Zoonotic spillover infections with Borna disease virus 1 leading to fatal human encephalitis, 1999–2019: an epidemiological investigation

Hans Helmut Niller, Klemens Angstwurm*, Dennis Rubbenstroth*, Kore Schlottau, Arnt Ebinger, Sebastian Giese, Silke Wunderlich, Bernhard Banas, Leonie F Forth, Donata Hoffmann, Dirk Höper, Martin Schwemmle, Dennis Tappe, Jonas Schmidt-Chanasit, Daniel Nobach, Christiane Herden, Christoph Brochhausen, Natalia Velez-Char, Andreas Mamilos, Kirsten Utpatel, Matthias Evert, Saida Zoubaa, Markus J Riemenschneider, Viktoria Ruf, Jochen Herms, Georg Rieder, Mario Errath, Kaspar Matiasek, Jürgen Schlegel, Friederike Liesche-Starnecker, Bernhard Neumann, Kornelius Fuchs, Ralf A Linker, Bernd Salzberger, Tobias Freilinger, Lisa Gartner, Jürgen J Wenzel, Udo Reischl, Wolfgang Jilg, André Gessner, Jonathan Jantsch, Martin Beer*, Barbara Schmidt**

Summary

Background In 2018–19, Borna disease virus 1 (BoDV-1), the causative agent of Borna disease in horses, sheep, and other domestic mammals, was reported in five human patients with severe to fatal encephalitis in Germany. However, information on case frequencies, clinical courses, and detailed epidemiological analyses are still lacking. We report the occurrence of BoDV-1-associated encephalitis in cases submitted to the Institute of Clinical Microbiology and Hygiene, Regensburg University Hospital, Regensburg, Germany, and provide a detailed description of newly identified cases of BoDV-1-induced encephalitis.

Methods All brain tissues from 56 encephalitis cases from Bavaria, Germany, of putative viral origin (1999–2019), which had been submitted for virological testing upon request of the attending clinician and stored for stepwise diagnostic procedure, were systematically screened for BoDV-1 RNA. Two additional BoDV-1-positive cases were contributed by other diagnostic centres. Positive results were confirmed by deep sequencing, antigen detection, and determination of BoDV-1-reactive antibodies in serum and cerebrospinal fluid. Clinical and epidemiological data from infected patients were collected and analysed.

Findings BoDV-1 RNA and bornavirus-reactive antibodies were detected in eight newly analysed encephalitis cases and the first human BoDV-1 isolate was obtained from an unequivocally confirmed human BoDV-1 infection from the endemic area. Six of the eight BoDV-1-positive patients had no record of immunosuppression before the onset of fatal disease, whereas two were immunocompromised after solid organ transplantation. Typical initial symptoms were headache, fever, and confusion, followed by various neurological signs, deep coma, and severe brainstem involvement. Seven of nine patients with fatal encephalitis of unclear cause were BoDV-1 positive within one diagnostic centre. BoDV-1 sequence information and epidemiological analyses indicated independent spillover transmissions most likely from the local wild animal reservoir.

Interpretation BoDV-1 infection has to be considered as a potentially lethal zoonosis in endemic regions with reported spillover infections in horses and sheep. BoDV-1 infection can result in fatal encephalitis in immunocompromised and apparently healthy people. Consequently, all severe encephalitis cases of unclear cause should be tested for bornaviruses especially in endemic regions.

Funding German Federal Ministry of Education and Research.

Lancet Infect Dis 2020; 20: 467–77

Published Online January 7, 2020 https://doi.org/10.1016/S1473-3099(19)30546-8

See Comment page 389

*Contributed equally

Institute of Clinical Microbiology and Hygiene (H H Niller MD, Prof J J Wenzel MD, Prof U Reischl PhD, Prof W Jilg MD, Prof A Gessner MD, Prof J Jantsch MD, Prof B Schmidt MD), **Department of Nephrology** (Prof B Banas MD), **Department of Neuropathology** (S Zoubaa MD, Prof M J Riemenschneider MD), **and Infectious Diseases** (Prof B Salzberger MD), **Regensburg University Hospital, Regensburg, Germany; Department of Neurology** (K Angstwurm MD, B Neumann MD, K Fuchs MD, Prof R A Linker MD), **Institute of Pathology** (Prof C Brochhausen MD, N Velez-Char MD, A Mamilos MD, K Utpatel MD, Prof M Evert MD), **University of Regensburg, Regensburg, Germany; Institute of Diagnostic Virology, Friedrich-Loeffler-Institut, Greifswald-Insel Riems, Germany** (D Rubbenstroth DVM, K Schlottau PhD, A Ebinger MSc, L F Forth PhD, D Hoffmann DVM, D Höper PhD, Prof M Beer DVM); **Institute of Virology, University Medical Center Freiburg, Faculty of Medicine, University of Freiburg, Freiburg, Germany** (D Rubbenstroth, S Giese PhD, Prof M Schwemmle PhD); **Department of Neurology,**

Introduction

Borna disease virus 1 (BoDV-1; species Mammalian 1 orthobornavirus, family Bornaviridae) is the causative agent of Borna disease, a mostly fatal neurological disorder of horses, sheep, and other domestic mammals in southern and eastern Germany, Austria, Switzerland, and Liechtenstein.[1,2] The bicoloured white-toothed shrew (*Crocidura leucodon*) is the only known natural reservoir host of BoDV-1.[3–7] In this host, BoDV-1 establishes a persistent infection with remarkably broad tissue tropism, but without apparent clinical disease.[4,5,7] By contrast, the virus is almost exclusively neurotropic and causes a T lymphocyte-mediated encephalitis in erroneous spill-over hosts, such as horses and sheep.[1,2,8]

The zoonotic potential of BoDV-1 has been a matter of an unresolved scientific dispute for decades. Worldwide distribution of human BoDV-1 infection had been postulated mostly in the context of affective or psychiatric disorders, such as depression or schizophrenia, whereas severe encephalitis cases were rarely investigated.[1,2,9–14] However, BoDV-1 sequences and isolates from these studies were demonstrated to be the likely result of

Klinikum rechts der Isar, School of Medicine (S Wunderlich MD), **Department of Neuropathology** (Prof J Schlegel MD, F Liesche-Starnecker MD), **Technical University of Munich, Munich, Germany; Bernhard Nocht Institute for Tropical Medicine, Hamburg, Germany** (Prof D Tappe MD, Prof J Schmidt-Chanasit MD); **Faculty of Mathematics, Informatics and Natural Sciences, University of Hamburg, Hamburg, Germany** (Prof J Schmidt-Chanasit); **Institute of Veterinary Pathology, Justus-Liebig-University Giessen, Giessen, Germany** (D Nobach DVM, Prof C Herden DVM); **Center for Neuropathology and Prion Research** (V Ruf MD, Prof J Herms MD) **and Section of Clinical & Comparative Neuropathology, Centre for Clinical Veterinary Medicine** (Prof K Matiasek DVM), **Ludwig-Maximilians-Universität München, Munich, Germany; Department of Neurology, Klinikum Traunstein, Traunstein, Germany** (G Rieder MD, M Errath MD); **Department of Neurology, Klinikum Passau, Passau, Germany** (T Freilinger MD, L Gartner MD); **and Hertie-Institute for Clinical Brain Research, University Tuebingen, Tuebingen, Germany** (T Freilinger)

Correspondence to:
Prof Martin Beer, Institute of Diagnostic Virology, Friedrich-Loeffler-Institut, 17493 Greifswald-Insel Riems, Germany
martin.beer@fli.de

Research in context

Evidence before this study

We searched PubMed for research articles published in English and German between inception and July 11, 2019, with the following search terms: "Bornaviridae" OR "Borna disease" OR "bornavirus". The search yielded 1042 results from 1931 to 2019. We focused on studies reporting animal and human bornavirus infections since 1985. The zoonotic potential of Borna disease virus 1 (BoDV-1) has been a matter of controversy for decades. Studies reporting the identification of BoDV-1 infection markers in humans were demonstrated by subsequent studies to be the likely result of laboratory contamination, and serological tests were often not validated or irreproducible. In addition, bornavirus-reactive antibodies were shown to be highly cross-reactive within the genus Orthobornavirus, and therefore serological findings alone could not be interpreted as proof of BoDV-1 infection. Thus, evidence for any zoonotic risk posed by bornaviruses had not been presented until 2015, when zoonotic transmission of the newly discovered variegated squirrel bornavirus 1 (VSBV-1) resulted in fatal encephalitis in at least four exposed people. Finally, three publications confirmed, by independent methods, for the first time that BoDV-1 infection induced fatal encephalitis in a cluster of solid organ transplant recipients and two additional unrelated cases in Germany. Thus, bornavirus infections have to be considered as hitherto unknown lethal zoonoses.

Added value of this study

Our study provides comprehensive data about the occurrence, course of infection, and epidemiology of zoonotic bornavirus infections, and the identification of eight further BoDV-1 infections in humans between 1999 and 2019 emphasises the zoonotic potential of BoDV-1 in endemic regions. We found BoDV-1 infection in two further immunocompromised patients and in six patients with no record of immunosuppression. Seven of nine fatal encephalitis cases in one diagnostic centre were BoDV-1 positive, whereas none of the surviving patients in this study tested positive. All patients lived within the known endemic area of BoDV-1 in central Europe. Phylogenetic analysis indicates multiple independent infections from a local reservoir without further transmission of the virus (so-called spillover infections to dead-end hosts).

Implications of all the available evidence

Based on these new data, BoDV-1 infection has to be considered as a severe and potentially lethal zoonosis. It does not represent a newly emerging entity in the known BoDV-1 endemic region but appears to have occurred unnoticed in humans for at least decades. Particularly in severe and fatal non-purulent encephalitis of unknown aetiology, bornaviruses have to be considered as potential causative agents. Our data confirm the role of BoDV-1 as a relevant zoonotic pathogen, provide the basis for a case definition, and emphasise the importance of differential diagnostics of severe and fatal encephalitis.

laboratory contamination because these sequences were almost identical to sequences of laboratory strains used in the respective institutions, and epidemiological links to the endemic area could not be presented.[14–16] Furthermore, reported bornavirus-reactive antibodies and viral antigens in serum samples could not be confirmed by independent methods.[1,13] In addition, bornavirus-reactive antibodies were shown to be highly cross-reactive within the genus Orthobornavirus, and therefore serological findings alone could not be interpreted as proof of BoDV-1 infection.[17]

The discovery of variegated squirrel bornavirus 1 (VSBV-1; species Mammalian 2 orthobornavirus) in Germany in 2015, which causes fatal encephalitis after transmission from exotic squirrels to humans, moved the zoonotic potential of mammalian bornaviruses back into focus.[18,19] In 2018–19, BoDV-1 was reported in three cases of encephalitis in solid organ transplant recipients infected via organs received from the same donor and two further transplantation-independent cases.[20–22] The organ donor had shown no signs of neurological disease and had died of suspected sudden cardiac arrest. Both kidney graft recipients died from BoDV-1-induced polyradiculoneuritis and encephalitis or encephalomyelitis, whereas the liver recipient survived and recovered with sequelae from leukencephalopathy and visual constraint due to optic nerve atrophy.[21] The donor and the patients who had not received transplants lived in known BoDV-1-endemic regions in Bavaria, southern Germany.[20–22]

In the first part of this study, we focused on the occurrence of BoDV-1-associated encephalitis cases in one large university hospital within the endemic area. In the second part, we provide a detailed description of eight newly identified cases of BoDV-1-induced encephalitis. We present and discuss the results of clinical and virological investigations as well as epidemiological and phylogenetic analyses.

Methods

Sample retrieval

The study included brain tissues that had been sent to the accredited diagnostic section of the Institute of Clinical Microbiology and Hygiene, Regensburg University Hospital (Regensburg, Germany), between January, 1995, and August, 2018, to clarify a possible viral cause of the encephalitis or encephalopathy. All brain samples from Bavaria, Germany, sent with an official request from the attending physician or neurologist to perform virological

PCR were stored immediately after arrival from the Department of Neurosurgery or Department of Neuropathology in the diagnostic repository of the Virology Department at either –80°C or at –20°C. A stepwise diagnostic procedure was done if requested by the attending clinician, starting with the more likely causes of viral encephalitis (eg, herpes simplex and varicella zoster virus) and followed by less frequent causes of the disease (eg, rabies). Samples in this diagnostic repository, which were obtained from patients submitted until August, 2018, were tested retrospectively for bornavirus infections (51 patients). This procedure was approved by the ethical commission of the Faculty for Medicine, University of Regensburg (reference number 18-1248-101). Starting in September, 2018, bornaviruses were included in the routine diagnostic panel for encephalitis cases in Regensburg (five patients). A detailed description of this sample panel is provided in the appendix (p 1).

Two additional BoDV-1 cases included in this study were independently identified at the Department of Neuropathology, Technical University of Munich (Munich, Germany), and at the Center for Neuropathology and Prion Research, Ludwig Maximilian University of Munich, in 2017 by bornavirus diagnostics done on brain autopsy samples of patients with suspected viral encephalitis, after the first cases of fatal BoDV-1 encephalitis had emerged in 2016.

RNA extraction and detection of BoDV-1 RNA

We extracted total RNA from native samples using TRIzol Reagent (Life Technologies, Darmstadt, Germany) in combination with RNeasy Mini Kit (Qiagen, Hilden, Germany) or NucleoMagVet kit (Macherey & Nagel, Düren, Germany) according to the manufacturers' instructions. Native samples for high-throughput sequencing were disintegrated with the Covaris cryoPREP (Covaris, Brighton, UK) before RNA extraction.[23] Nucleic acids from formalin-fixed paraffin-embedded (FFPE) sections were extracted using miRNeasy FFPE Kit (Qiagen). BoDV-1 RNA was detected using two real-time RT-qPCR assays: mix 1 was used for BoDV-1 phophoprotein (P) gene RNA and mix 6 for matrix protein (M) gene RNA, following procedures described in detail elsewhere.[21] The RT-qPCR mix 6 is particularly suited to detect degraded RNA because of a very short amplicon of 75 bp. All precautions of accredited laboratories, particularly separate rooms for nucleic acid extraction, amplification, and detection, were taken to exclude any cross-contamination.

High-throughput sequencing

Library preparation and sequencing of BoDV-1-positive samples were done depending on their RNA quality, as determined by the RNA 6000 Pico Kit (Agilent Technologies, Waldbronn, Germany). Libraries from high-quality RNA (patients P5, P7, and P8) were processed following the protocol of Wylezich and colleagues,[23] and a 400 bp sequencing run was done on an Ion Torrent S5XL instrument using a 530 chip (Thermo Fisher Scientific, Darmstadt, Germany). For degraded RNA (patients P1, P2, P3, P4, and P6), a modified protocol was used (appendix p 1) and a 200 bp sequencing run was done using a 540 chip. BoDV-1 consensus sequences were determined by an iterative mapping and assembly approach with the 454 software suite (version 3.0; Roche, Mannheim, Germany), using the complete genome of BoDV-1 strain V (GenBank accession number U04608)[24] as the reference sequence. All sequences are available from the International Nucleotide Sequence Database under accession numbers LR722641 to LR722647.

Phylogenetic analysis

Partial human BoDV-1 nucleoprotein (N) gene sequences (1056 bp, representing positions 137–1192 of the complete BoDV-1 genome; GenBank accession number U04608) were analysed together with sequences from naturally infected shrews and agricultural animals from the endemic regions in Germany, Austria, Switzerland, and Liechtenstein. Phylogenetic trees were built using Neighbor-Joining algorithm and Jukes-Cantor distance model in Geneious R10 and rooted with sequence BoDV-2 No/98 (AJ311524).

See Online for appendix

Detection of bornavirus-reactive antibodies

Patient sera and cerebrospinal fluid (CSF) samples were screened for the presence of bornavirus-reactive IgG by indirect immunofluorescence assays (iIFAs) using BoDV-1-infected Vero cells according to previously described procedures.[17,21,25] Antibodies directed against recombinant BoDV-1 phosphoprotein were detected as described previously using a bornavirus IgG immunoblot (line immunoassay system, EUROIMMUN AG, Lübeck, Germany).[19] The BoDV-1-reactive samples were independently tested by two or three specialised laboratories (University Medical Center Freiburg, Freiburg, Germany; Friedrich-Loeffler-Institut, Greifswald-Insel Riems, Germany, and Bernhard Nocht Institute for Tropical Medicine, Hamburg, Germany), which regularly participated in interlaboratory comparisons.

Immunohistochemistry and in-situ hybridisation

FFPE tissue sections were stained for bornavirus antigens by use of a standard immunohistochemistry staining protocol with monospecific polyclonal and monoclonal antibodies targeting BoDV-1 nucleoprotein, phosphoprotein, and X protein.[18,21,26] In-situ hybridisation was done following protocols for the detection of genomic RNA and mRNA of the BoDV-1 nucleoprotein N gene.[21,27]

Virus isolation

Infectious BoDV-1 was isolated from brain samples of patient P8 following standard procedures. Briefly, fresh tissue was homogenised in cell culture medium and subsequently ultrasonicated using a Branson Sonifier 450 (Emerson, St Louis, MO, USA). Following

centrifugation for 10 min at 10 000×*g* to remove tissue debris, 100 µL of supernatant was mixed with single-cell suspensions of Vero cells and incubated at 37°C and 5% carbon dioxide. After 24 h, the inoculum was replaced by fresh cell culture medium. Inoculated cell cultures were passaged at approximately 4-day intervals. At approximately weekly intervals (appendix p 9), a sub-culture was stained by an immunofluorescence assay to visualise BoDV-1-infected cells[17] and viral RNA was quantified with RT-qPCR. Virus isolation was done in parallel in three laboratories in two independent diagnostic centres (two laboratories in two independent biosafety units at the Friedrich-Loeffler Institute and one laboratory at the Institute of Clinical Microbiology and Hygiene, University Hospital Regensburg).

Role of the funding source

The funder of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. MB and BSc had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

Brain biopsies or post-mortem brain tissue taken during a symptomatic phase of disease were available for 56 patients (figure 1). 28 (50%) of these patients had been diagnosed with malignant neoplasia (n=15), infectious diseases (ten), autoimmune disorders (one), intracranial haemorrhage (one), and cerebral infarction (one), but the cause of the neurological disease had not been identified for the remaining 28 patients (figure 1). Nine (32%) of 28 patients without a definite diagnosis had died from encephalitis, whereas 15 (54%) patients in this group survived the disease. Clinical records were not available for samples submitted by external institutions for four (12%) patients (figure 1).

Brain tissues from all 56 patients were tested for BoDV-1 RNA by two independent RT-qPCR tests. Samples of seven of the nine fatal encephalitis cases of unknown cause were positive for BoDV-1 RNA (figure 1). By contrast, none of the samples from the 28 patients with a definite diagnosis or those from the 19 remaining cases with unclear aetiology and with non-fatal or unknown course of disease tested positive (figure 1).

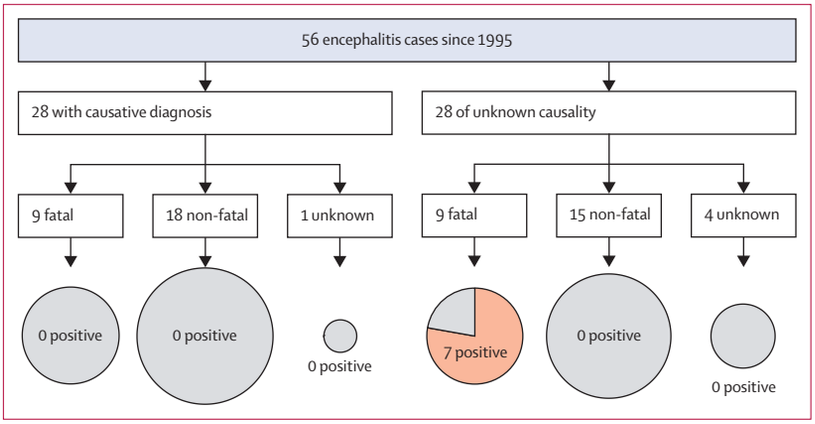


Figure 1: **Retrospective and diagnostic screening of putative viral encephalitis cases for BoDV-1 infection at the University Hospital Regensburg (Regensburg, Germany) since 1995**
BoDV-1=Borna disease virus 1.

The seven BoDV-1-positive patients from Regensburg included the previously published kidney transplant recipient[21] and six newly identified patients. These six newly discovered cases are presented together with two additional fatal BoDV-1 infections that had been identified independently by diagnostic testing for bornaviruses in other centres in Bavaria (table 1).

The eight newly discovered BoDV-1-infected patients died between 1999 and 2019. They were white European and 17–65 years old (mean 38·6 years [SD 15·0]), and included six women and two men. Six patients had no known record of immunosuppression before the onset of BoDV-1-associated symptoms, whereas two patients had received immunosuppressive therapy after solid organ transplantation 16 or 3 months before hospital admission (table 1).

In seven cases, the disease started with both headache and fever. In all patients, the disease continued with neurological signs, including unsteady gait, confusion, memory deficits, seizures, and progressive loss of consciousness (table 1). Following hospital admission, the clinical state progressively deteriorated, leading to deep coma and loss of brainstem reflexes. Patients died within 16–57 days after hospital admission (mean 39·5 days [SD 14·2]).

Initial MRI of the brain did not reveal any lesions in six of the patients, whereas in the remaining two patients non-gadolinium-enhancing lesions were observed in the temporal lobe and in one case also in the frontal lobe. Later MRI showed involvement of the frontal and temporal lobes, peri-insular cortex, basal ganglia, and brainstem in all but two patients, with gadolinium enhancement observed in four patients (table 1; appendix p 7).

Electroencephalography showed diffuse slowing in all individuals, seen in seven cases within 1–6 days of the disease. In seven patients, initial CSF analysis revealed increased leucocyte counts (9–343 leucocytes per µL; normal: <5 leucocytes per µL), lactate concentration (2·3–3·8 mmol/L; normal: ≤2·1 mmol/L), and protein concentrations (473–1016 mg/L; normal: <450 mg/L). These parameters further increased during the course of disease (table 1). With the exception of patient P4, all patients also showed intrathecal immunoglobulin synthesis. Three patients had hyponatraemia and one patient had central diabetes insipidus.

All patients received antibiotics, or aciclovir or ganciclovir. Steroids were administered in four patients. Additional treatments, such as cidofovir, cyclophosphamide, or plasma exchange, were given to some patients without any apparent success (table 1).

Autopsy revealed panencephalitis (five patients) or meningoencephalitis (two) of suspected viral origin, accompanied by hypophysitis in one patient and myelitis in all three patients for whom the spinal cord was examined (table 1).

Very high to moderate levels of BoDV-1 RNA (quantitation cycle [Cq] values 10–28) could be detected by RT-qPCR in available frozen or FFPE brain tissues from seven patients. In the remaining patient (P2), the brain biopsy was only weakly positive (Cq 35), presumably because of severe RNA degradation in this sample (table 2; appendix p 2). In one patient (P8), the brachial plexus and sural nerve also tested positive (Cq 32 and 29, respectively; appendix p 3). CSF was inconsistently found weakly positive for BoDV-1 RNA (Cq values ≥34) in four of seven tested patients (table 2; appendix p 4). Viral RNA was not detected in fresh non-neuronal tissue available from one patient or in any serum sample tested from seven patients (table 2; appendix p 3).

BoDV-1 antigen and RNA were detected in FFPE brain slices from patient P5 by immunohistochemistry and in-situ hybridisation, which confirmed presence of the infection (appendix p 8). FFPE brain slices were not available for infection confirmation for the other patients.

Infectious virus was independently isolated in three diagnostic laboratories from the frontal and temporal lobes and medulla oblongata of patient P8. Persistent infection was established in Vero cell cultures and 100% of cells were infected after about 6 weeks (appendix p 9). Virus replication was further confirmed by RT-qPCR (appendix p 9), and a partial BoDV-1 genome sequence (2272 bp) was found to be identical to the original sequence obtained from patient P8. Comparisons of the sequence from patient P8 with available BoDV-1 sequences from other human infections and laboratory strains are shown in the appendix (p 5).

Further viral, bacterial, parasitic, or fungal infections were not detected by diagnostic PCR in these patients (data not shown).

Sera or CSF samples were available for testing from all eight patients with detectable BoDV-1 RNA and from 44 BoDV-1 RNA-negative patients included in this analysis. BoDV-1-reactive IgG antibodies were detectable in sera or CSF of all patients with evidence of BoDV-1 RNA but not in any tested sample from BoDV-1 RNA-negative patients. Antibody levels reached peak iIFA titres of 4000–16 000 in seven infected patients (figure 2; appendix p 6). For patient P6, only two CSF samples were available, which showed low anti-BoDV-1 titres of 20 and 80 (appendix p 6). Antibodies directed against BoDV-1 phosphoprotein were confirmed by a bornavirus immunoblot assay for all patients with high antibody titres (figure 2; appendix p 6). In agreement with previously published cases,[15,16] antibody levels were sometimes undetectable when the patients were admitted to hospital but rapidly increased thereafter (figure 2).

	Patient P1	Patient P2	Patient P3	Patient P4	Patient P5	Patient P6	Patient P7	Patient P8
Clinical								
Preceding medical state	Healthy	Healthy	Diabetic nephropathy, arterial hypertension, congestive heart disease, solid organ transplantation 16 months and reactive depression 5 months before admission	End-stage diabetic nephropathy, solid organ transplantation 3 months and pneumonia 2 weeks before admission	Healthy, herpes zoster infection 9 months before admission	Healthy	Healthy	One relapse of multiple sclerosis 8 years earlier, no immunotherapy, arterial hypertension, obesity
Previous immunosuppression	None	None	Tacrolimus, azathioprine	Cyclosporin A, mycophenolate mofetil, everolimus, tacrolimus, steroids	None	None	None	None
Initial symptoms	Headache, fever, night sweats, weight loss for 10 days, gait ataxia, confusion, progressive loss of consciousness	Headache, meningeal signs, dysphagia, confusion, high fever for 2–3 weeks, progressive loss of consciousness	Dizziness for 1 week, blurred speech, optical hallucinations	Fever, headache, meningism, confusion, epileptic seizures	Headache, fever, epileptic seizure, meningism, coma	Fever for 1 week, headache, apathy, memory deficits, seizures, loss of consciousness	Fever for 1–2 weeks, headache, confusion, mild hemiparesis, gait ataxia, seizures	Fever, headache, mild aphasia, confusion, coma within 6 days of hospital admission
Late symptoms	Deep coma	Deep coma, loss of brainstem reflexes	Vegetative state	Deep coma, loss of brainstem reflexes	Brain oedema, deep coma, brain death	Deep coma, loss of brainstem reflexes	Deep coma, loss of brainstem reflexes	Brain oedema, deep coma, loss of brainstem reflexes
Treatment	Antibiotics, aciclovir	Antibiotics, aciclovir	Ganciclovir for 3 days	Antibiotics, aciclovir, cidofovir (single dose)	Antibiotics, intravenous immunoglobulins, aciclovir, steroids, immune adsorption, cyclophosphamide	Aciclovir, antibiotics, steroids, intravenous immunoglobulins, plasma exchange	Aciclovir, antibiotics, steroids, plasma exchange, surgical decompression	Aciclovir, antibiotics, steroids, immune absorption
Autopsy findings	Panencephalitis	Panencephalitis	Meningoencephalitis, frontal atrophy	Panencephalitis	Panencephalitis, myelitis	Panencephalitis, myelitis	Meningoencephalitis, hypophysitis	Brainstem-accentuated meningoencephalomyelitis

(Table 1 continues on next page)

	Patient P1	Patient P2	Patient P3	Patient P4	Patient P5	Patient P6	Patient P7	Patient P8
(Continued from previous page)								
Imaging and electrophysiology								
Brain MRI*	Day 6, normal; day 22, symmetrical cortical and subcortical lesions in frontal and temporal lobes, peri-insular cortex, mesencephalon, pons, and medulla oblongata with partial gadolinium enhancement	Day 3, normal; day 46, involvement of mesial frontal and temporal lobes, insular cortex, basal ganglia, mesencephalon, and medulla oblongata with strong gadolinium enhancement	Day 11, normal; day 39, mild diffuse atrophy without focal abnormalities	Day 5, symmetrical lesions in frontal and temporal lobes, insular cortex, and basal ganglia without gadolinium enhancement; day 38, progressive involvement of brainstem	Day 5, swelling of left temporal lobe without gadolinium enhancement; day 12, progressive involvement of mesial frontal and temporal lobes, peri-insular cortex, basal ganglia, internal capsule, mesencephalon, and medulla oblongata with gadolinium enhancement, generalised oedema with herniation, spinal cord oedema	Day 2, normal; day 6, lesion in right hippocampus; day 14, symmetrical subcortical lesions in parietal and occipital lobes, splenium, and tractus corticospinalis without gadolinium enhancement; day 26, involvement of whole cortex, basal ganglia, thalamus, pons, and medulla oblongata without gadolinium enhancement	Day 1, normal; day 26, symmetrical cortical and subcortical lesions in frontal, parietal, and temporal lobes, basal ganglia, peri-insular cortex, mesencephalon, pons, and medulla oblongata with partial gadolinium enhancement, partially haemorrhagic	Days 2 and 9, normal
Electroenceph-alography*	Days 6 and 32, diffuse slowing	Days 1 and 26, diffuse slowing	Day 6, moderate diffuse slowing; day 16, moderate diffuse and left temporal slowing	Day 13, diffuse slowing; day 37, isoelectric	Day 4, diffuse slowing, intermittent rhythmic delta activity; day 12, isoelectric	Days 2, 5, 9, 13, and 23, severe diffuse slowing; days 16 and 18, burst suppression	Day 3, bifrontal slowing; day 7, diffuse slowing, left-sided PLEDs, late severe slowing; day 27, severe diffuse slowing	Day 1, diffuse slowing; day 14, isoelectric
Laboratory examination findings								
Cerebrospinal fluid	Maximum leucocyte count 30 cells per µL, lactate 4·0 mmol/L, protein 800 mg/L; intrathecal IgG synthesis†	Maximum leucocyte count 586 cells per µL, lactate 3·0 mmol/L, protein 1500 mg/L; intrathecal IgG synthesis†	Maximum leucocyte count 19 cells per µL, lactate 1·9 mmol/L, protein 470 mg/L; intrathecal IgG synthesis; 14-3-3 protein positive	Maximum leucocyte count 133 cells per µL, lactate 10·7 mmol/L, protein 5270 mg/L	Maximum leucocyte count 633 cells per µL, lactate 8·7 mmol/L, protein 5670 mg/L; intrathecal IgG synthesis; NSE 27·3 µg/L	Maximum leucocyte count 20 cells per µL, lactate 4·9 mmol/L, protein 2100 mg/L, intrathecal IgG synthesis, tau-protein >2200 pg/mL, 14-3-3 protein positive	Maximum leucocyte count 33 cells per µL, lactate 4·4 mmol/L, protein 1800 mg/L, intrathecal IgG or IgM synthesis; tau or phospho-tau protein elevated	Maximum leucocyte count 180 cells per µL, lactate 7·2 mmol/L, protein 2300 mg/L; intrathecal IgG, IgA, or IgM synthesis
Serum abnormalities	Hyponatraemia	Initial C-reactive protein 247 mg/L	None	None	Central diabetes insipidus	Initial hyponatraemia	Hyponatraemia	None
Epidemiology								
Season at onset of symptoms	Midsummer	Midsummer	Summer	Midsummer	Mid-spring	Winter	Autumn	Winter
Contact with animals	Dogs	Outdoor cats	Cats and dogs	Not reported	Outdoor cats, dogs, pet rodents	Horses, cattle, outdoor cats, rabbits	Unknown	Outdoor cats
Environment	Suburban, extensive outdoor activities	Rural, professional farming	Rural	Rural	Rural	Extensive outdoor activities, farming	Rural, farming	Rural, former farmer

BoDV-1=Borna disease virus 1. NSE=neuron-specific enolase. PLEDs=periodic lateralised epileptiforme discharges. *Indicated as days after admission. †Intrathecal immunoglobulin synthesis is either cerebrospinal fluid-specific oligoclonal bands or elevated IgG index.

Table 1: **Clinical, imaging, laboratory, and epidemiological characteristics of patients with fatal BoDV-1 encephalitis**

	Brain*			Peripheral nerves*			Non-neural tissues*			Cerebrospinal fluid*		Sera	
	n	Type	Cq†	n	Type	Cq†	n	Type	Cq†	n	Cq†	n	Cq†
Patient P1	2	Frozen	25–27	Not tested	..	..	Not tested	..	..	2	Negative	3	Negative
Patient P2	1	Frozen	35	Not tested	..	..	Not tested	..	..	2	Negative	3	Negative
Patient P3	2	FFPE	20–21	Not tested	..	..	Not tested	..	..	3	Negative	5	Negative
Patient P4	1	Frozen	19	Not tested	..	..	Not tested	..	..	2	34	6	Negative
Patient P5	3	Frozen	18–23	Not tested	..	..	Not tested	..	..	5	34 to negative	7	Negative
Patient P6	1	FFPE	20	Not tested	..	..	Not tested	..	..	2	34–35	Not tested	Not tested
Patient P7	8	Frozen	19–28	Not tested	..	..	Not tested	..	..	Not tested	Not tested	1	Negative
Patient P8	7	Frozen	10–23	5	Frozen	29 to negative	11	Frozen	Negative	1	35	2	Negative

BoDV-1=Borna disease virus 1. Cq=quantitation cycle. FFPE=formalin-fixed paraffin-embedded specimen. *Detailed results are presented in the appendix (p 3 for brain, peripheral nerves, and non-neuronal tissues, and p 4 for cerebrospinal fluid). †Cq values >37 are considered negative.

Table 2: **Detection of BoDV-1-specific RNA by RT-qPCR in tissue samples, cerebrospinal fluid, and sera**

Complete or partial BoDV-1 sequences were generated by high-throughput sequencing for seven cases. All human BoDV-1 sequences obtained clearly differed from each other as well as from widely used laboratory strains (figure 3; appendix pp 10–11), further excluding the possibility of cross-sample contamination. Sequences from four southeastern Bavarian patients (P5, P6, P7, and P8) belonged to the same subclade within BoDV-1 cluster 1A and were closely related to equine BoDV-1 sequences from this region (figures 3, 4). Patients P1 and P4 were residents of central Bavaria and their BoDV-1 sequences clustered with equine sequences of BoDV-1 cluster 2 from the same region (figures 3, 4). The BoDV-1 sequence of patient P3, who lived in northeastern Bavaria, belonged to cluster 4. Sequences of the same cluster were found also in two further human cases[20,22] and a sheep originating from northern Bavaria (figures 3, 4). Thus, phylogenetic analysis argued in clear favour of independent zoonotic transmission events from local sources for each of these cases. Sequence information was not available for case P2, but the patient originated likewise from a region where BoDV-1 is known to be endemic (figure 4).

Further epidemiological information gathered for all cases was analysed for potential sources of infection, including potential contact with bicoloured white-toothed shrews as well as with other animals. Seven patients lived in rural or suburban areas, and several were working in agriculture or were reported to perform other outdoor activities that might have brought them in contact with infected shrews. Three patients kept dogs and five owned cats or had otherwise close contact with them. Notably, two patients' cats were reported to regularly bring small mammals, including shrews. One patient owned pet rodents that tested negative for BoDV-1 and VSBV-1 in a broad range of organs.

Two patients had received solid organ transplants from two different donors several months before the onset of disease. In the case of patient P4, the donor originated from northern Germany, but the phylogenetic analysis of the patient-derived BoDV-1 cluster 2 sequence indicated an infection source close to the home of the recipient in Bavaria. Furthermore, the recipient of another organ of this donor has not developed neurological disease within 10 years following transplantation. In the case of patient P3, the origin of the donor remained unknown, but two further transplant recipients from this donor did not show neurological disorders for at least 12 years (German Organ Transplantation Foundation [DSO],

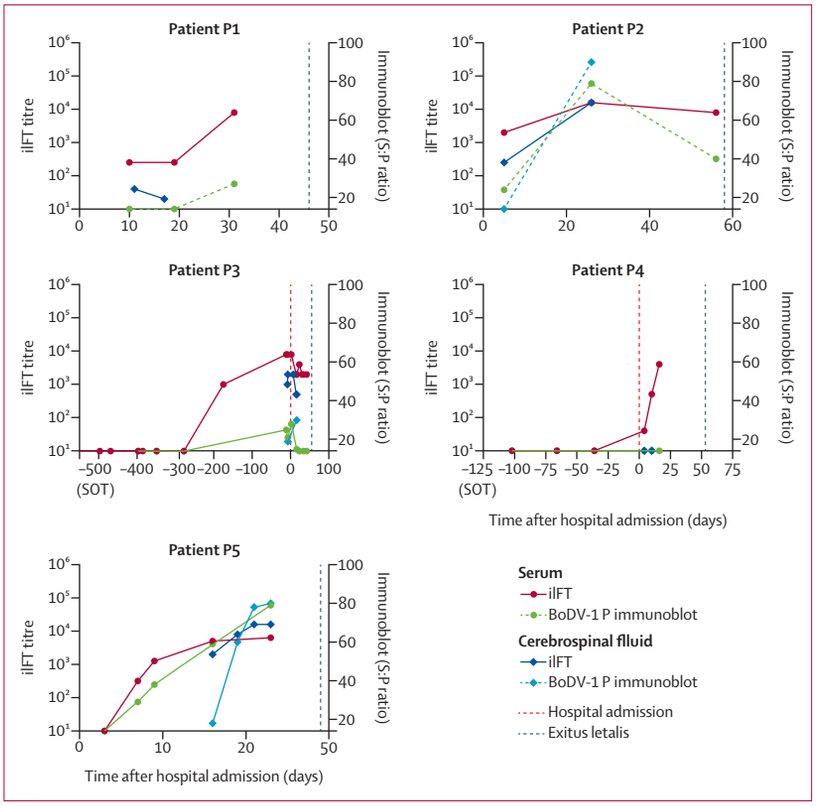
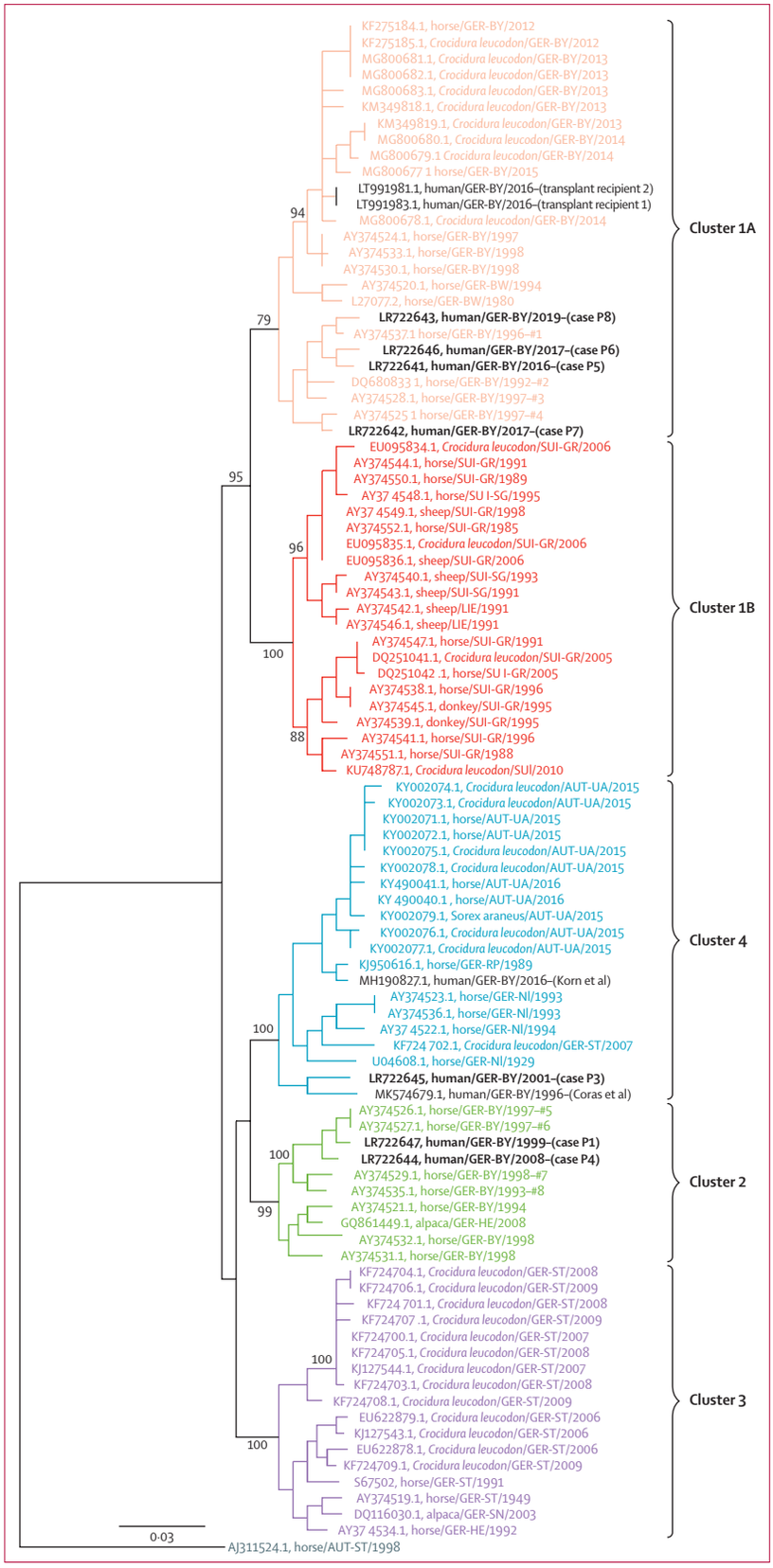


Figure 2: **Kinetics of BoDV-1-reactive antibodies in serum and cerebrospinal fluid samples**
Borna disease virus 1 (BoDV-1)-reactive antibodies were detected by either indirect immunofluorescence assay (iIFA; left Y axis) or the immunoblot assay (recombinant BoDV-1 phosphoprotein; right Y axis). The lower end of the Y axis represents the detection limit of the respective assay. Cases with less than three samples per sample type (P6, P7, and P8) are presented in the appendix (p 6). SOT=time patient received a solid organ transplantation. S:P ratio=sample:positive ratio.



personal communication). Thus, in both cases, the allograft has to be considered an unlikely source of BoDV-1 infection.

Discussion

We report eight newly discovered cases of fatal encephalitis associated with zoonotic BoDV-1 infection in southern Germany between 1999 and 2019. The presented cases raise the number of unequivocally confirmed and published human BoDV-1 infections in the endemic area to 14. The six previously published cases included BoDV-1 transmission from an infected solid organ transplant donor to three organ recipients[21] and two further unrelated cases.[20,22] All patients except for the transplant donor had severe encephalitis, which was lethal in eleven cases. One patient was released without recovery for palliative care, whereas only the published liver recipient recovered from the disease.[21] Seven of the fatal BoDV-1 infections had been diagnosed at the virology section of the Regensburg University Hospital. Strikingly, these seven cases constituted seven of nine fatal encephalitis cases of unknown cause tested in this diagnostic centre, whereas none of the tested surviving patients was bornavirus-positive. Thus, our data suggest that, although BoDV-1-associated encephalitis seems to be a relatively rare event in absolute numbers, it might constitute a high proportion of severe to fatal encephalitis cases of unknown cause within BoDV-1-endemic areas, in particular in immunosuppressed patients. Therefore, BoDV-1 has to be included in future differential diagnostic strategies for cases of suspected viral or autoimmune encephalitis.

Since iatrogenic transmission via solid organ transplantation had been demonstrated previously,[21] the same route of infection had to be considered for the two infected organ transplant recipients identified in this study. However, the unremarkable health status of additional graft recipients from the same donors argues strongly against transplant-associated transmission and in favour of local infection sources. In the case of patient P4, this argument was further supported by the close homology of the patient's BoDV-1 sequence to equine and human sequences from the respective region and the fact that the donor originated from outside the known endemic area.

***Figure 3*: Phylogenetic analysis of partial BoDV-1 N gene sequences of human and animal origin**

Human BoDV-1 sequences are depicted in black, and sequences generated during this study are in bold. Cluster designations, host, and geographical origin were adapted from previously published work.[4–6,15,20,21,28] Values at branches represent support in 1000 bootstrap replicates. Only bootstrap values of 70 or more at major branches are shown. BoDV-1=Borna disease virus 1. GER=Germany. BY=Bavaria. BW=Baden-Wuerttemberg. HE=Hesse. NI=Lower Saxony. RP=Rhineland-Palatinate. SN=Saxony. ST=Saxony-Anhalt. SUI=Switzerland. GR=Grisons. SG=St Gallen. LIE=Liechtenstein. AUT=Austria. UA=Upper Austria. ST=Styria.

BoDV-1 whole-genome sequences are now available from ten patients. With the exception of the previously published transplant cluster, all sequences were different from each other. In agreement with their origin from residents of southern Germany, all sequences belonged to BoDV-1 clusters 1A, 2, and 4. For each patient, including transplant recipient P4, the most closely related sequences were derived from spillover animal hosts, such as horses or sheep, from the home region of the respective patient or the transplant donor, clearly indicating independent local infection sources. This finding contrasts with previously published, supposedly human BoDV-1 isolates and sequences, which were found to be almost identical to sequences of widely distributed laboratory strains, regardless of their geographical origin (appendix pp 10–11).[15,16]

One of the limitations of our study is that the route (or routes) of BoDV-1 transmission from its reservoir host, the bicoloured white-toothed shrew, to humans and other accidental dead-end hosts remains elusive. For horses, infection is assumed to occur via feed contaminated with shrew excretions, and rats have been experimentally infected via intranasal and subcutaneous routes.[5,29,30] Potential direct or indirect contacts between humans and shrews are difficult to verify, particularly during retrospective case analysis. However, our analysis suggested several potential risk factors for infection, such as living in rural or suburban areas, agricultural work, or other outdoor activities and animal contacts. Notably, close contacts to cats had been reported for at least seven of the nine non-iatrogenically infected patients with available information, including the previously published Bavarian case[20] and the infected organ transplant donor (DSO, personal communication).[21] Cats are known to hunt shrews and bring them into their homes, which might facilitate the exposure of humans to BoDV-1-infected reservoir hosts. Alternatively, cats might passively carry the virus after preying on infected shrews and thereby mediate transmission of the virus. Although rare natural infections of cats have been described, zoonotic transmission by BoDV-1-infected cats seems to be unlikely because cats are considered as dead-end hosts.[31]

Likewise, we have found no indication of natural human-to-human transmission. Phylogeographic data and the detection of high BoDV-1 RNA levels only in human brain and neuronal tissue argue against the possibility of infection chains within human populations.

The incubation period of BoDV-1-induced disease in humans is unknown. In naturally infected horses, the incubation period is assumed to last from several weeks to months, whereas it can be as short as 2 weeks in experimentally infected animals depending on the route of infection.[2] Symptoms in the three infected transplant recipients started around 3 months after transplantation.[21] However, it remains elusive whether this time period is representative for non-iatrogenic natural infection of immunocompetent humans.

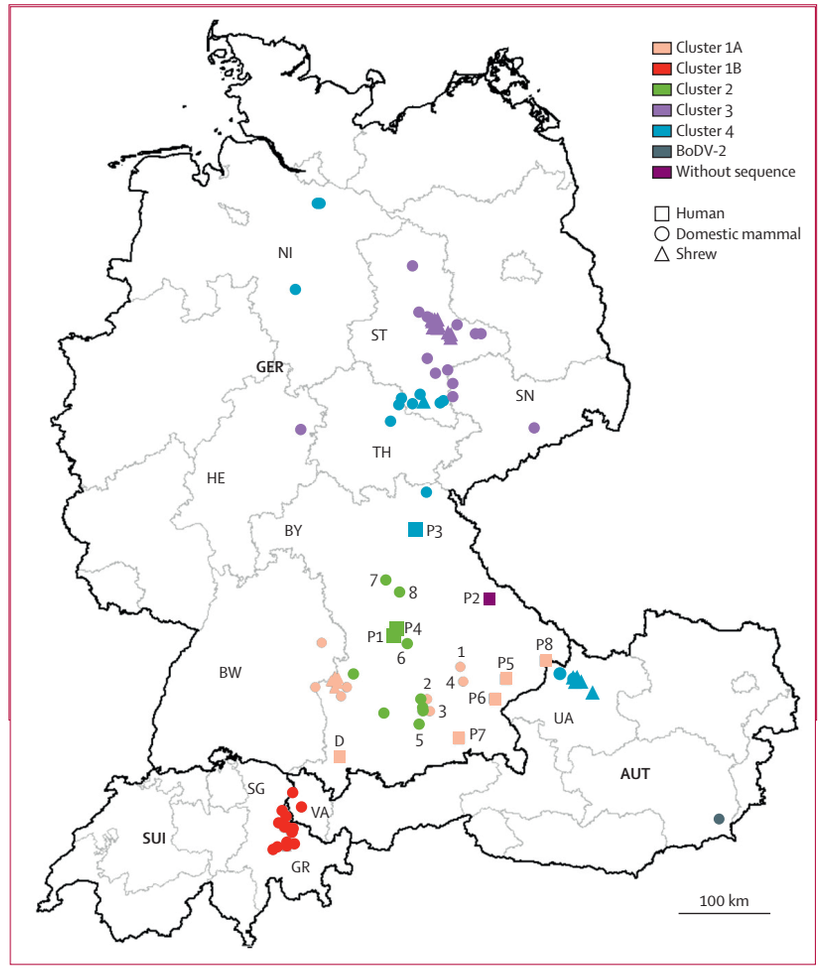


Figure 4: **Geographical mapping of reported human BoDV-1-infections in the endemic area**
Human infections are presented together with published sequence-confirmed BoDV-1 infections of reservoir and accidental animal hosts with available geographical localisation.[4–6,15,28] Colours represent regional BoDV-1 sequence clusters. Localisations of human cases are marked according to the county of origin. D represents the donor of the published transplantation-associated BoDV-1 cluster.[21] Black numbers mark selected animal sequences with a close genetic association with human sequences. The map was generated using Karten-Explorer 1.3 (Friedrich-Loeffler-Institut, Greifswald-Insel Riems, Germany), and is based on the EuroBoundaryMap. BoDV-1=Borna disease virus 1. GER=Germany. BY=Bavaria. BW=Baden-Wuerttemberg. HE=Hesse. NI=Lower Saxony. RP=Rhineland-Palatinate. SN=Saxony. ST=Saxony-Anhalt. TH=Thuringia. SUI=Switzerland. GR=Grisons. SG=St Gallen. AUT=Austria. UA=Upper Austria. VA=Vorarlberg.

Diagnosis of accidental BoDV-1 infection in dead-end hosts is challenging because mammalian bornaviruses are known to be strongly neurotropic and cell associated in naturally and experimentally infected non-reservoir hosts.[1,8,20,21] In agreement with previously published zoonotic VSBV-1 and BoDV-1 infections, we detected high bornavirus RNA loads only in CNS tissues.[18–21] Lower viral RNA counts were also present in the kidney of an immunocompromised patient,[21] but not in previously healthy patients in this or previous studies.[19,20] Furthermore, we detected viral RNA in peripheral nerves of an immunocompetent patient. BoDV-1 RNA was not detected in serum samples from any of the patients reported here, whereas CSF samples were only inconsistently and weakly

positive for viral RNA. Although the detection of BoDV-1 RNA in CSF is highly suggestive of BoDV-1 infection, the negative predictive value of CSF analysis by RT-qPCR appears to be low. Thus, intra-vitam diagnosis relies heavily on brain biopsies and on the detection of virus-reactive antibodies in serum or CSF samples. In agreement with findings of previous studies,[20,21] almost all infected patients developed high serum antibody titres, whereas CSF samples became positive later and usually reached lower titres. However, at the time of hospital admission, antibody titres were sometimes low or undetectable, emphasising that serology might yield negative results particularly in the early phase of disease.

Therapies for BoDV-1 infection have not been established so far. Ribavirin and favipiravir (T-705) were shown to be effective against a broad spectrum of bornaviruses in cell culture and animal experiments,[32–35] whereas a postulated effect of amantadine could not be confirmed.[36–38] Experience in antiviral treatment of BoDV-1-infected humans is scarce. The infected liver recipient had been treated with ribavirin during the late phase of disease, but it is unknown whether this treatment contributed to his survival.[21] Thus, more substances with anti-bornaviral activity need to be identified to facilitate effective treatment of BoDV-1 infections in the future.

Initial clinical symptoms of the patients included fever, headache, and cognitive impairment, followed by progressive brain disease leading to deep coma and death. Myelitis was evident in all three patients for whom the spinal cord was examined during autopsy. Seven of the eight supposedly immunocompetent patients reported here or in the literature died within 16–57 days after hospital admission (mean 32·9 days [SD 13·1]; appendix p 13).[20,22] By comparison, disease progression was significantly slower in the four lethally infected immunocompromised organ transplant recipients (p=0·035, Student's t test with assumption of unequal sample variance), with courses ranging from 53 to 99 days (mean 75·8 days [SD 21·8]; appendix p 13).[21] However, patients who had received transplants were also considerably older (mean 61·3 years [SD 12·8]) than patients who had not received transplants (32·7 years [12·2]).[20–22] Therefore, we cannot exclude the possibility that age and comorbidities beyond immunosuppressive treatment of the transplant patients were covariates for disease progression. As demonstrated in experimentally infected rodents,[8] the immunosuppressive medication might have restrained the T cell-mediated immunopathology. In line with this assumption, the longest survival times were observed for the two published kidney transplant recipients, for whom intensified immunosuppression including antithymocyte globulin was required because of organ rejection.[21] Notably, in these two cases the disease started with peripheral neuropathy mimicking Guillain-Barré syndrome,[21] which was not observed during this study. The two affected kidney transplant recipients differed from the other transplant patients in that intensified immunosuppression including antithymocyte globulin was required.[21] However, in another patient, Guillain-Barré syndrome-like disease occurred without reported immunosuppression.[22]

In summary, we report eight newly detected independent cases of fatal zoonotic BoDV-1 encephalitis and provide a detailed description of common clinical, diagnostic, and epidemiological characteristics. We identified BoDV-1 infection in the majority of fatal encephalitis cases of unknown cause, whereas we did not discover non-fatal infections in the same sample collection. However, as a result of the selective analysis of cases with a high probability of BoDV-1 detection instead of an unbiased sampling, the prevalence and incidence of BoDV-1 infections in our study region and in the whole endemic area remain unclear, representing a further limitation of our study. The possibility of mild, asymptomatic, or oligosymptomatic courses of BoDV-1 infections cannot be excluded and requires further investigations. Although all reported BoDV-1 infections in the endemic area so far originated from the south of Germany, it seems conceivable that similar cases occur in other endemic regions in Germany, Austria, Switzerland, and Liechtenstein. In addition, VSBV-1 or other, yet unknown, mammalian orthobornaviruses might be responsible for similar diseases in other parts of the world.

On the basis of the clinical signs and courses of disease that we have described in this study, clinicians should consider bornavirus infection in patients matching the following criteria: febrile episodes with rapidly evolving central or peripheral nervous system affections of unknown origin; increased leucocyte, protein, and lactate concentrations in CSF; diffuse slowing in electroencephalography in the early disease course; bilateral involvement of the frontal and temporal lobes, basal ganglia, insular cortex, and brainstem in MRI, at least at later timepoints; living in rural or suburban environment in a known BoDV-1 endemic area; and potential direct or indirect contact with the wild animal reservoir (eg, as a farmer or cat owner).

Contributors

KA, SW, GR, and BN collected clinical data. HHN, DR, KS, AE, SG, LFF, DHof, DHöp, MS, DT, JS-C, DN, CH, KA, JJW, and JJ did molecular, serological, and classic virological analyses. KA, SW, BN, KF, RAL, TF, LG, GR, MEr, and JJ treated the patients and contributed samples and data to the study. SZ, NV-C, AM, and KU did autopsies. MR, CB, MEv, JH, KM, FL-S, VR, and JS provided macroscopic and microscopic (neuro)pathological diagnoses. BB and BSa contributed transplant data. JJW and UR validated nucleic acid testing of BoDV-1. WJ and AG established the diagnostic repository. HHN, JJ, KA, DR, KS, MB, and BSc designed the study. HHN, KA, and BSc obtained ethical approval. DR, HHN, KA, KS, JJ, MB, and BSc wrote the manuscript. All authors critically evaluated and approved the manuscript.

Declaration of interests

BSa reports personal fees from GlaxoSmithKline, Sanofi, and Roche, and grants from Pfizer, Merck Sharp & Dohme, and Gilead, all outside of this work. All other authors declare no competing interests.

Acknowledgments

The study was funded by the German Federal Ministry of Education and Research within the research consortium ZooBoCo (grant number 01KI1722A). We thank André Freudenberg, Susanne Kessler, Elke Kreuzpaintner, Heiko Siegmund, Kathrin Steffen, Doris Junghans, Mareen Lange, and Patrick Zitzow for their excellent technical assistance. Timo Homeier-Bachmann and Patrick Wysocki assisted with the geographical mapping of BoDV-1 sequences. We are very grateful to Gerhard Dobler for indicating a possible case to us and for organising the sample transfer. We thank Ana Barreiros from the German Organ Transplantation Foundation for information about transplant-related cases, Stephan Finke for contributing immunofluorescence images of the human BoDV-1 isolate, and Christina Wendl for contributing neuroradiological images.

Editorial note: The *Lancet* Group takes a neutral position with respect to territorial claims in published maps and institutional affiliations.

References

1 Lipkin WI, Briese T, Hornig M. Borna disease virus—fact and fantasy. *Virus Res* 2011; **162:** 162–72.

2 Richt JA, Rott R. Borna disease virus: a mystery as an emerging zoonotic pathogen. *Vet J* 2001; **161:** 24–40.

3 Hilbe M, Herrsche R, Kolodziejek J, Nowotny N, Zlinszky K, Ehrensperger F. Shrews as reservoir hosts of Borna disease virus. *Emerg Infect Dis* 2006; **12:** 675–77.

4 Dürrwald R, Kolodziejek J, Weissenböck H, Nowotny N. The bicolored white-toothed shrew *Crocidura leucodon* (HERMANN 1780) is an indigenous host of mammalian Borna disease virus. *PLoS One* 2014; **9:** e93659.

5 Nobach D, Bourg M, Herzog S, et al. Shedding of infectious Borna disease virus 1 in living bicolored white-toothed shrews. *PLoS One* 2015; **10:** e0137018.

6 Weissenböck H, Bagó Z, Kolodziejek J, et al. Infections of horses and shrews with Bornaviruses in Upper Austria: a novel endemic area of Borna disease. *Emerg Microbes Infect* 2017; **6:** e52.

7 Puorger ME, Hilbe M, Müller JP, et al. Distribution of Borna disease virus antigen and RNA in tissues of naturally infected bicolored white-toothed shrews, *Crocidura leucodon*, supporting their role as reservoir host species. *Vet Pathol* 2010; **47:** 236–44.

8 Stitz L, Bilzer T, Planz O. The immunopathogenesis of Borna disease virus infection. *Front Biosci* 2002; **7:** d541–55.

9 Hornig M, Briese T, Licinio J, et al. Absence of evidence for bornavirus infection in schizophrenia, bipolar disorder and major depressive disorder. *Mol Psychiatry* 2012; **17:** 486–93.

10 Bode L, Dürrwald R, Rantam FA, Ferszt R, Ludwig H. First isolates of infectious human Borna disease virus from patients with mood disorders. *Mol Psychiatry* 1996; **1:** 200–12.

11 Nakamura Y, Takahashi H, Shoya Y, et al. Isolation of Borna disease virus from human brain tissue. *J Virol* 2000; **74:** 4601–11.

12 Watanabe Y, Ibrahim MS, Hagiwara K, et al. Characterization of a Borna disease virus field isolate which shows efficient viral propagation and transmissibility. *Microbes Infect* 2007; **9:** 417–27.

13 Lieb K, Staeheli P. Borna disease virus—does it infect humans and cause psychiatric disorders? *J Clin Virol* 2001; **21:** 119–27.

14 Rubbenstroth D, Schlottau K, Schwemmle M, Rissland J, Beer M. Human bornavirus research: back on track! *PLoS Pathog* 2019; **15:** e1007873.

15 Dürrwald R, Kolodziejek J, Herzog S, Nowotny N. Meta-analysis of putative human bornavirus sequences fails to provide evidence implicating Borna disease virus in mental illness. *Rev Med Virol* 2007; **17:** 181–203.

16 Schwemmle M, Jehle C, Formella S, Staeheli P. Sequence similarities between human bornavirus isolates and laboratory strains question human origin. *Lancet* 1999; **354:** 1973–74.

17 Zimmermann V, Rinder M, Kaspers B, Staeheli P, Rubbenstroth D. Impact of antigenic diversity on laboratory diagnosis of avian bornavirus infections in birds. *J Vet Diagn Invest* 2014; **26:** 769–77.

18 Hoffmann B, Tappe D, Höper D, et al. A variegated squirrel bornavirus associated with fatal human encephalitis. *N Engl J Med* 2015; **373:** 154–62.

19 Tappe D, Schlottau K, Cadar D, et al. Occupation-associated fatal limbic encephalitis caused by variegated squirrel bornavirus 1, Germany, 2013. *Emerg Infect Dis* 2018; **24:** 978–87.

20 Korn K, Coras R, Bobinger T, et al. Fatal encephalitis associated with Borna disease virus 1. *N Engl J Med* 2018; **379:** 1375–77.

21 Schlottau K, Forth L, Angstwurm K, et al. Fatal encephalitic Borna disease virus 1 in solid-organ transplant recipients. *N Engl J Med* 2018; **379:** 1377–79.

22 Coras R, Korn K, Kuerten S, Huttner HB, Ensser A. Severe bornavirus-encephalitis presenting as Guillain-Barré-syndrome. *Acta Neuropathol* 2019; **137:** 1017–19.

23 Wylezich C, Papa A, Beer M, Höper D. A versatile sample processing workflow for metagenomic pathogen detection. *Sci Rep* 2018; **8:** 13108.

24 Cubitt B, Oldstone C, de la Torre JC. Sequence and genome organization of Borna disease virus. *J Virol* 1994; **68:** 1382–96.

25 Rubbenstroth D, Brosinski K, Rinder M, et al. No contact transmission of avian bornavirus in experimentally infected cockatiels (*Nymphicus hollandicus*) and domestic canaries (*Serinus canaria* forma domestica). *Vet Microbiol* 2014; **172:** 146–56.

26 Piepenbring AK, Enderlein D, Herzog S, et al. Pathogenesis of avian bornavirus in experimentally infected cockatiels. *Emerg Infect Dis* 2012; **18:** 234–41.

27 Werner-Keiss N, Garten W, Richt JA, et al. Restricted expression of Borna disease virus glycoprotein in brains of experimentally infected Lewis rats. *Neuropathol Appl Neurobiol* 2008; **34:** 590–602.

28 Kolodziejek J, Dürrwald R, Herzog S, Ehrensperger F, Lussy H, Nowotny N. Genetic clustering of Borna disease virus natural animal isolates, laboratory and vaccine strains strongly reflects their regional geographical origin. *J Gen Virol* 2005; **86:** 385–98.

29 Carbone KM, Duchala CS, Griffin JW, Kincaid AL, Narayan O. Pathogenesis of Borna disease in rats: evidence that intra-axonal spread is the major route for virus dissemination and the determinant for disease incubation. *J Virol* 1987; **61:** 3431–40.

30 Kupke A, Becker S, Wewetzer K, Ahlemeyer B, Eickmann M, Herden C. Intranasal Borna disease virus (BoDV-1) infection: insights into initial steps and potential contagiosity. *Int J Mol Sci* 2019; **20:** e1318.

31 Bornand JV, Fatzer K, Melzer K, Gonin Jmaa D, Caplazi P, Ehrensperger F. A case of Borna disease in a cat. *Eur J Vet Pathol* 1998; **4:** 33–35.

32 Mizutani T, Inagaki H, Araki K, Kariwa H, Arikawa J, Takashima I. Inhibition of Borna disease virus replication by ribavirin in persistently infected cells. *Arch Virol* 1998; **143:** 2039–44.

33 Jordan I, Briese T, Averett DR, Lipkin WI. Inhibition of Borna disease virus replication by ribavirin. *J Virol* 1999; **73:** 7903–06.

34 Reuter A, Horie M, Höper D, et al. Synergistic antiviral activity of ribavirin and interferon-α against parrot bornaviruses in avian cells. *J Gen Virol* 2016; **97:** 2096–103.

35 Tokunaga T, Yamamoto Y, Sakai M, Tomonaga K, Honda T. Antiviral activity of favipiravir (T-705) against mammalian and avian bornaviruses. *Antiviral Res* 2017; **143:** 237–45.

36 Cubitt B, de la Torre JC. Amantadine does not have antiviral activity against Borna disease virus. *Arch Virol* 1997; **142:** 2035–42.

37 Hallensleben W, Zocher M, Staeheli P. Borna disease virus is not sensitive to amantadine. *Arch Virol* 1997; **142:** 2043–48.

38 Stitz L, Planz O, Bilzer T. Lack of antiviral effect of amantadine in Borna disease virus infection. *Med Microbiol Immunol* 1998; **186:** 195–200.