Therefore, we initiated a retrospective case-finding study and analyzed human samples stored in the biobank of the Center for Virology in Vienna to address the question whether there are so far undetected human BoDV-1 infections in Austria.

* Corresponding author.
E-mail address: markus.bauswein@ukr.de (M. Bauswein).

https://doi.org/10.1016/j.meegid.2025.105806
Received 8 May 2025; Received in revised form 16 July 2025; Accepted 28 July 2025
Available online 29 July 2025

Table 1
Frequency of clinical symptoms in the study cohort.

Clinical symptoms	n	%
(suspected) encephalitis/meningoencephalitis/viral meningitis	654	76
(suspected) Guillain-Barré syndrome	44	5
cranial nerve palsy	32	4
ataxia	23	3
altered mental status/deep coma	18	2
paresis	18	2
encephalopathy	11	1
aphasia	8	1
suspected but ruled-out tick-borne encephalitis (TBE)	51	6

2. Materials and methods

2.1. Biobank and ethics

The retrospective analysis for BoDV-1 infections of cerebrospinal fluid (CSF) and serum samples stored in the biobank of the Center for Virology of the Medical University Vienna was approved by the Ethics Committee of the Medical University Vienna (reference number 1231/2023). All samples were pseudonymized and processed according to the standards of our diagnostic laboratories. All procedures performed in this study were in accordance with the Helsinki declaration as revised in 2013 and its later amendments.

2.2. BoDV-1 RT-qPCR

RNA extraction for BoDV-1 RT-qPCR was performed using the TANBead Nucleic Acid Extraction Kit (TANBead, Taoyuan City, Taiwan) according to the manufacturer's instructions. For RNA extraction, 200 µL of CSF samples were used (eluted volume 50 µL).

The RT-qPCRs were performed using Invitrogen SuperScript III One-Step RT-PCR System with Platinum Taq DNA Polymerase (Invitrogen Thermo Fisher Scientific, Waltham, MA, USA).

Each reaction contained 5.1 µL RNase-free water, 12.5 µL 2× SuperScript III One-Step RT-PCR ReactionMix, 1.0 µL of the primer-probe mix, 1.0 µL SuperScript III One-Step RT-PCR EnzymeMix, and 0.4 µL SuperScript III One-Step RT-PCR $MgSO_4$ in a total volume of 20 µL. The thermal program consisted of 1 cycle of 55 °C for 20 min and 94 °C for 3 min, followed by 45 cycles of 94 °C for 15 s and 58 °C for 30 s. All RT-qPCRs were performed with the CFX96 Real-Time PCR Detection System (Bio-Rad, Hercules, CA, USA). A concentration of 300 RNA copies/mL was determined as lower limit of detection by titrating an externally quantified positive sample provided by the Institute of Clinical Microbiology and Hygiene of the University Hospital Regensburg. Primer and probe sequences targeting the x/p gene region of BoDV-1 were as follows (Schindler et al., 2007):

Forward Primer: 5`-TCCCTGGAGGACGAAGAAGAT-3`
Reverse Primer: 5`-CTTCCGTGGYCTTGGTGACC-3`
Probe: 5`-FAM-CCAGACACTACGACGGGAACGA-TAMRA-3`

Table 2
Location of medical facilities collecting CSF samples (demographic data for 2019 retrieved from www.statistik.at).

Location of medical facility colleting the sample	Inhabitants in the year 2019 [range]	Federal State of Austria	CSF samples [n]	CSF samples [%]
Vienna	1,910,000—1,915,000	Vienna	469	54.6
Eisenstadt	10,000—15,000	Burgenland	228	26.5
Linz	205,000—210,000	Upper Austria	38	4.4
Mistelbach	10,000—15,000	Lower Austria	34	4.0
Wels	60,000—65,000	Upper Austria	27	3.1
Amstetten	20,000—25,000	Lower Austria	20	2.3
Tulln an der Donau	15,000—20,000	Lower Austria	17	2.0
Scheibbs	1000—5000	Lower Austria	7	0.8
Klagenfurth am Wörthersee	100,000—105,000	Carinthia	3	0.3
Vöcklabruck	10,000—15,000	Upper Austria	3	0.3
Graz	290,000—295,000	Styria	2	0.2
Salzburg	155,000—160,000	Salzburg	2	0.2
St. Pölten	55,000—60,000	Lower Austria	2	0.2
Wiener Neustadt	45,000—50,000	Lower Austria	2	0.2
Friesach	1000—5000	Carinthia	1	0.1
Melk	5000—10,000	Lower Austria	1	0.1
Schwarzach im Pongau	1000—5000	Salzburg	1	0.1
Steyr	35,000—40,000	Upper Austria	1	0.1
Villach	60,000—65,000	Carinthia	1	0.1

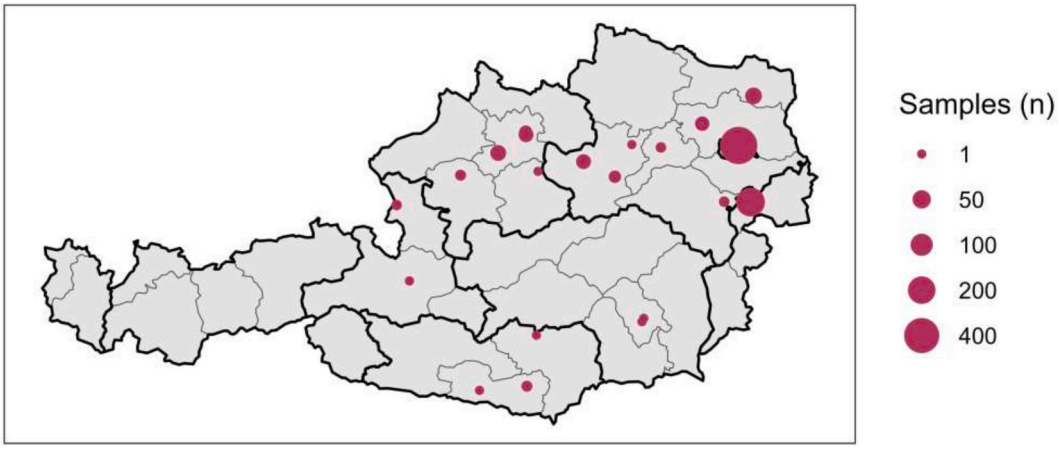


Fig. 1. Location of medical facilities where CSF samples were collected.
Dots in the map of Austria show the location of medical facilities collecting CSF samples that were included in the study, the diameter represents the number of samples associated with a settlement.

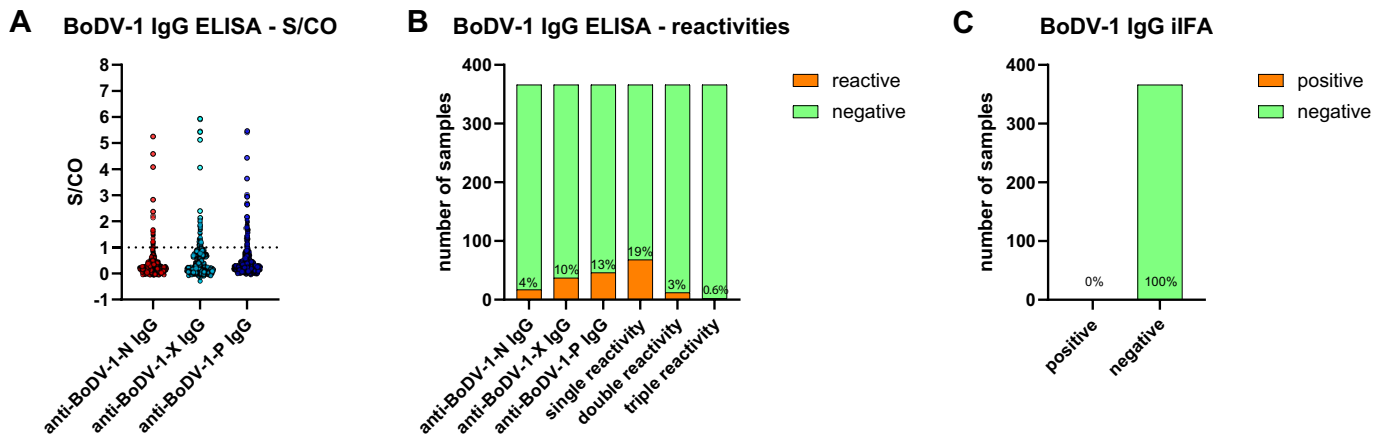


Fig. 2. Screening by BoDV-1 serology.
All serum samples ($n = 366$) were tested by a triple BoDV-1 IgG ELISA system (anti-BoDV-1-N-IgG, anti-BoDV-1-X-IgG, anti-BoDV-1-P-IgG). A shows sample-to-cut-off ratios (S/CO) for all individual samples, while absolute numbers and percentages of sample reactivities (S/CO >1.00) in the different single ELISA assays as well as an overview of single, double, and triple reactivites of samples are depicted in B. All ELISA-reactive (and all ELISA-negative) samples were tested negative by a BoDV-1 iIFA (C). Thus, all ELISA reactivites were interpreted as false reactive. In conclusion, in none of the 366 serum samples confirmed anti-BoDV-1 IgG antibodies were detected.

2.3. BoDV-1 serology

In accordance with the workflow for diagnostic samples established at the Institute of Clinical Microbiology and Hygiene of the University Hospital Regensburg, a BoDV-1 IgG enzyme-linked immunosorbent assay (ELISA) system using three different recombinant BoDV-1 proteins and a BoDV-1 IgG indirect immunofluorescence assay (iIFA) were performed as previously described (Neumann et al., 2022; Bauswein et al., 2023). For all tests, a well-characterized positive sample was used as positive control.

2.4. Data analysis

Data were analyzed and figures were created using R software (The R Foundation for Statistical Computing, Vienna, Austria) and GraphPad Prism (GraphPad Software, San Diego, CA, USA).

3. Results

3.1. Screening with BoDV-1 RT-qPCR

Stored CSF samples ($n = 859$) from the biobank of the Center for Virology of the Medical University Vienna were selected for subsequent screening by BoDV-1 RT-qPCR based on certain clinical characteristics (see Table 1).

CSF samples were collected between January 2019 and August 2022, in median four days after symptom onset (range 0–310 days). The location of the medical facilities where CSF samples were collected is shown in Fig. 1 and Table 2.

In none of the 859 CSF samples BoDV-1 RNA was detected.

3.2. Screening with BoDV-1 serology

For 366 of the 859 PCR-tested CSF samples, corresponding serum samples were available. Serum samples were collected between January 2019 and August 2022, in median four days after symptom onset (range 0–212 days). In accordance with the workflow for diagnostic samples, the serum samples were examined by both a previously published BoDV-1 ELISA system using recombinant viral N, X, and P protein and by an iIFA to test for anti-BoDV-1 IgG antibodies (Neumann et al., 2022; Bauswein et al., 2023; Korn et al., 2018).

In none of the tested serum samples confirmed anti-BoDV-1 IgG antibodies were detectable (Fig. 2). False ELISA-reactivities were in the range of previous serological studies (Bauswein et al., 2023; Bauswein et al., 2024; Böhmer et al., 2024).

4. Discussion

No human BoDV-1 infections have been reported from Austria so far, while a circumscribed region in Upper Austria is considered to be an endemic region based on cases in sentinel animals (Ebinger et al., 2024).

Therefore, we initiated a retrospective case-finding study in Austria using human samples from the biobank of the Center for Virology of the Medical University Vienna. The pre-test probability was increased by the selection of 859 CSF samples which were stored from patients with neurological symptoms potentially resembling those of a BoDV-1 infection. The identification of a patient suffering from BoDV-1 encephalitis within a cohort of patients with requested diagnostics for tick-borne encephalitis (TBE) in a recently published study from an endemic region in Germany was the rationale to additionally include patients with requested but ruled-out TBE in the present study (Bauswein et al., 2023).

However, neither in any of the 859 CSF samples screened by BoDV-1 RT-qPCR nor in any of the corresponding 366 sera screened by BoDV-1 serology, the diagnostic constellation of a suspected or confirmed BoDV-1 infection was detected.

RNA degradation in the samples cannot be completely ruled out as a contributing factor for negative BoDV-1 RT-qPCRs in a diagnostic material such as CSF, for which the expected RNA copy numbers are very low and often close to the lower limit of detection. Furthermore, the timing of sample collection after symptom onset may have influenced the validity of BoDV-1 serology, as seroconversion regularly occurs only several days after hospitalization when severe symptoms are already present (Neumann et al., 2022). However, in combination with the BoDV-1 RT-qPCR performed from CSF samples, the negative serology is valuable because it increases the negative predictive value of PCR-negative CSF results, as the sensitivity of PCR from CSF decreases after seroconversion (Allartz et al., 2024).

69 (8 %) of CSF samples included in this study were collected in medical facilities with location in Upper Austria, the only so far known endemic region of BoDV-1 in Austria. Rural residence within endemic regions represents the main risk factor for human BoDV-1 infections according to a recent case-control study (Pörtner et al., 2023), while there is only one published case in which travelling to an endemic region

resulted in infection (Meyer et al., 2022). Data on the actual residence of patients (urban or rural) and on potential travel activity to the circumscribed BoDV-1 endemic in Upper Austria are unfortunately lacking for the biobank CSF samples screened in this study. Further research using samples with a more distinct geographical link to the circumscribed endemic rural region in Upper Austria, however, will be necessary to complement the preliminary finding of this present study.

CRediT authorship contribution statement

Markus Bauswein: Writing – review & editing, Writing – original draft, Visualization, Validation, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Lisa Arnold:** Writing – review & editing, Methodology, Investigation, Formal analysis, Data curation. **David N. Springer:** Writing – review & editing, Visualization, Methodology, Investigation, Formal analysis, Data curation. **Monika Redlberger-Fritz:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Consent for publication

Not applicable (pseudonymized retrospective data).

Ethics approval and consent to participate

The retrospective analysis for BoDV-1 infections of CSF and serum samples stored in the biobank of the Center for Virology of the Medical University Vienna was approved by the Ethics Committee of the Medical University Vienna (reference number 1231/2023). All samples were pseudonymized and processed according to the standards of our diagnostic laboratories. All procedures performed in this study were in accordance with the Helsinki declaration as revised in 2013 and its later amendments.

Funding

The study was in part funded by the Bavarian State Ministry of Health, Care, and Prevention (StMGPP), project "Zoonotic Bornavirus Focal Point Bavaria – ZooBoFo" (to M.B.).

Declaration of competing interest

The authors declare no competing interests.

Acknowledgements

We thank Sylvia Malik and Michaela Binder for their excellent technical assistance.

Data availability

The data supporting the finding of this study are available on Mendeley Data (http://data.mendeley.com; doi: 10.17632/cd5zrngjh4.1).

References

Allartz, P., Hotop, S.-K., Muntau, B., Schlaphof, A., Thomé-Bolduan, C., Gabriel, M., et al., 2024. Detection of bornavirus-reactive antibodies and BoDV-1 RNA only in encephalitis patients from virus endemic areas: a comparative serological and molecular sensitivity, specificity, predictive value, and disease duration correlation study. Infection 52, 59–71. https://doi.org/10.1007/s15010-023-02048-1.

Bauswein, M., Eidenschink, L., Knoll, G., Neumann, B., Angstwurm, K., Zoubaa, S., et al., 2023. Human infections with Borna disease virus 1 (BoDV-1) primarily Lead to severe encephalitis: further evidence from the Seroepidemiological BoSOT study in an endemic region in southern Germany. Viruses. https://doi.org/10.3390/v15010188.

Bauswein, M., Knoll, G., Schmidt, B., Gessner, A., Hemmer, B., Flaskamp, M., 2024. No evidence of an association of multiple sclerosis (MS) with Borna disease virus 1 (BoDV-1) infections in patients within an endemic region: a retrospective pilot study. Infection 52, 243–247. https://doi.org/10.1007/s15010-023-02099-4.

Böhmer, M.M., Haring, V.C., Schmidt, B., Saller, F.S., Coyer, L., Chitimia-Dobler, L., et al., 2024. One health in action: investigation of the first detected local cluster of fatal Borna disease virus 1 (BoDV-1) encephalitis, Germany 2022. J. Clin. Virol. 171, 105658. https://doi.org/10.1016/j.jcv.2024.105658.

Bourg, M., Herzog, S., Encarnação, J.A., Nobach, D., Lange-Herbst, H., Eickmann, M., Herden, C., 2013. Bicolored white-toothed shrews as reservoir for Borna disease virus, Bavaria, Germany. Emerg. Infect. Dis. 19, 2064–2066. https://doi.org/10.3201/eid1912.131076.

Dubey, S., Cosson, J.-F., Vohralík, V., Krystufek, B., Diker, E., Vogel, P., 2007. Molecular evidence of Pleistocene bidirectional faunal exchange between Europe and the near east: the case of the bicoloured shrew (Crocidura leucodon, Soricidae). J. Evol. Biol. 20, 1799–1808. https://doi.org/10.1111/j.1420-9101.2007.01382.x.

Dürrwald, R., Kolodziejek, J., Weissenböck, H., Nowotny, N., 2014. The bicolored white-toothed shrew Crocidura leucodon (HERMANN 1780) is an indigenous host of mammalian Borna disease virus. PLoS One 9, e93659. https://doi.org/10.1371/journal.pone.0093659.

Ebinger, A., Santos, P.D., Pfaff, F., Dürrwald, R., Kolodziejek, J., Schlottau, K., et al., 2024. Lethal Borna disease virus 1 infections of humans and animals - in-depth molecular epidemiology and phylogeography. Nat. Commun. 15, 7908. https://doi.org/10.1038/s41467-024-52192-x.

Haring, V.C., Litz, B., Jacob, J., Brecht, M., Bauswein, M., Sehl-Ewert, J., et al., 2024. Detection of novel orthoparamyxoviruses, orthonairoviruses and an orthohepevirus in European white-toothed shrews. Microb. Genom. https://doi.org/10.1099/mgen.0.001275.

Kolodziejek, J., Dürrwald, R., Herzog, S., Ehrensperger, F., Lussy, H., Nowotny, N., 2005. Genetic clustering of Borna disease virus natural animal isolates, laboratory and vaccine strains strongly reflects their regional geographical origin. J. Gen. Virol. 86, 385–398. https://doi.org/10.1099/vir.0.80587-0.

Korn, K., Coras, R., Bobinger, T., Herzog, S.M., Lücking, H., Stöhr, R., et al., 2018. Fatal encephalitis associated with Borna disease virus 1. N. Engl. J. Med. 379, 1375–1377. https://doi.org/10.1056/NEJMc1800724.

Meyer, T., Tappe, D., Hasan, D., Rust, M., Schulz, J.B., Schiefer, J., Tauber, S.C., 2022. "Borna disease virus 1" (BoDV-1)-Enzephalitis eines 18-Jährigen außerhalb des bisher bekannten Endemiegebietes. DGNeurologie 5, 300–304. https://doi.org/10.1007/s42451-022-00440-z.

Neumann, B., Angstwurm, K., Linker, R.A., Knoll, G., Eidenschink, L., Rubbenstroth, D., et al., 2022. Antibodies against viral nucleo-, phospho-, and X protein contribute to serological diagnosis of fatal Borna disease virus 1 infections. Cell Rep. Med. 3, 100499. https://doi.org/10.1016/j.xcrm.2021.100499.

Niller, H.H., Angstwurm, K., Rubbenstroth, D., Schlottau, K., Ebinger, A., Giese, S., et al., 2020. Zoonotic spillover infections with Borna disease virus 1 leading to fatal human encephalitis, 1999–2019: an epidemiological investigation. Lancet Infect. Dis. 20, 467–477. https://doi.org/10.1016/S1473-3099(19)30546-8.

Nobach, D., Bourg, M., Herzog, S., Lange-Herbst, H., Encarnação, J.A., Eickmann, M., Herden, C., 2015. Shedding of infectious Borna disease virus-1 in living bicolored white-toothed shrews. PLoS One 10, e0137018. https://doi.org/10.1371/journal.pone.0137018.

Nowotny, N., Kolodziejek, J., Jehle, C.O., Suchy, A., Staeheli, P., Schwemmle, M., 2000. Isolation and characterization of a new subtype of Borna disease virus. J. Virol. 74, 5655–5658. https://doi.org/10.1128/jvi.74.12.5655-5658.2000.

Pörtner, K., Wilking, H., Frank, C., Böhmer, M.M., Stark, K., Tappe, D., 2023. Risk factors for Borna disease virus 1 encephalitis in Germany - a case-control study. Emerg. Microbes Infect. 12, e2174778. https://doi.org/10.1080/22221751.2023.2174778.

Schindler, A.R., Vögtlin, A., Hilbe, M., Puorger, M., Zlinszky, K., Ackermann, M., Ehrensperger, F., 2007. Reverse transcription real-time PCR assays for detection and quantification of Borna disease virus in diseased hosts. Mol. Cell. Probes 21, 47–55. https://doi.org/10.1016/j.mcp.2006.08.001.

Schlottau, K., Forth, L., Angstwurm, K., Höper, D., Zecher, D., Liesche, F., et al., 2018. Fatal encephalitic Borna disease virus 1 in solid-organ transplant recipients. N. Engl. J. Med. 379, 1377–1379. https://doi.org/10.1056/NEJMc1803115.

Weissenböck, H., Bagó, Z., Kolodziejek, J., Hager, B., Palmetzhofer, G., Dürrwald, R., Nowotny, N., 2017. Infections of horses and shrews with Bornaviruses in Upper Austria: a novel endemic area of Borna disease. Emerg. Microbes Infect. 6, e52. https://doi.org/10.1038/emi.2017.36.