

**Borna disease virus:
new aspects on infection, disease,
diagnosis and epidemiology** pages 259-288

H. Ludwig ⁽¹⁾ & L. Bode ⁽²⁾

(1) Institute of Virology, Free University Berlin, Königin-Luise-Strasse 49, 14195 Berlin, Germany

(2) Robert Koch-Institut, Nordufer 20, 13353 Berlin, Germany

Summary

A 'disease of the head' affecting horses, as described in the 17th Century is now known as Borna disease. Research over the past 100 years has established that the aetiological agent, Borna disease virus (BDV), is an unsegmented, single- and negative-stranded, enveloped ribonucleic acid (RNA) virus which represents the family *Bornaviridae* in the order *Mononegavirales*. The virus exists worldwide in horses, sheep, cattle, cats, dogs and ostriches. The infection can be fatal, but the majority of carriers are persistently infected without showing symptoms. The association with psychiatric diseases in humans led to an international explosion of research on BDV, with centres established in Germany, the United States of America and Japan. Experimental infections of tree shrews and rats served to examine the effects of persistent and overt disease, most excitingly, virus-induced behavioural changes, and emotional and learning deficits. This 'emerging' virus infection shows complex pathogenetic mechanisms in the nervous system, but also spreads through myelomonocytic cells. Diagnosis can be made serologically, but detection of antigen markers in peripheral white blood cells, combined with nucleic acid amplification is more profitable. Comparative RNA studies reveal an unusually high genetic homology of viruses. Isolates recovered from humans and equines suggest species-specificity. Vaccination is not an advisable strategy, but antiviral therapy, especially with amantadine sulphate, promises efficacy in human mood disorders, and is effective *in vitro*. Infections with BDV follow

a vulnerability principle to cause disease. Although cross-species transmission of this commensal virus has not been proven, zoonotic aspects of BDV should be carefully considered.

Keywords

Borna disease – Diagnosis – Epidemiology – Public health – Viruses – Zoonoses.

Introduction and historical development

Like the disease itself, interest in Borna disease virus (BDV) occurs in waves. The 1990s saw a dramatic increase in interest in the disease, due to the elucidation of the biological features, the morphology, and aspects of the molecular biology of the virus. World-wide interest arose from the implication of BDV in psychiatric diseases of humans and the isolation of BDV from infected people (21, 22). Veterinary interest was overshadowed by medical aspects, but has recently been stimulated by the zoonotic aspects of the virus. This review will cover the highlights of the development of research over the last century, a development which has invariably been linked with veterinary medical problems. New perspectives of this infection will also be presented, with an emphasis on current knowledge and the altered importance of this virus in veterinary medicine. The relevance of the virus in terms of public health is also highlighted.

Attention attracted by a disease of horses now known to be associated with BDV, dates back several hundred years. The oldest reports describing typical characteristics of Borna disease (BD) are found in 1660, speaking of pain which pushes the horses or makes them totally dull and dumb (60). A book published in 1716 records sleepiness, melancholia and agitation in the course of such head pain (195). Similar and more precise reports followed later in the 18th Century (202), and a detailed description of this brain inflammation was published in 1822 (200). A short summary of these historical aspects of BDV research, and a comprehensive bibliography covering the last 100 years, has recently been published (53).

'Bornasche Krankheit', named Borna disease (BD) since 1970, is principally a disease of horses and sheep. Around 1900, the disease became of great economic importance, because severe outbreaks occurred in the area around the city of Borna (near Leipzig, Germany) (Fig. 1), not only in farm animals, but also in military horses (206), leading to enforced political and veterinary investigations. During this time the name 'Borna Krankheit' was coined. The famous investigations by Joest and Degen demonstrated that the disease was paralleled by inflammatory reactions, mainly in the midbrain. These authors also discovered the pathognomonic appearance of intranuclear inclusion bodies, named Joest-Degen bodies (84). A summary of intensive clinical investigations of more than 500 cases presented the classical clinical symptomatology and progress of the disease in horses (165). The early knowledge of the veterinary public health aspects helped to broaden the scope of investigations. In the 1920s and 1930s, Zwick succeeded in adapting the virus to rabbits (216), which then allowed vaccination with brain suspension (215, 217). The disease was endemic in central Europe and was regulated by state surveillance and control measures (53). In France, Nicolau and Galloway collected an immense amount of infection data, new pathological findings, and early pathogenetic interpretations which also concentrated on infection of small animals (142, 143) (Fig. 2).

Publications in the 1950s and 1960s, overwhelmingly by veterinary scientists in the German Democratic Republic (e.g. Heinig, Hiepe, Matthias, Ihlenburg, von Sprockhoff and Nitzschke), extended the knowledge on clinical and pathogenetic aspects of the disease in horses, sheep, rabbits and rats (71, 74, 79, 78, 125, 126, 145, 166, 203, 204).

When the concept of slow virus infections was presented by Sigurdsson (176), based on rida in sheep, the theory seemed to be applicable to other infections of animals and man, including BD (127). With the slow growth of BDV in tissue culture, the theory was strengthened (105, 106), but subsequent research showed the picture to be more complicated (110).

Between 1970 and 1980, only two groups (in Munich and Giessen, Germany) were involved in basic research. The virus could be grown in sheep and rabbit cells and, after co-cultivation, in many permanent cell lines (41, 106). New pathogenetic studies in the rabbit eye pointed to immune pathological events, and outlined the capacity of the agent to spread intra-axonally in the central nervous system (CNS) and via the optic nerve (97, 98). The presence of BDV in different brain areas and in the cerebrospinal fluid (CSF) led to the detection of a local immune response with oligoclonal immunoglobulins in brain fluid (107, 108). The assumption that BDV caused a persistent infection (3) could be demonstrated in infected tree shrews (*Tupaia glis*) which showed no clinical symptoms, but nevertheless altered their natural behaviour (180).

In the 1980s and 1990s, research was concentrated in groups in Berlin and Giessen. Assays on tissue culture adaptation of the virus were performed (72, 110) in addition to adaptation of BDV strain V to the rat (75), and also to the mouse (86), where persistent, tolerant infections in premature animals could be induced (75). Similar data with inbred rats (Lewis strain) were achieved, with an emphasis on the role of immune pathology (140, 141). These experiments later led to pathogenetic studies on the spread of BDV intra-axonally to the brain (31). In addition to animal experimentation in rabbits, rats and mice, important findings on persistency and neutralisation of the agent were achieved (110). The tolerant infection of rats without clinical symptoms allowed for the first time to psychologically analyse parameters of learning and mood (50). These experiments pointed to the interference of BDV with regulatory centres of normal instinctive behaviour (the limbic system), and allowed measurement of deficiencies. The neuropathological substrates for these experiments showed antigen, inflammatory reactions, and evidence for pharmacological reactivity in limbic areas (64, 110, 178). Research performed over the same period demonstrated the immune response against BDV in humans (10, 12, 160).

During the 1990s, interest in BDV increased world-wide. With the finding of specific nucleic acid, and the release of BDV from cells, the

morphology of the virus as an enveloped icosaheder was revealed and molecular characterisation could be performed (28, 37, 214). Two reference strains were sequenced, strain V and strain He-80, both horse-derived (28, 37). Analysis of the genome structure demonstrated that BDV is a single-stranded, non-segmented, negative ribonucleic acid (RNA) virus. This, together with the unusual properties of BDV (44, 96, 110, 167), led to the grouping of this virus in the order *Mononegavirales*, and the creation of a new family, the *Bornaviridae* (154). The discovery of BDV-specific antigen and nucleic acid in peripheral blood mononuclear cells (PBMCs) of animals and humans stimulated new research world-wide (18, 20). Infection with BDV could now be monitored by detection of genomic and messenger RNA (mRNA) in PBMCs, preferentially of open reading frames (ORF) I and II (35, 45, 89, 135, 162).

The isolation of BDV from blood samples of psychiatric patients (22, 151) opened up another dimension in BDV research (114).

After characterisation of structural elements of the virus and clarification of the biological and molecular properties, inhibition of BDV infection by amantadine sulphate (25) and other drugs (85, 130), or biological material (185) suggested the possibility of treating this persistent infection.

The discoveries of BDV-nucleic acid by Lipkin *et al.* (104) and the involvement of the virus in psychiatric diseases with isolations from humans by Bode *et al.* (22) built the foundations for an explosion in interest world-wide. This review will examine the latest advances in this field with an emphasis on BD in animals and its relevance for public health.

Infection of animals and humans

Borna virus has a remarkably broad host range including the principal types of livestock, pets and even wildlife mammals in zoos (1, 53, 128, 171). Studies in horses and sheep originally suggested that infection is always associated with a high mortality rate. The opposite is now known to be true, many animals carry the virus without

showing disease. Therefore, the progressing pathological signs of BD and the status of BDV infection without disease must be clearly distinguished. Natural infection is found principally in horses.

Horses

Initial infection is often signalled by behavioural changes which can be observed prior to overt disease signs, a characteristic reported a hundred years ago (165). Observation of the horse from some distance can reveal unusual ear positions, fibrillar trembling of skin areas, often with the head lowered, and an anxious look in the eyes. The animals show head shaking or ataxia followed by lowered consciousness and somnolence. High frequencies of yawning and disturbances in movement are observed, often accompanied by a wall-pushing behaviour. The disease lasts from one to three weeks, and when symptoms worsen, death is the likely outcome. Such behaviour was characterised in the literature using names such as *Hitzige Kopfkrankheit*, *Hirnwuth*, *Nervenkrankheit*, *rasender Koller*, encephalomyelitis enzootica and polioencephalomyelitis non purulenta infectiosa (53).

Studies based on several thousand horses which had been clinically diagnosed (Fig. 3), and from which material had been submitted for detailed virological investigations, have confirmed the predominance of such changes to normal behaviour early in the course of the disease. The illness might progress with symptoms such as cholic, alterations in the eyes, paralysis of the hindlimbs and manege-movements (19, 109). Generally, early signs of disease can be categorised in three complexes, as follows:

- a) depression or excitation
- b) centrally-caused sensoric disturbances in motility, and
- c) hyperkinesis or ataxia.

Some aspects of the history of the epidemiology of BD in horses will be described below. It is now clear that the disease process leading to death is not the usual course of the disease. Many more cases of

asymptomatic disease are observed in the horse population, occasionally with slight behavioural changes, or atactic movements, followed by recovery.

The disease is endemic mainly in eastern Germany (Saxony), but also in Bavaria, Baden-Württemberg and Hesse (53), Switzerland (30) and Alpine regions in Austria (211). Evidence of BDV infection in horses in coastal areas of Israel seems to be more prominent than in mountainous regions (124; M. Malkinson, personal communication). Endemic areas such as that in central Europe have not been reported elsewhere in the world. However, in Australia, Bornavirus was recovered after inoculation of tissue cultures with samples taken from horses with neurological symptoms. The RNA sequences were almost identical to those of the p40 and p24 genes of strain V (4). The classical clinical picture of BD can also be seen in other parts of Germany, especially in the north. Cases of typical BD have also been found in southern Sweden (L. Bode and J. Skidell, unpublished findings).

In over 3,000 horses investigated in Germany, a relatively high prevalence of infection was detected (more than 20%) (H. Ludwig and L. Bode, unpublished findings). In France, a group of several hundred horses monitored by new laboratory parameters showed an infectivity rate of more than 50% (L. Bode, H. Ludwig and J. Brugère-Picoux, unpublished findings). Significant prevalence, based on antibody detection and nucleic acid studies, has also been described in horses from Japan and Iran, although no reports on clinical cases and endemic areas are available from these countries (6, 30). The infection has also been reported from the United States of America (USA) (88).

The evidence above suggests that the world-wide infection rate in healthy animals may be surprisingly high. Animals can remain healthy over many years but still maintain markers for BDV. Such animals (depending on their genetic vulnerability) are prone to present with subtle alterations in normal behaviour when stress factors and/or other diseases threaten their health. Such latently infected horses may show

diminished health, signs of somnolence or ataxia and other clinical problems (19, 24).

Work by Bode *et al.* has specifically focused on the relevance of BDV antigen (e.g. proteins in PBMCs, which are an easily measurable diagnostic parameter) in otherwise unaffected horses (18, 19). Some selected, but representative, comparative results on antibodies and antigen are illustrated in Figure 4.

In herds where sporadic cases of disease had been diagnosed, more than 70% of animals tested positive for antigen. Antibody studies of other groups have given similar results (6, 101). Equine isolates from post-mortem tissues (63, 120), and PBMCs of diseased animals (156) support the view of phasic expression of BDV infections.

In this context, a recent finding on the genetic background of BDV should be mentioned. A genetic analysis was performed on the BDV p40 gene obtained from sixteen horses which died following clinical disease. The data clearly show that the further apart the animals were located, the higher the genetic variability in the N-protein gene (120). These results show that molecular epidemiology can allow differentiation between diseased horses from different locations (D. Lüscho and L. Bode, unpublished findings), and support similar studies on horses in Austria (211) and Australia (4).

Sheep

Disease in sheep has also been intensively studied (7, 71), and these animals serve as another natural host (111). In the early 20th Century, reports from several areas of Europe (7, 53, 215) demonstrated that BDV was considered an economically important disease in endemic areas, and this is still the case today (30, 51, 52, 129).

The clinical picture is governed by CNS disturbances in motility, although these may be less significant than in the horse. The early phases of altered behaviour and ataxia with clinical symptoms may resemble those in scrapie. In later stages, a clear differential diagnosis can be made. In progressing cases of BD the animals deteriorate

between days four and ten, showing the typical wall-pressing position (a sign of headache and increased CSF pressure) as well as staggering and atactic movements (Fig. 5) and might succumb. The disease period of scrapie is considerably longer (several months) and the disease always ends with death. Differential diagnosis of visna, and infections with *Listeria* or parasites must be considered (111).

Latent infections in sheep, and the possible epidemiological relevance of these infections, were first reported in the 1950s (125). Such data were also deduced from experimental infection of sheep. In the 1970s, intracerebral infection of sheep was performed using strain V as inoculum (0.5 ml). In three of the animals, no disease symptoms could be induced, however, one sheep died suddenly. Surprisingly, all three asymptomatic sheep had virus in the brain and retina, as demonstrated by inoculation of rabbits and immunoelectrophoresis (H. Ludwig and R. Rott, unpublished findings). Modern *intra vitam* assays allow the monitoring of latent infections in healthy sheep (68). Diagnostic surveys of two herds in Germany revealed that more than 20% of the animals showed significant antibody titres (L. Bode and H. Ludwig, unpublished findings). Borna disease has also been reported in sheep and goats in Switzerland (30, 129). However, the latter species either seems to be less sensitive to the virus or does not express overt clinical infection (170).

On the island of Hokkaido in Japan, the prevalence rate of BDV infection in sheep was found to exceed 50%. These investigations were based on the presence of antibodies and the demonstration of specific RNA (68).

Cattle

Disease and infection in cattle seems to be rare compared to horses and sheep, although a few cases have been reported (92, 125, 215). However, the disease is not known to be endemic in cattle. Infected and diseased cattle have been reported in Switzerland (29, 30, 56). At two locations in Germany, a study followed severe clinical, laboratory confirmed cases with neurological symptoms (17), where heifers died under progressive paralysis (Fig. 6). The clinical picture partially

resembled that of bovine spongiform encephalopathy (BSE). Investigations to detect latent carriers in other parts of Germany showed that occasionally up to 50% of animals had been in contact with the virus. In a survey at two locations, 50% of animals without clinical signs were either positive for antigen or antibodies (L. Bode and P. Reckwald, unpublished findings). In Japan, the presence of BDV RNA in healthy dairy cattle has also been reported (67).

Cats

The discovery of BD in cats was based on a clinical disease of unknown aetiology which was reported in 1974 (100). The animals showed prominent staggering movements (the disease was later named staggering disease [SD]) with ataxia and paralysis, principally in the hind limbs. Subtle and progressing changes in behaviour were obvious. The cats mewed more than usual, were anxious, and presented with depressive symptoms. The animals stopped eating, showed hyperaesthesia and hypersensitivity to light and noise. A staring gaze was often observed (showing mydriasis), and the cats could not withdraw their claws. Most of these cats died in one to four weeks or were euthanised in a stage of severe neurological illness (100).

After such clinical cases had been intensively investigated, a neuropathological picture of an inflammatory reaction (perivascular and parenchymal lymphoid infiltrations) became clear (116), similar to the neuropathology of BD (65, 215).

At this point, A.-L. Lundgren (of the University of Uppsala) contacted researchers in Berlin, and in a series of new discoveries, BD in cats was explained. In half of a group of twenty-four cats with SD, antibodies with high titres were present. The typical clinical picture, together with the neuropathological evaluations and the serological findings, pointed to BDV infection. This was the first time that the virus had been detected in a natural host other than horses and sheep (117). In the 1950s, Ihlenburg and co-workers had shown that cats were susceptible hosts, using experimental infection (78). In addition, it is now clear that several reports with an unclear diagnosis and

characteristic neuropathological patterns could have represented BDV infections (198).

A final proof that Bornavirus was indeed involved in this cat disease was provided by the demonstration of BDV-specific antigens and the amplification of p40 and p24 gene products from eleven cats, including several brain regions. In 100% of the brains, BDV antigens were demonstrable by enzyme-linked immunosorbent assay (ELISA), whereas only 75% of the brains were nucleic acid positive (118).

In contrast to studies with coronal sections of horses, only 50% of cat brains (seven out of thirteen) showed antigen positive neurons detectable with an anti-p24 monoclonal antibody. The expression of viral antigens in cat BD is therefore considerably lower than in the horse (65). The comparatively small amounts of viral products in cats with a clinical picture of SD might explain the questionable data obtained from diseased cats in Austria, where serologically positive data had been obtained (147, 209).

Without doubt, BDV is aetiopathogenetically involved in this kind of disease; inoculation of cat brain material into rats provides additional evidence for this suggestion. Borna virus could be passaged and titred, especially in new-born rats. The same material caused disease in adult rats. The *in vitro* cultivation of a cat virus, however, required several passages (118). A final proof that SD is the feline form of BD was provided by experimental infection of specific-pathogen-free cats, using cat isolate or strain V. Only a few cats showed acute symptoms, whereas several cats changed their behaviour for several weeks and recovered without permanent damage. Surprisingly, all cats showed inflammatory reactions in different brain areas at a time when behaviour had returned to normal (119).

Borna disease virus infections in cats are not only present in Sweden, but have been demonstrated in Germany (76), and Australia (4) on a serological and molecular basis. Approximately 7% (12/173) of sera from diagnostic investigations of cats with non-neurological symptoms had BDV antibodies, although titres were considerably lower than in cats with SD. Recent data obtained from cats in animal

homes with gastrointestinal problems or neurological symptoms, gave a positive rate of approximately 13%. In three cases with unclear symptoms, BDV antigen titres could be followed over weeks. A simultaneous occurrence of disease with antigen expression was clearly seen. All three cats recovered from disease but continuously tested positive for BDV markers (J. Hübner and L. Bode, unpublished findings). Extensive studies have been presented on the correlation between the incidence of immunosuppressive diseases, such as feline immunodeficiency virus, and the presence of BDV markers in cats (serological markers and antigens) (76).

Infection has also been reported in house cats from Japan with or without clinical signs, where serological and nucleic acid studies from PBMCs showed approximately 13% positivity (reverse transcriptase polymerase chain reaction [RT-PCR], using the p24 gene) (136, 137, 144). Another study performed on cats in the United Kingdom found a surprisingly high prevalence of BDV positivity in neurologically diseased cats. The amplification of the p24 gene products suggests that the cat is a natural host for BDV (157).

Borna virus infections of cats are of considerable epidemiological importance, because cats (like horses) live in close proximity to humans and BDV-positive cats therefore remain a possible source of human infection. Although cross-species transmission has not been demonstrated, the infection of cats has to be regarded as a potential hazard to human health (53).

Dogs

Dogs had been disregarded in respect to BDV infection, although transfer of infectious virus was demonstrated by Nicolau and Galloway under experimental conditions (142, 143).

However, clear antigen positivity and p40 and p24 amplicates were demonstrated in 1994 in a diseased dog from Sweden which had been euthanised because of aggressiveness. Neuropathologically, the diagnosis 'non suppurative meningoencephalitis' had been made (L. Bode and A.-L. Lundgren, unpublished findings). Investigations

performed over the following three years with dogs treated by practitioners in south Germany (an area endemic for BD) revealed approximately 40% seropositivity (13/34). The infection was activated during the investigation period in only half of the seropositive animals. Of thirty-four animals, two presented with neurological disease. This means that the total infection rate reached almost 50% of such clinically overt, preselected cases. The majority of the clinical symptoms pointed to allergic skin disease, myositis or were undefined (L. Bode and P. Pick, unpublished findings). In a case in Austria where a dog with neurological symptoms was euthanised, BDV antigen and specific RNA were demonstrated (210).

These data clearly prove that natural infection also occurs in dogs. It is currently not clear whether a species-specific strain exists, or whether a virus with a wide host range remains responsible. For the clinician, however, all dogs with neurological symptoms should not only be considered suspicious for rabies or canine distemper (infectious agents which also belong to the order *Mononegavirales*), but also for BDV infection. This scenario is of additional interest, because of the close contact between dogs and humans.

Birds

The susceptibility of birds such as chickens was not uniformly accepted after experimental inoculation of animals by Zwick (215) and Nicolau and Galloway (142). However, the embryonated egg appeared to accept the infection and propagate virus (146). After infection of one-day-old chicks, strain V was able to multiply in the chicken brain, and cause a persistent infection in older chickens. Some of the birds died, but usually only a few showed atactic neurological symptoms and even these birds survived the disease (105, 109). These experiments were repeated and confirmed, showing that the chicken is susceptible to experimental infection. Infected chicks and one-day-old quail produced significant amounts of BDV-specific antibodies, and some of the animals rendered high titres of neutralising antibodies (109, 110, 112). However, natural infections in birds are unknown.

In 1993, a neurologic disease was observed in ostriches on farms in Israel. Mainly young birds presented with spastic paresis and progressing disease leading to death. Thirteen brains from dead ostriches were tested using an antigen-ELISA (22), and seven were found to be positive for the major immunogenic components (p40 and/or p24 proteins). Furthermore, one out of ten healthy animals which had been in contact with the diseased animals harboured antigen in the brain (122). Proof that BDV was the cause of disease was provided by co-cultivation experiments of infected tissue with different cell lines. After the fourth passage it was possible to demonstrate p40 antigen using either ostrich immune serum or a defined monoclonal antibody (123). Although many ostriches died, some birds recovered after infusion of immune serum taken from recovered adult birds. Since 1993, endemic BD in ostrich farms has been significantly reduced, although disease occasionally occurs (208, M. Malkinson and Y. Weisman, personal communication). Natural infection with BDV has not been proven in any other species of bird.

A condensed summary of the seroprevalence data for several species of animals from different countries is presented in Figure 7.

Infection and disease in humans

Comparison of the inflammatory reaction during the BD process in the brains of animals with undefined neurological diseases and similar infiltration patterns in human brains (e.g. Heine-Medin disease and encephalitis lethargica) (173) pointed to the possibility of human infections. The neuropathology of BD stimulated interest in human and primate infections in general, and these observations initiated several independent investigations on the susceptibility of rhesus monkeys (*Macaca mulatta*) to brain suspensions containing BDV, including rabbit-adapted virus (142, 149, 215), and spread of virus in the body (148). Further studies using the laboratory strain V (182), and another series of intracerebral and intranasal injections of rhesus monkeys were performed (H. Ludwig and L. Stitz, 1981, unpublished findings). Only some neuropathological and ophthalmological data have been published to date (33, 99). In these experiments, primates

showed subtle behavioural changes which in most of the cases progressed into an overt neurological symptomatology, leading to an early decision to euthanise the severely diseased animals. Most of the animals showed the classical picture of BD, except where intraperitoneal infection was performed (a few of these monkeys survived after an early phase of changed behaviour). Serological data have recently been reported (11) and some conclusions discussed (114). In addition to these experiments on monkeys, early studies with tree shrews infected with strain V had attracted attention as a BDV model. As outlined later, these animals acquired a persistent infection and showed significant alterations in principal patterns of natural behaviour (179, 180).

The discovery of human infections occurred in four steps, as follows:

- a)* antibodies were detected in a few psychiatric patients (10, 160, 199) and in a large cohort of patients with various diseases, suggesting widespread infection (15)
- b)* antigens were detected in PBMCs (18)
- c)* BDV proteins and nucleic acid were found in PBMCs of defined patients with mood disorders (19), and nucleic acid was detected in PBMCs (89)
- d)* isolates were obtained from patients with severe mood disorders (22), and recently, from schizophrenic patients in Japan (134), and Germany (151)
- e)* BDV RNA was detected in human autopsy brain samples (46, 161)
- f)* BDV antigens were present in CSF of depressed patients (47).

A vulnerability model, with BDV activation involved in the symptomatology of some psychiatric diseases, is favoured by some groups (9, 22), whereas others have found BDV parameters, but do not believe that this provides proof of a link to certain diseases. However, substantial data suggest that a correlation exists between BDV infection and mood disorders in vulnerable individuals. Mood

disorders are classified according to the *Diagnostic and statistical manual of mental disorders* (DSM IV) (2). Other mental disorders, such as schizophrenia and chronic fatigue syndrome (CFS) are under discussion but data require further confirmation (13, 55, 139).

Antibodies

Antibodies are measured using the indirect immune fluorescence antibody assay (IFA), Western blots, ELISAs, or modified ELISAs. Several groups world-wide have described antibodies in people with psychiatric disease, but also in people without symptoms, and in blood donors (5, 8, 34, 47, 55, 57, 59, 80, 90, 91, 96, 110, 160, 162, 193, 207, 212). Point-prevalence data show rates of 10% to 15% positivity in psychiatric patients, rising to 20% and 30%, when long-term investigations were applied (8).

Although BDV serology is of limited diagnostic value, antibody detection has revealed much information over the last fifteen years. Antibody carriers have been found to occur world-wide and, as is the case in animals, the antibodies are mainly directed to the soluble antigen complex (p40 and p24). Antibodies have low titres, as in naturally infected animals, and are not permanently present. In healthy individuals, seroprevalence is approximately 2%. Neuropsychiatric patients with basic immunological diseases reach 15%, and patients with acute affective disorders 30%, depending upon the severity of the disease (15). Neutralising antibodies have never been found in humans (H. Ludwig and L. Bode, unpublished findings).

Antigens

Despite accumulating data in this field, the presence of antibodies remained only an indirect proof of BDV infection. The antigen expression of the two major immunogens (p40 and p24) in PBMCs has been regarded as an important parameter (Fig. 8). Summarising several studies, in general, the antigen prevalence in ambulant, slightly depressive patients reaches approximately 15%, and in stationary severe cases, approximately 40% (9, 23). Long-term investigations showed that antigens may be detectable within a period

of three weeks, but occur intermittently. This is similar to horses with BD where antigen positivity has been measurable for up to two months (24). A variety of cohort studies have been performed comparing antigen presence in white blood cells with the pattern of the psychiatric symptoms and disease, these studies clearly show that BDV antigen expression periods correlate with disease periods (24, 47).

A further link between BDV replication in the brain and disease is provided by recent investigations of 128 samples of CSF from a retrospective study with neuropsychiatric patients. Approximately 10% of the depressed patients (3/32), and two out of nineteen patients with multiple sclerosis (MS), showed high BDV antigen titres in the CSF. Further studies will be required to determine whether this can be related to the depression which is a frequently described co-morbidity of MS patients (47).

Nucleic acid

Demonstration of BDV-specific mRNA or genomic RNA has become a widely-used tool to investigate human BDV infections. The RNA is detected in PBMCs, using a technique originally described by Bode *et al.* (20), many researchers have found specific amplicates in diseased patient groups, as well as in blood donors.

There are also some study reports in psychiatric patients with no BDV RNA. However, this appears to be due to use of the wrong cohort of patients, the wrong time of investigation or an alternative RT-PCR technique (102, 158), as the world-wide confirmation of positive nucleic acid data demonstrated (20, 35, 45, 80, 81, 89, 91, 139, 150, 162, 197).

Many molecular biological investigations using PBMCs have concluded that a higher BDV RNA prevalence can be found in psychiatric patients, compared to controls. Based on general virological knowledge it also becomes clear that a link between seropositivity and the presence of BDV RNA cannot be made.

Of interest, although not an aetiopathogenetic proof, is the fact that BDV RNA is also found in post-mortem examinations of brains obtained from psychiatric patients (134, 161), or elderly sclerotic patients (over 80 years of age) (46). In addition, in Japan, specific RNA was obtained from normal brains (66) and from brain tumours (138).

Virus isolates

In contrast to laboratory strains of BDV, the cat virus (112), and a few horse isolates (63), no genetically defined wild-type viruses existed until the mid-1990s.

Based on the detection of antigen and nucleic acid in PBMCs of severely diseased psychiatric patients (18, 20), virus isolation experiments were performed on a subcohort of thirty-two patients. Applying double blind studies, randomly selected PBMCs were co-cultivated with human oligodendroglia cells (oligo cells) which were routinely carried through approximately twenty passages. These efforts resulted in the first four human BDV isolates (21, 22). Two of these were derived from bipolar patients and one from a patient with obsessive compulsive disorder. The fourth strain was isolated from a patient who suffered from chronic fatigue and depression and was participating in the CFS programme at Harvard Medical School, Boston, USA (21). Three of these strains were characterised biologically by sequencing most of the genes and by experimental infection of rabbits and rats; the strains obtained from humans clearly behaved differently from laboratory strains in these experiments (22). Significantly, the sequences from the original PBMCs and the isolate sequences were identical (45). Important mutations in several genes differentiated these viruses from animal strains (24). In 1998, workers in Japan succeeded in recovering a BDV strain from a schizophrenic patient through inoculation of gerbils (134), and more recently, another human isolate was obtained from granulocytes of a patient with chronic schizophrenia by inoculation of a guinea-pig cell line (151). This patient had continuously shown BDV RNA in white blood cells (150). The human strains are abbreviated as Hu-H1, Hu-H2, Hu-

H3 and Hu-USA1 (21, 22), and RW98 (151). These data show that humans also serve as a natural host (and possibly reservoir) for this virus.

The aetiological agent: characterisation and classification

Morphology

The morphological structure of BDV has recently been characterised. Particles of 90 nm or 50 nm-60 nm were found in cell culture-released purified virus preparations. These icosahedral particles represent the infectious units. They can be neutralised by high-titre anti-BDV sera, and cause BD in experimentally infected rabbits, from which the virus can be re-isolated (214). Whether or not part of these particle preparations are defective remains to be clarified (114). A group in Japan essentially confirmed these data and demonstrated similar particles by electron microscopy (95). It remains uncertain whether virion morphogenesis occurs via budding (95) or assembly under the plasma membrane (114).

Physico-chemical properties and genome characteristics

The bouyant density of partially purified virus lies between 1.16 g/cm³ and 1.22 g/cm³, and in sucrose, is 1.22 g/cm³ (110). Infectivity is destroyed by heat treatment at 56°C for three days or at 37°C for five days, or alternatively, by exposure to pH below 5.0 or above 12.0, and by treating suspensions with organic solvents, detergents and ultraviolet irradiation. Disinfectants containing chlorine or formaldehyde rapidly destroy infectivity (71, 110).

Two different BDV strains were originally isolated from horses (112). Strain V was established by Zwick and co-workers (216), and has been passaged through rabbits (105), rats (145), and tissue culture (110), whereas Hessen-80 (strain He-80) has a more or less undefined history (72). Presumably, the He-80 strain has had less passages in rabbits and more in rats and tissue culture than strain V. Strain He-80 was employed by Narayan (140) and taken to the USA. In the

meanwhile, the strain has been distributed to many laboratories outside Europe (31, 37, 168).

Strain V, regarded as the reference virus for the family *Bornaviridae* was purified after release from cells (214), and genomic RNA was extracted and sequenced (28). This genome was the basis for taxonomic grouping. Furthermore, the He-80 strain was sequenced after isolation of ribonucleoprotein (RNP) (36).

The viral genome codes for at least six ORFs starting from the 3' end with the following genes: N (nucleoprotein), P (phosphoprotein), X (p10-protein), M (matrixprotein), G (glycoprotein), L (large polymerase), and is represented by a linear single-stranded unsegmented RNA of 8.915 kb with a negative polarity and molecular weight of 3×10^6 Da. The nucleic acid is not polyadenylated, and extracistronic sequences have been detected at 3' (leader) and 5' (trailer) ends of the genome. The termini of the RNA show partially inverted complementarity. Antigenomic RNAs (positive-stranded, and of full length) are present in infected cells (44, 167).

Despite different origins, separation over almost half a century, and different cultivation and tropism properties, strains V and He-80 have the same genomic organisation and share a surprisingly close genetic relationship (more than 95% at the nucleotide level).

In addition to these two full-length 'reference' sequences from Bornaviruses which had undergone interspecies passaging, gene stretches or total genes from natural virus sources have been examined. All these sequences display a high degree of genetic homology. Particularly in the p40 and p24 genes, very few significant differences were found. This high stability of BDV genomes supports the hypothesis that present genomes are the result of a long-term evolutionary process and that the virus has already had a long period of time to adapt to different human and animal hosts.

The outstanding properties of the virus together with the property of nuclear replication, and the demonstration of the virion-morphology, led to the establishment of a new family, named *Bornaviridae*.

Replication occurs in a similar manner to other viruses grouped in the order *Mononegavirales* (e.g. rabies, canine distemper and measles viruses), with one exotic and outstanding feature reminiscent of influenza viruses, replication in the nucleus. This was observed by early pathologists describing the important role of the intranuclear Joest-Degen bodies (83), and was over the years demonstrated by different groups which detected the typical intranuclear antigen (40, 106).

Both the negative-stranded genomic RNA and strands with positive polarity are present in the nucleus, where transcription and replication occurs. Information obtained by *in situ* hybridisation demonstrated that the Joest-Degen bodies (84) represent the major areas of replication (65). Such bodies have been stained, using histological methods, in the nuclei of neurons in horse brains, and were regarded to be pathognomonic for the typical disease (84).

The mechanism of BDV transcription is not assumed to be the cap-snatching mechanism which has been described for influenza virus. The N gene gives rise to monocistronic mRNA, and P and X proteins are coded by a bicistronic (gene) unit, whereas the M and G proteins and L polymerase gene products originate from polycistronic messages. Two of the BDV transcripts were found to be processed by a cellular RNA splicing mechanism in the post-transcriptional phase. The BDV genome carries two introns (I and II), at nucleotide positions 1932-2025 (intron I) and 2410-3703 (intron II), if the antigenomic sequence is taken as a basis. Ribonucleic acid species coming from the splicing of introns I and II might predict an ORF which could encode a different BDV L-polymerase species which is 150 amino acids longer at the N terminus. Certainly, RNA splicing contributes to the regulation of expression of several gene products, such as the M gene, the G gene, and most probably the L gene (44, 167).

Proteins

Prior to the availability of genetic data, major proteins were defined (e.g. the 40 kDa protein representing the nucleoprotein N and the 24

kDa protein representing the phosphoprotein P). Early experiments demonstrated that the p24 protein is often found as an aggregate banding at 60 kDa (110), showing up as a disulphide-linked dimeric form (94). These proteins have been found in an association which forms the soluble antigen (s-antigen). In the 1950s and 1960s, this antigen was demonstrated to represent the major antigenic component in BDV infection (145, 204) to which the humoral immune response is directed. Protein chemical studies have shown that s-antigen is produced in excess. Recently, a 10 kDa protein (X protein) was suggested to function as a nuclear transport protein (121).

The protein of 14.5 kDa molecular weight which was detected by Schädler *et al.* (164) has been shown to represent the matrix (M) protein which is the backbone of the glycoprotein gp17. Several groups have shown that the M protein (gp17) is present in the envelope and induces antibodies which neutralise the virus (70, 192). This viral protein is also possibly involved in virus attachment (93). The M protein aggregates to a tetrameric form by strong hydrophobic interactions representing a molecular mass of 68 kDa (191).

According to ORF IV, the p56 represents the backbone of a heavily glycosylated protein (G protein) which is also found analogously in other negative- and single-stranded RNA viruses, such as *Rhabdoviridae* and canine distemper virus. Native G protein has a molecular mass of 94 kDa. However, this protein form is metastable and has at least one natural cleavage site (furine-like), leading to 43 kDa polypeptides (61, 192). This glycoprotein is associated with the infectious particle, participating, like the gp17, in neutralisation of the virion (192). This process appears to be similar to other *Mononegavirales* (49), although the exact mechanism of the inactivation process is not known.

The ORF V codes for the 180 kDa protein which represents the L polymerase. This protein has a high genetic homology with polymerases of other *Mononegavirales*. Another ORF predicted in the mRNA species which might be generated by RNA splicing possibly

codes for an additional BDV polymerase of molecular weight 190 kDa (28).

The proteins N, P, M and G are the basis for the antigenicity of BDV, but N and P play the principal role. The N protein is expressed early during the infectious cycle, and exists in two isoforms (38/40 kDa) (155). The protein covers the RNA, probably together with the p24 and the L polymerase. It is not clear whether gp17 participates in RNP structures. Ribonucleoproteins have been found and isolated from the nucleus and shown to be infectious (36). A protein complex formed by p40 and p24 represents the principal immunogen in BDV infections. This complex, usually named the s-antigen, is not only found in brains of infected animals but also in tissue culture, and can be isolated after ultra-sonication of the tissue and centrifugation at 100,000 g. In the supernatant of such preparations, the s-antigen is enriched. This complex structure gives rise to a strong humoral immune response but is not involved in neutralisation (see below). Characterisation has shown that over 90% of the s-antigen consists of p40 and p24 (112).

Within this framework, the glycoproteins of BDV and BDV-infected cells require special observation. The M protein (gp17), as a product of ORF III, and the G (gp94) glycoprotein, derived from ORF IV, are part of the outer membrane of the virion. Both glycoproteins have two hydrophobic stretches in each of their amino acid sequences. The carbohydrate side chains are N-linked with α -D-mannose and N-acetyl- β -D-glucosamine as terminal residues (188). These particular structures play an essential role in the neutralisation process of the virion, and may be involved in virus entry (189, 192). It is clear that the M protein aggregates to a tetrameric form as a consequence of strong hydrophobic interactions (191). Evidence suggests that the M protein participates in forming the infectious RNP by binding to the P protein dimer (paper by Stoyloff *et al.* in preparation). In contrast, the G protein is metastable, with a hot spot region accessible to trypsin-like endoproteases. In other *Mononegavirales*, such glycoproteins play a major role in immunogenicity and protective processes.

During long-term BDV infection in animals, neutralising antibodies against these glycoproteins are usually generated. The further role of these neutralising antibodies is not well defined. They were not only measured in sera and CSF of naturally infected horses, but also after experimental infection of rabbits, rats, mice, chicken and quails (110, 112).

In the rat obesity model, all obese rats produced neutralising antibodies (110). It was assumed that this humoral response inhibited virus spread (112). Recent data could confirm that virus spread can, in fact, be inhibited through neutralising antibodies (187).

The fact that neutralising antibodies have not been detected in naturally infected cats, dogs, and humans remains an interesting but unexplained puzzle.

Recent findings on the molecular biological properties and structural elements of Borna virus as member of the family *Bornaviridae* (154) will be published by the International Committee on Taxonomy of Viruses.

Epidemiology and public health aspects

The epidemiology of BD must be studied in close relation to the epizootiology. The infection in animals is known to be widespread. A principal feature of BDV infection is the large number of animals within a population that are carriers, and can therefore, at least at certain times, spread the virus. This can be assumed to be true for horses, sheep, cats, dogs and, unequivocally, for humans.

Major questions regarding the public health risk posed by these animal viruses remain unanswered. The uniformity in biological and molecular properties of BDV-isolates underlines the close relationship between animal and human viruses (114).

Two hypothetical models exist, either each species is infected by a species-adapted virus, or BDV easily crosses species barriers. Borna disease virus is undoubtedly an old pathogen. However, it is known from animal experiments (and this is in line with general biological

knowledge) that several passages alter the host range and clinical picture of BDV infection. This has been demonstrated with the rat virus (75, 145) which infects the mouse when passaged more than six times (86). When this rat virus is passaged up to ten times, the capacity to tolerantly and persistently infect one-day-old rats is lost, instead the rats are killed in eight to ten days (65). Similar observations have been made for BDV isolates recovered from infected human PBMCs by co-cultivation with oligo cells. Only three further passages of these strains in young rabbit brain cells worsen the pathogenicity for rabbits. The same situation occurs after one *in vivo* passage in rat brain (22).

Based on studies with human virus and laboratory strains, the few mutations observed are obviously sufficient to differentiate the strains (24). It is conceivable that protein-altering nucleotide changes (e.g. in the glycoprotein genes) are responsible for individual biological properties which are expressed as tissue culture, neutralisation, virulence and drug resistance properties. Therefore, BDV might be able to adapt relatively rapidly to other hosts. These questions and speculations, however, need further elucidation on a molecular level.

In comparison with other *Mononegavirales*, the high stability of the BDV genome was unexpected. Although only a few strains or genes from different sources have been sequenced, this high stability seems to be a general pattern. The hypothesis which has not yet been falsified suggests that BDV represents an old pathogen with a history of possibly millions of years of adaptation. In this respect, separate evolutionary processes in different hosts are likely. However, this would not exclude occasional interspecies transmission, especially between humans and pets. The zoonotic aspect is still unsolved. More wild-type isolates are required to elucidate this aspect.

Biology of infection

Infection of cells

When discussing target cells of BDV, differentiation between cells infected under natural conditions and *in vitro* infection of cell lines

and primary cells is important. *In vivo*, BDV preferentially infects neurons of the limbic system, and leaves markers named Joest-Degen bodies (84). *In vitro*, the first tissue culture growth in different cell lines was reported after co-cultivation of primary hippocampal rabbit brain cultures with permanent cells which outgrew the secondary and tertiary cells (105, 110, 112). Similar reports followed on BDV growth in primary cultures (41, 43). Several outstanding biological properties of BDV soon became clear, including virus replication without causing a cytopathic effect (CPE), whatever cell was infected. This also meant that assays of this virus always required cell-antigen-detection-tests (focus tests) which complicated cell biological and diagnostic investigations (Fig. 9). As early as 1926, Joest suspected that the nucleus was involved in replication (83). More recent studies demonstrated that fine or large antigen accumulations (depending upon the cells used) appeared in the nucleus, a pattern which allowed infectivity to be monitored (106). Recent molecular studies confirmed that BDV replicates in the nucleus (27, 36). Furthermore, in the 1970s, Ludwig and Becht obtained evidence that BDV is a cubic RNA virus (106), pointing to the differences with other RNA viruses, the replication of which was known to occur in the cytoplasm. The growth curve closely linked to the cell cycle showed that the virus spread from cell to cell, forming a focus of a certain size with a characteristic number of antigen carrying cells (110). This discovery enabled titration of the virus in a focus assay, analogously to the titration of other viruses by plaque assays, and demonstration of the neutralisation of BDV. Soon neutralising antibodies were detected in sera and CSF of naturally and experimentally infected animals (75, 112).

Another outstanding property of BDV is the broad cell spectrum, whether established cell lines or primary cells in culture are used. The following lines were persistently infected (TL indicates infection with strain V): Vero/TL, MDBK/TL, MDCK/TL, CRFK/TL, ED/TL, PK15/TL, C6/TL and, from human cells, oligo/TL. Other laboratories grew BDV in mouse neuroblastoma cells (175) and guinea-pig cells (151). The oligodendroglia cell line originally established by Y. Iwasaki was kindly made available to H. Ludwig at the end of the 1970s. This cell line has proved to be especially useful for cultivation

of human viruses (22). However, the cell line should be handled with precaution insofar as undefined passages (in which contaminations cannot be excluded) which have been distributed illegally to some other laboratories are concerned. The only supplier of original oligo cells is the Institute of Virology at the Free University in Berlin.

Primary cells of rabbit origin (e.g. young rabbit brain, spleen and retina cells) (112, 196) show a predilection for this virus, and are appropriate for virus titration (110).

Antigen extracted from infected cells reacted in Ouchterlony or immunoelectrophoresis tests with prominent precipitation lines demonstrating the s-antigen (105). Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) showed major components with apparent molecular weights of 42 kDa and 25 kDa (108), representing p40 and p25 (110, 112).

It is now known that p40 and p24 are actively transported from the cytoplasm into the nucleus and participate in the antigen accumulation which has been investigated using IFA (72, 81, 105, 108, 112).

No detailed protein and molecular studies have, as yet, investigated whether BDV antigens in cell lines are expressed differently. In persistently infected cell lines, antigen localisation in the nucleus is predominant, whereas primary cells, such as young rabbit brain (YRB) cells, either grown from whole organs or selectively from neural tissue show characteristic antigen expression in both compartments (nucleus and cytoplasm) (Fig. 9). This field of study requires further research, since viral functions might depend upon the different expression of singular proteins and their cleavage products.

Borna disease virus antigens are not usually found on the plasma membrane. However, the glycoproteins reach the cell membrane and, neutralising antibodies especially, bind to infected cell membranes (110). The virus growth curve in freshly infected cells reaches saturation after three or four days (110). However, the complete replication cycle of BDV is not known. A schematic presentation of

replication events, including the involvement of the nucleus, was outlined by Stoyloff (188).

Experimental infection of animals

In vivo, the virus is detectable in both the brain and blood (8, 18). All studies of human and animal infections now take advantage of this *intra vitam* property of BDV to also use PBMCs as target cells.

Whether this neurotropic virus establishes foci outside the brain during the course of natural infection (e.g. in the bone marrow or the spleen) has not been critically studied, however, splenectomy alters the clinical picture (112).

In vivo studies of adult or immature (1-3 days old) experimentally infected animals, such as rabbits, rats, or mice, demonstrate that patterns of clinical infection are different from those seen in nature (114). This has been overlooked in experimental studies performed recently (185). Differences might be explained by the much greater virus load and the non-natural (intracerebral) route of infection.

The rabbit appears to be the animal of choice for experimental infection (114, 215). Intracerebral infection induces the classical symptomatology (215), with a selective accumulation of antigen and infectious virus in limbic brain areas (M. Furuya and H. Ludwig, unpublished findings) (Fig. 10).

A solid interpretation of *in vivo* infections of rats commenced with basic investigations by Nitzschke using strain V (145). Passaging the virus altered and stabilised the incubation time and presented evidence of immune tolerance in young animals, as previously demonstrated by lymphocytic choriomeningitis virus infections in mice (194). Studies on the adaptation of BDV to the embryonated egg, performed before tissue culture studies became available, were important in this context (146). With this background knowledge, groups in Berlin (75) and Giessen (140) developed an animal model based on the rat. The model describes the clinical outcome depending on the age of the animal at the time of infection, as follows:

- one-day-old rats become infected, replicate the virus and acquire a persistent infection
- animals do not show clinical symptoms
- such animals with high BDV titres and a heavy antigen load in the brain were used in controlled psychological experiments to demonstrate learning deficiencies (50)
- rats infected as adults acquire chronic disease and die with varying incubation times (Fig. 11), or become obese and survive (110).

Other interesting neonatal rat models, e.g. for the study of autism or play, have recently been presented (152, 153).

When adult rats were infected, BD could be induced, and inflammatory reactions occurred in this chronic stage of infection. The animals showed behavioural changes (aggressiveness and somnolence) and, depending on the number of virus passages, more or less severe clinical symptoms. As already demonstrated in rabbits (97), alterations in the retina were obvious (65, 140). Based on the rat experiments, BDV was later adapted to the mouse (86). Obesity could be induced in the rat and, with less evidence, in the mouse (65, 87). In the rat, BDV caused hypothalamic lesions, and dysfunctions are the probable reasons for this clinical picture of obesity.

The scope for investigation of experimental infection is immense. Such studies have provided evidence that immune pathological reactions appear (even in animals which do not show clinical signs, such as tree shrews [180], or experimentally infected cats [119]) and might be involved in the progression of the disease with fatal outcome (75, 141). These experiments also led to the hypothesis that cluster of differentiation antigen 8 (CD8) cells are involved in immunopathological patterns, and that CD4- and CD8-cells induce disease. Much of this information has recently been summarised (185).

However, these data do not explain the various forms of natural disease in horses, cats or humans, and therefore their significance

regarding BDV infection in nature is questionable. Findings have helped to define the pathogenesis of the progression of disease, and have demonstrated that naturally infected animals succumb under the load of antigen in the brain, and possibly secondarily due to the protracted immune response. However, subtle behavioural changes await more sophisticated explanations and hypotheses, for example, that of a neurotransmitter change due to BDV activation (22, 23, 64, 103).

Behavioural changes as the principal clinical signs were first observed in infected tree shrews, animals with primate-like morphological organ-structures (e.g. hand, ear) (179). Clear alterations in major behavioural patterns of communicative and reproductive behaviour were recognised (e.g. cleaning, resting and sexual behaviour). The sensitive co-ordination of sexual behaviour was critically disturbed. Despite increased social contact between the partners (tree shrews are usually aggressive, solitary animals), reproduction became a problem. Notably, the alterations in social behaviour over the course of this persistent infection led to unusual interactions between infected parents and offspring, which have never been observed under natural conditions. This basic, revolutionary finding was reported in the mid-1970s (180), and raised much discussion about the effect of BDV on limbic structures.

Additionally, learning experiments which demonstrated significant deficiencies in persistently infected healthy rats represented another outstanding discovery in Borna research. The infected rats behaved less cautiously in open field experiments (changes in emotional behaviour) and showed other significant learning deficits when submitted to established test systems (50). Recent studies on altered play behaviour in rats report comparable results (153).

These induced behavioural changes in tree shrews and rats, supported by antigen studies in limbic structures (65, 114) and the accumulating knowledge about BDV in human mood disorders (9, 22, 23), marked the development of a new field in biological psychiatry. Virus infection is assumed to modulate the function of brain structures

involved in learning and emotions. The functionally connected brain areas, called the limbic system (177), are known to control the ability to co-ordinate behaviour, and to learn and follow natural instinctive patterns (180). These are exactly the same brain areas that are preferred by BDV for replication and antigen expression.

Knowledge of the biology of natural infections, often deduced from experimental infection, is at present only elementary (183, 184, 185). Information has been collected from naturally infected horses and cats, and recently, also from humans. However, all these biological and immunological aspects must to be subjected to further scrutiny.

Infection in horses has already provided some answers. Natural infection can present in three forms, as follows:

- subtle behavioural changes
- severe neurological disease (rare), occasionally with a fatal outcome, or
- infection without symptoms (the more widespread type of infection).

At present, the extent to which the outcome of infection is due to strain differences or to the genetic resistance of the animal or species is not known.

It has been suggested that once the horse enters the phase of overt disease, the load of antigen together with immune reactions in the brain cause a functional breakdown; the carnivore (e.g. the cat) may be better prepared to cope with the infection. Exceptions might be present in endemic areas (the region of Borna, Germany, or for cats, the area north of Stockholm [116]) where the load or virulence of the circulating virus in the population could lead to super-infections which finally induce the severe endemics with high mortality.

In humans, the knowledge of the biology of infection seems to stop at the level of neurotransmitter alterations expressed in clinical forms, like psychiatric diseases, due to limbic functional alterations. No reports exist of severe inflammatory processes in the brains of patients with encephalitis who are infected with BDV, or even patients who

died as a result of BDV infection. However, these clinical and pathological pictures may be observed in the future and brought into context with infectious processes. Relationships between BDV infections, encephalitis lethargica and Economos' disease have as yet not been proved using modern laboratory techniques.

Pathogenesis

Pathogenetic effects in the body are governed by the mode of spread of the infectious units, originally thought to occur intra-axonally along the nerves (31, 98, 148), and now also accepted to follow viraemia (18, 20).

From the numerous neuropathological studies in naturally and experimentally infected animals, a certain picture of the pathogenesis becomes clear. Post-mortems of horses, sheep and cats which died from BD usually show no significant pathological alterations in the body and brain. In some cases, hyperaemia and/or haemorrhages in the brain were observed during single endemic outbreaks of BD in horses. Such unusual pictures may point to highly virulent strains, or may indicate that this virus, whilst neurotropic, also attacks the endothelium of arterioles and venules in the brain (109).

In classical cases of BD, most of the reports describe inflammatory reactions accumulating in the limbic system (hippocampus, amygdala, thalamus, hypothalamus). There is evidence of neuronal lesions, degeneration of neurons and neuronophagia, a common picture given in the early literature (84, 173).

Types of cells involved in the BDV infection processes include astrocytes, oligodendrocytes, glial (65) and Schwann cells (32), in addition to the neurons carrying the Joest-Degen inclusion bodies (65). The picture became clearer when antigen studies were included in the pathogenetic considerations. Immune histology shows that horse brains harbour many neurons filled with antigen. A characteristic pattern is visible in the hippocampus (110). Clearly, a correlation exists between antigen and nucleic acid (detectable by *in situ* hybridisation) (32, 65). These pictures are quite different in cats

with SD, where only a few antigen-carrying neurons were observed (118). In the view of the authors, the significance of immunopathology in natural infections has been overstated. Severe inflammatory reactions have been reported in the brains of horses which show no symptoms (125), and in experimentally infected cats (119) without clinical disease. In the experimental systems (using rats or mice), especially in one-day-old infected animals, the brain is loaded with antigen (64, 110), again showing the typical distribution of antigen layers developing with time in the hippocampus (64, 110). Such antigen pictures have been reproduced by several groups. The eye seems to be specifically prone to immune reactions, as demonstrated in rabbits (97) and rhesus monkeys (99).

Pathogenetic views may be broadened and further complicated by the recent discoveries that BDV leaves the brain (18) and not only enters the peripheral nervous system, but also infects cells of the myeloid stem line (20, 89). These events seem to occur in parallel with severe neurological or psychiatric disease which would indicate that the virus replicates in the periphery. This does not mean that in such cases the brain is not involved, especially as BDV in animals is known to replicate in the brain under natural conditions. These observations may indicate that BDV infection is dormant or latent in the reticulo-endothelial system. It is possible that the bone marrow stem cells carry a persistent infection, only shedding virus in cases of stress or other immunosuppressive, activating processes. A coincidence between mood disorders or behavioural alterations with increasing severity, and presence of antigen in the periphery is clear (114). Although the neuropathogenesis of BDV infections is vaguely understood, the primary pathomechanisms are still debated. In full blown natural BD, the fatal outcome can be explained by excessive virus production and immune pathology, secondary to these events (immune reactive cells and/or antibodies could attack the antigen-carrying neuron) (Fig. 12). Experimental infections in rats or mice may mimic such processes, but do not clarify natural infections. Unknown mechanisms during BDV infection, such as cytokine release and interaction of infected neurons with mediators in the brain (81, 152, 174), may pathogenetically contribute to phasic behavioural and

clinical alterations (24). As deduced from preliminary experimental data in the rat, neurotransmitter disturbances, especially in the glutamatergic system may play a key role in the understanding of neuropathogenesis (64, 65; G. Gosztonyi and H. Ludwig, unpublished findings).

The hypothesis put forward by the authors to explain neuropathogenetic events in human infections (which may be similar in cats and horses) is the following: a transient functional interaction of viral antigens with neurotransmitter receptors in the limbic system (occurring during BDV activation phases) represents a primary pathomechanism. This would explain the lifelong relapsing disease episodes and also the intervals without symptoms which are characterised by a form of 'latent' infection (24, 39, 113). This hypothesis means that some severe human psychiatric diseases may be caused by a BDV infection triggered by individual genetic vulnerability (22), including stress sensitivity (48, 115). It is known that such unbalanced neurotransmitters induced by stress events and disturbances of the hypothalamic-pituitary-axis (HPA), facilitate virus infections and morbidity in general (62). In addition, it is assumed that BDV infection reduces the flexibility of the neurotransmitter equilibrium in the limbic system (114). On the other hand, resistant individuals exist who are not harmed by this virus.

Diagnosis and surveillance

The classical diagnosis of BD is made by the clinician (74, 109, 133, 165). The typical symptoms in the initial stage and the progressing course of BD have been described. These symptoms allow a differentiation from infections like rabies, canine distemper, equine herpes virus type 1, and BSE or scrapie.

Post-mortem neuropathology can confirm the diagnosis through the presence of the typical inflammatory reactions in central areas of the brain, in the hippocampus, hypothalamus, amygdala with perivascular infiltrates and, most importantly, the intranuclear Joest-Degen inclusion bodies.

The detection of BDV antigens in smear preparations or in cryosections of horse and rabbit brains (205) considerably facilitated post-mortem diagnosis (63, 73, 159). Immune-histology is the current method of choice, and this can be supported by *in situ* hybridisation techniques (32, 65) or by specific nucleic acid amplification from suspicious brain material (46, 161, 213). The simplest diagnostic tool is the monitoring of BDV antigens by ELISA (22). The Western blot analysis for BDV antigens has also been used, however, this method should use a set of defined antibodies (15, 59, 207), and is less sensitive than other tests.

Additionally, virus can relatively easily be grown in tissue culture using brain suspensions (63, 72, 105, 120, 156), although several tissue culture passages might be necessary. Intracerebral inoculation of suspicious tissue material into rabbits or new-born rats still has considerable confirmatory importance (109, 110).

Intra vitam diagnosis has successfully been performed since the mid-1950s (42, 109, 145, 203) through demonstration of antibodies using different systems, such as Ouchterlony, complement fixation and IFA tests, and later on, the ELISA (22, 72, 110, 118). The IFA is still a valuable method, if performed with a monoclonal-counterstain for specificity, but antibody testing using ELISA is now used more often. Unlike other virus infections (e.g. human immunodeficiency virus [HIV] infections), Western blot has not gained significance as a confirmatory test. Until recently, the demonstration of antibodies was the principal approach to diagnosis of BDV infection, especially in relation to psychiatric diseases in humans (mostly with the wrong interpretation). The recombinant proteins often used as the defined antigen in such tests (5, 34, 55, 91) remain a weak tool with regard to specificity and sensitivity (114). Neutralising antibodies, whether in natural or experimental infections, are only of limited diagnostic value (112), in sharp contrast to their important role in other *Mononegavirales* where protective immunity is often involved (49).

Antibodies found in the CSF of naturally (107) and experimentally infected animals (108) have also been used as a diagnostic tool.

Detailed investigations showed that such immunoglobulins are of oligoclonal character (107, 108), a sign of intrathecal, local production (110). This phenomenon is known to occur in various inflammatory infections of the brain of parasitic, bacterial or viral origin, and as in MS, these oligoclonal immunoglobulins are used as a diagnostic marker. In BD, oligoclonal immunoglobulins have not taken on great importance, although it is of interest that, when present in high titres in CSF, they always have neutralising capacity. This means that the brain probably also produces antibodies against the glycoproteins (112, 188).

Antigens

The demonstration of BDV antigens has become the major tool for monitoring infection. The fluorescence activated cell sorting method was introduced to differentiate virus antigen-carrying cells in PBMC sub-populations (181). The specificity was achieved by a set of monoclonal antibodies against the p40 and p24 proteins (18). Cells carrying BDV antigen outside the brain have now become a valuable marker for diagnosis of BDV infection (20, 80, 162).

Based on this knowledge, ELISA tests applicable to different species were developed. The method is based on binding BDV antigens from concentrated blood samples through the use of specific anti-p40 and anti-p24 monoclonal antibodies, and demonstration of the positive reaction with a heterologous hyperimmune serum (preferentially of rabbit origin). The rabbit immunoglobulin G can then be monitored by anti-rabbit antibodies (coupled to enzyme) in the ELISA. The same principle has been applied to monitor antibodies in the ELISA (see above). In this case, native antigen from tissue culture, bound to monoclonal antibodies, was used as a specific target to measure antibodies in suspect samples ('double-sandwich test'). Bound sample antibodies were monitored by enzyme-linked anti-species antibodies (22). However, this assay does not measure the same antibody quality as the IFA due to acetone-fixed, versus native, soluble antigen. The antibody ELISA is a valuable assay if used together with ELISA

antigen assays. As mentioned above, native antigens are preferable to recombinant antigens for the detection of antibodies.

Borna disease virus genome pieces and/or mRNA

Nucleic acid amplification using a nested polymerase chain reaction (PCR) has become another powerful tool to detect infection (20, 137, 213). Parts of the p40 or p24 genes are used as targets, and with the appropriate primers, BDV-specific information can be amplified (24, 163). Nevertheless, this test has been applied world-wide (4, 35, 89, 162). However, this method is limited by low replication rates and low numbers of infected cells.

Due to these original difficulties, BDV diagnostics is still performed by a few specialised laboratories with long-term experience in this field. Blind evaluation studies performed by several groups in Germany accumulated many discrepancies, most probably due to technical problems, such as lack of sensitivity of test systems, use of the wrong primers and contamination problems. Discussions regarding the validity of test results were supported by two groups claiming that no BDV RNA exists in peripheral cells (102, 158), opposing the overwhelming majority of research world-wide which has found BDV-specific RNA in white blood cells of healthy and psychiatrically diseased humans. Recently, Schwemmle *et al.* speculated that all human isolates are due to contamination (172), based on data taken from BDV RNA sequences collected by several other groups (22, 46, 81, 151). Approximately 18% of animal and 'human' BDV genome stretches (but not all RNA sequences published) were selectively chosen for comparison, thus leading to considerable misinterpretation. Furthermore, the data disregard the biology (22) and detailed molecular studies on the individual nucleotide identity of isolates and original PBMCs (45), as well as the origin of the isolates. This controversial view exemplifies the danger and difficulty of a simplified interpretation of genetic data, and neglects the known complexity of the BDV system as a persistent infection found in mammals world-wide, with a surprisingly genetically stable negative-stranded RNA virus (168).

It is of diagnostic relevance that the presence of nucleic acid (positive PCR) proves only the presence of genetic material, but no information is obtained on the expression of BDV proteins, the production of which usually runs parallel with disease processes (20, 22, 24, 47, 76).

Live virus

In vitro virus isolation from animals and humans has not yet achieved diagnostic importance, because of the difficulties in recovering the agent from infected animals and from humans. Although definitive statements on the spread of different BDV strains in animal and human populations cannot be made, it is necessary to concentrate future studies on more wild-type isolates which, in turn, will make molecular epidemiological studies possible (120, 156).

The diagnostic test battery needed to acquire a comprehensive picture of the state of a BDV infection, and also allowing some prognostic statements, is summarised in Figure 13. The 'gold standard' to date is determining at least two infection markers in not less than two follow-up samples (preferably three samples), during the acute phase of disease.

Surveillance

Surveillance of BDV infection in animals and humans still suffers from poor standardisation of diagnostic methods. Better antigen and nucleic acid assays may help to explain the molecular epidemiology and spread of BDV infections, thus allowing improved surveillance of activated infections in individuals who may be shedding the virus.

The development of sensitive antibody and antigen detection methods has permitted the screening of populations. Unexpectedly high levels of infection were reported in horses, sheep, cattle and cats, especially in otherwise healthy groups where diseased animals with activated infections had previously been observed (19, 76, 120). A similar finding, which was based on the presence of antibodies has been reported in horse stables throughout Germany (19, 24, 101, 109). In general, cohabitation of sheep and horses enhances the equine disease

rate, therefore, future surveillance must concentrate on the detection of animals (and humans) with activated infections which may be shedding virus.

Borna disease can be classed with the recently established group of emerging diseases, in the sense that the real importance of the disease relates to its possible involvement in psychiatric diseases of humans, and in this way a new view of this virus has 'emerged'. In this respect, efforts must be made to detect and survey silent shedders of the virus.

Additional activities should concentrate on meat and blood products of animals used for medication of humans. Although a report excluded the possibility of infection through eating raw horse meat in Japan (77), medical products (not inactivated) used in human reproduction as well as in transplantation medicine are not controlled, and therefore remain a source for human infection. This emphasises the need for surveillance of this persistent infection in such animals. Given the possibility that BDV could represent a zoonotic agent, greater knowledge of its transmission is urgently required.

Prophylaxis and treatment

Two approaches have been applied to cope with BD in the past, namely: hygienic measures and vaccination. Long-term empirical data collected in the endemic areas predicted that separation of sheep and horses would prevent the spread of BD. Furthermore, the sanitary situation seemed to be a critical factor. Severe losses were mostly observed in farms where several animal species were kept together in one stable. Therefore, the recommendation to improve hygiene and prevent contact between susceptible species had some effect, but did not lead to elimination of BD.

Threatened by the severe losses of horses in rural areas of Saxony, Bavaria and Hessa, in Germany, at the turn of the 20th Century, vaccination was envisaged (54, 217). When killed vaccines were shown to be ineffective, filtered brain suspension from diseased horses with living virus were applied subcutaneously. Only when lapinised live vaccine became available (Zwick vaccine, based on

strain V) (217) and later a vaccine based on strain Dessau (131) in some areas (especially the former German Democratic Republic), was rigorous vaccination made compulsory (from 1962 to 1992). Due to the change in political situation and progress of knowledge on the ineffectiveness of the vaccine, these campaigns were ceased.

Given present knowledge on the stability of BDV genomes, it is assumed that the widespread infection rate found in Germany (at least in horses) is partly due to these vaccination campaigns. From a modern perspective, any vaccination against this persistent infection located in the nervous system cannot be recommended. Based on the epidemiological situation, even modern tailored DNA vaccines are unjustified at present.

Of great importance is the possibility of antiviral treatment. Researchers in France in the 1920s introduced hexamethylenetetramine treatment of BD in horses with some success (132). This 'therapy' was later recommended by von Ostertag in parts of Germany (201). However, the above vaccination programmes abrogated the development of these therapeutic approaches.

Recent *in vitro* studies with the antiviral substance amantadine sulphate (a chemical relative of hexamine) highlighted treatment of BDV infection. A patient who gave positive results to tests for BDV infection, and suffered from Parkinson's disease with underlying mood disorder, improved and returned to a state of good health after such a treatment, even clearing the BDV genome (although the Parkinsonism remained) (25). Despite controversial issues raised by several groups on the effectiveness of the drug (38, 69, 186), the authors recently resolved these discrepancies by showing that the drug effectively inhibited all wild-type BDV strains from humans and horses. *In vitro* studies clarified that the laboratory strains (vaccine strains) are insensitive to the drug (26). This means that antiviral therapies are an alternative treatment for patients suffering from activated BDV (57, 58). In two open trials, a clinical responder rate of approximately 70% supported the view that infected patients benefit

from this therapy (58). In the blood, a virus-static effect was achieved. Systematic investigations have yet to be performed in animals.

Other antiviral substances interact with viral glycoprotein, as described for gp17 (190), or interfere with BDV replication (e.g. ribavirin) (130, 85). However, the latter almost universal antiviral compound, is known to cause considerable side-effects in treatment of humans for other virus infections.

Conclusions and perspectives

Borna disease virus infections are included in the group of 'emerging diseases' because of the possible emerging zoonotic threat of this old disease. Biological and molecular properties of BDV, the only representative virus of the family *Bornaviridae* have revealed that this negative-stranded, enveloped RNA virus, spread world-wide, has an unusually broad host spectrum. Natural infections and disease have been noted in horses, sheep, cats, dogs and ostriches. Since the mid-1990s, interest has been stimulated by human infection and the role of this agent in biological psychiatry. The quantity of research data on BDV published world-wide, embedded in numerous findings and conclusions drawn from infected animals, together with the recent detection of BDV antigen in brain fluid at peaks of clinical illness in psychiatric patients (47) (Fig. 14) underlines the interference of this virus with the well-being of humans.

Conceptually, BDV infection is diametrically different from lytic virus infections which are known and accepted to cause disease. To understand the persistently infecting BDV which induces no CPE and replicates preferentially in limbic structures of the brain, a new concept is required. The authors favour the possibility of transient functional (instead of structural) alterations, of brain neurotransmitter circuits that would explain phasicity and symptoms (at least in mood disorders). The disease potential is similar to that of *Helicobacter pylori* and *Chlamydia pneumoniae* infections, two agents which are known or are increasingly thought to be involved in gastric and vascular diseases. Bornavirus appears to complicate the picture, because of the effects on normal behaviour in animals and on mood

regulations (or intellectual abilities in humans), but also the potential to rarely induce encephalitis.

In animals, the role of BDV in producing this spectrum of disease symptoms is common knowledge. In humans, the possibility of an infection is accepted but the link with certain psychiatric disorders is still under discussion. More thorough longitudinal studies and new isolates will help to reach a better understanding of the virus. It is unlikely that humans are not affected by the pathogenic potential of this agent.

For the veterinarian, this infection may not appear to require urgent attention, but the zoonotic potential of the virus has initiated rising interest in medical fields, especially in psychiatry.

The perfect adaptation of this virus to animals has raised concern over whether transmission may occur through animal products. Risk assessments of blood products of animal origin should be performed in the near future to answer these questions.

The lack of knowledge on the epidemiology of BDV, due to the lack of solid molecular epidemiological data on virus sources and methods of spread in nature, will certainly influence future research. Borna disease virus infection and BD no longer cause epizootics in the classical sense, but because of the perfect adaptation of this virus, it appears to be a potentially dangerous commensal in mammals (14, 16, 114).

No methods of eradication of the virus are available at present. All biological and molecular properties suggest that the virus is an evolutionary old pathogen, which is perfectly adapted to a highly specialised cell, the neuron. This survival strategy has continued to fuel interest in this agent from veterinary and medical quarters. In medicine, with 1%-5% of the population suffering from mood disorders, investigation of the properties of such an agent opens up a broad new field of study.

The unknown zoonotic potential of Bornavirus and the association and involvement in psychiatric diseases are fascinating areas for further research. The devastating consequences of mood disorders for the individual, and in public health terms (62), which often touch highly integrative functions in the brain (82), make the Borna agent a modern challenge for research in human and veterinary medicine, world-wide.

Acknowledgements

The authors are grateful to G. Gosztonyi, R. Stoyloff, P. Reckwald, T. Leiskau, D. Lüschoff and J. Hübner for continuous encouragement and co-operation, American colleagues I.W. Lipkin and J.C. de la Torre for previous fruitful joint studies, and Israeli colleagues M. Malkinson and Y. Weisman for their co-operation on work on diseased ostriches. Thanks are also extended to J. Brugère-Picoux, Paris, and many colleagues world-wide for providing blood samples. Special thanks go to R. Ferszt, D.E. Dietrich and H. Emrich for care of the psychiatric patients in special study programmes, and to T. Komaroff, Boston, for initial studies on CFS patients. Long-term studies were supported by the Deutsche Forschungsgemeinschaft and the European Community.

Virus de la maladie de Borna : nouveaux aspects de l'infection, de la maladie, du diagnostic et de l'épidémiologie

H. Ludwig & L. Bode

Résumé

La maladie de Borna est une affection des chevaux connue depuis le XVII^e siècle (on l'appelait alors une « maladie de la tête »). Les recherches effectuées au cours des cent dernières années ont montré que l'agent causal de la maladie de Borna était un virus à acide ribonucléique (ARN) enveloppé, non segmenté, à brin simple et négatif, qui représente la famille des *Bornaviridae* dans l'ordre des

Mononegavirales. Le virus, dont la distribution est mondiale, affecte les chevaux, les ovins, les bovins, les chiens, les chats ainsi que les autruches. La maladie peut être mortelle, mais la plupart des individus porteurs du virus présentent une infection persistante et asymptomatique. Chez l'homme, l'infection est associée à des troubles mentaux, ce qui a justifié les très nombreuses recherches effectuées sur le virus de la maladie de Borna ainsi que la création de centres spécialisés en Allemagne, aux États-Unis d'Amérique et au Japon. L'infection expérimentale de tupaïas et de rats a permis d'observer les effets de la maladie persistante et clinique, notamment une perturbation du comportement et une baisse des capacités émotionnelles et d'apprentissage. Cette infection virale « émergente », qui met en jeu des mécanismes pathogéniques complexes dans le système nerveux, se propage également *via* les cellules myélomonocytaires. Le diagnostic peut être effectué par examen sérologique, mais la recherche de marqueurs d'antigène dans les leucocytes périphériques, associée à l'amplification de l'acide nucléique, donne de meilleurs résultats. Des études comparées de l'ARN révèlent une homologie génétique des virus exceptionnellement élevée. L'étude des virus isolés chez l'homme et le cheval semble indiquer que le virus est spécifique. La vaccination n'est pas conseillée, mais la thérapie antivirale utilisant notamment du sulphate d'amantadine, dont l'efficacité a été prouvée *in vitro*, pourrait permettre de traiter les troubles émotionnels chez l'homme. L'infection par le virus de la maladie de Borna est consécutive d'un état de fragilité. Bien que la capacité de ce virus commensal de se transmettre d'une espèce à l'autre n'ait pas été démontrée, le caractère zoonotique de l'infection qu'il entraîne doit être examiné avec la plus grande attention.

Mots-clés

Diagnostic – Épidémiologie – Maladie de Borna – Santé publique – Virus – Zoonoses.

El virus de la enfermedad de Borna: nuevos aspectos concernientes a la infección, la enfermedad, su diagnóstico y su epidemiología

H. Ludwig & L. Bode

Resumen

La patología que hoy denominamos enfermedad de Borna es una "enfermedad de la cabeza" descrita en el caballo desde el siglo XVII. Los más de cien años de investigación sobre esta enfermedad han establecido que su agente etiológico (el virus de la enfermedad de Borna) es un virus ARN unicatenario, de hebra negativa, no segmentado y con envoltura, representante de la familia *Bornaviridae*, orden *Mononegavirales*. Este virus, presente en el mundo entero, afecta a caballos, ovinos, bovinos, gatos, perros y avestruces. Aunque a veces resulta fatal, la mayoría de los portadores presentan una infección persistente y asintomática. El hecho de que la enfermedad se presente en el ser humano asociada a desórdenes psiquiátricos motivó un gran auge internacional de las investigaciones dedicadas a su agente vírico, con la creación de centros de investigación en Alemania, Estados Unidos de América y Japón. La infección experimental de ratas y musarañas arborícolas sirvió para estudiar los efectos de la enfermedad en su forma clínica y persistente, entre ellos, sin duda el más interesante, los cambios de comportamiento y los déficits emocionales y de aprendizaje inducidos por el virus. Esta infección vírica "emergente" presenta mecanismos patogénicos complejos en el sistema nervioso, pero también se difunde por células mielo-monocíticas. Aunque el diagnóstico serológico es posible, resulta más rentable la detección de marcadores antigénicos en glóbulos blancos periféricos, combinada con la amplificación de ácidos nucleicos. Los estudios comparativos de ARN han revelado un alto nivel de homología genética entre los virus. Las cepas aisladas en el hombre y el caballo hacen pensar en la existencia de estirpes específicas para cada especie. La vacunación no es un procedimiento muy recomendable, contrariamente a la terapia antivírica (sobre todo con sulfato de amantadina), que no sólo encierra grandes posibilidades

para combatir los desarreglos psicológicos en el ser humano sino que además resulta eficaz *in vitro*. Las infecciones por el virus de la enfermedad de Borna obedecen a un esquema de vulnerabilidad. Aunque de momento no está demostrada su capacidad de franquear la barrera interespecífica, conviene prestar la mayor atención a las posibles derivas zoonóticas de este virus comensal.

Palabras clave

Diagnosis – Enfermedad de Borna – Epidemiología – Salud pública – Virus – Zoonosis.

References

1. Altmann D., Kronberger H., Schüppel K.-F., Lippmann R. & Altmann I. (1976). – Bornasche Krankheit (Meningo-Encephalomyelitis simplex enzootica equorum) bei Neuwelttylopoden und Equiden. *In* Verh.bericht XIII Int. Symp. Erkrankg Zootiere (R. Ippen & H.-D. Schröder, eds), 16-20 June, Innsbruck. Institut für Zoo- und Wildtierforschung, Berlin, 127-132.
2. American Psychiatric Association (1994). – Diagnostic and statistical manual of mental disorders, 4th Ed. (DSM IV). American Psychiatric Association, Washington, DC, 208 pp.
3. Anzil A.P., Blinzinger K. & Mayr A. (1973). – Persistent Borna virus infection in adult hamsters. *Arch. ges. Virusforsch.*, **40** (1), 52-57.
4. Arunagiri C., McGrath J., Wright D., Dean M., Barbero R., Studdert M. & Gowans E. (1999). – Identification of Borna disease virus in horses and cats in Australia (Abstract). *In* Abstracts, Proc. 11th International Congress of Virology, 9-13 August, Sydney. International Union of Microbiological Societies, Sydney, 72.
5. Auwanit W., Ayuthaya P.I., Nakaya T., Fujiwara S., Kurata T., Yamanishi K. & Ikuta K. (1996). – Unusually high seroprevalence of Borna disease virus in clade E human immunodeficiency virus type 1-

infected patients with sexually transmitted diseases in Thailand. *Clin. diagn. Lab. Immunol.*, **3** (5), 590-593.

6. Bahmani M.K., Nowrouzian I., Nakaya T., Nakamura Y., Hagiwara K., Takahshi H., Rad M.A. & Ikuta K. (1996). – Varied prevalence of Borna disease virus infection in Arabic, thoroughbred and their cross-bred horses in Iran. *Virus Res.*, **45** (1), 1-13.

7. Beck A. & Frohböse H. (1926). – Die enzootische Encephalitis des Schafes. Vergleichende experimentelle Untersuchungen über die seuchenhafte Gehirnrückenmarksentzündung der Pferde und Schafe. *Arch. Tierheilkd.*, **54**, 84-110.

8. Bode L. (1995). – Human infections with Borna disease virus and potential pathogenic implications. *In* Borna disease (H. Koprowski & W.I. Lipkin, eds). *Curr. Top. Microbiol. Immunol.*, **190**, 103-130.

9. Bode L. (1996). – Bornavirus – ein Faktor bei endogenen Affektstörungen? (Editorial). *Bundesgesundheitsblatt*, **8**, 281.

10. Bode L., Riegel S., Ludwig H., Amsterdam J.D., Lange W., Koprowski H. (1988). – Borna disease virus-specific antibodies in patients with HIV infection and with mental disorders. *Lancet*, **2** (8612), 689.

11. Bode L. & Ludwig H. (1989). – Borna disease virus infections and immune responses in primates and man. *In* Proc. 3rd Annual Symposium of the European Society of Veterinary Neurology (ESVN), 10-12 April, Bern. ESVN, Bern, 89-90.

12. Bode L., Riegel S., Reckwald P. & Ludwig H. (1990). – Improved and rapid serodiagnosis of Borna disease virus infections in animals and man (Abstract). *In* Proc. 8th International Congress of Virology, 26-31 August, Berlin. International Union of Microbiological Societies, Berlin, 302.

13. Bode L., Komaroff A.L. & Ludwig H. (1992). – No serologic evidence of Borna disease virus in patients with chronic fatigue syndrome. *Clin. infect. Dis.*, **15** (6), 1049.
14. Bode L., Czech G., Ferszt R. & Ludwig H. (1992). – Borna-Virus-Infektion beim Menschen: Eine neue Zoonose? In Bericht des 4. Hohenheimer Seminars 'Aktuelle Zoonosen', 16-17 September, Stuttgart-Hohenheim (Deutsche Veterinärmedizinische Gesellschaft [DVG], ed.). DVG, Giessen, 138-147.
15. Bode L., Riegel S., Lange W. & Ludwig H. (1992). – Human infections with Borna disease virus: seroprevalence in patients with chronic diseases and healthy individuals. *J. med. Virol.*, **36** (4), 309-315.
16. Bode L., Ferszt R. & Czech G. (1993). – Borna disease virus infection and affective disorders in man. *Arch. Virol. Suppl.*, **7**, 159-167.
17. Bode L., Dürrwald R. & Ludwig H. (1994). – Borna virus infections in cattle associated with fatal neurological disease. *Vet. Rec.*, **135** (12), 283-284.
18. Bode L., Steinbach F. & Ludwig H. (1994). – A novel marker for Borna disease virus infection. *Lancet*, **343** (8892), 297-298.
19. Bode L., Dürrwald R., Koeppel P. & Ludwig H. (1994). – Neue Aspekte der equinen Borna-Virus-Infektion mit und ohne Krankheit. Diagnoseschema und Einsendeprotokoll. *Prakt. Tierarzt*, **75**, 1065-1068.
20. Bode L., Zimmermann W., Ferszt R., Steinbach F. & Ludwig H. (1995). – Borna disease virus genome transcribed and expressed in psychiatric patients. *Nature Med.*, **1** (3), 232-236.
21. Bode L., Dürrwald R., Rantam F.A., de la Torre J.C., Ferszt R., Komaroff A.L. & Ludwig H. (1996). – Isolation of human Borna disease virus. In Proc. 10th International Congress of Virology, 11-16

August, Jerusalem. International Union of Microbiological Societies, Jerusalem, 52.

22. Bode L., Dürrwald R., Rantam F.A., Ferszt R. & Ludwig H. (1996). – First isolates of infectious human Borna disease virus from patients with mood disorders. *Molec. Psychiatry*, **1** (3), 200-212.

23. Bode L. & Ludwig H. (1997). – Bornavirus-Infektion und psychiatrische Erkrankungen. *Z. Allg. Med.*, **73**, 621-627.

24. Bode L. & Ludwig H. (1997). – Clinical similarities and close genetic relationship of human and animal Borna disease virus. *Arch. Virol. Suppl.*, **13**, 167-182.

25. Bode L., Dietrich D.E., Stoyloff R. Emrich H.M. & Ludwig H. (1997). – Amantadine and human Borna disease virus *in vitro* and *in vivo* in an infected patient with bipolar depression. *Lancet*, **349** (9046), 178-179.

26. Bode L., Stoyloff R., Dietrich D.E. & Ludwig H. (1999). – Human Bornavirus and amantadine (Abstract). *In Proc. 11th International Congress of Virology, 9-13 August, Sydney. International Union of Microbiological Societies, Sydney, 98.*

27. Briese T., de la Torre J.C., Lewis A., Ludwig H. & Lipkin W.I. (1992). – Borna disease virus, a negative-strand RNA virus, transcribes in the nucleus of infected cells. *Proc. natl Acad. Sci. USA*, **89** (23), 11486-11489.

28. Briese T., Schneemann A., Lewis A.J., Park Y.-S., Kim S., Ludwig H. & Lipkin W.I. (1994). – Genomic organization of Borna disease virus. *Proc. natl Acad. Sci. USA*, **91** (10), 4362-4366.

29. Caplazi P., Waldvogel A., Stitz L., Braun U. & Ehrensperger F. (1994). – Borna disease in naturally infected cattle. *J. comp. Pathol.*, **111** (1), 65-72.

30. Caplazi P., Melzer K., Goetzmann R., Rohner-Cotti A., Bracher V., Zlinszky K. & Ehrensperger F. (1999). – Die 'Bornasche

Krankheit' in der Schweiz und im Fürstentum Liechtenstein. *Schweizer Arch. Tierheilkd.*, **141**, 521-527.

31. Carbone K.M., Duchala C.S., Griffin J.W., Kincaid A.L. & Narayan O. (1987). – 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.*, **61** (11), 3431-3440.

32. Carbone K.M., Moench T.R. & Lipkin W.I. (1991). – Borna disease virus replicates in astrocytes, Schwann cells and ependymal cells in persistently infected rats: location of viral genomic and messenger RNAs by in situ hybridization. *J. Neuropathol. experim. Neurol.*, **50** (3), 205-214.

33. Cervós-Navarro J., Roggendorf W., Ludwig H. & Stitz L. (1981). – Die Borna-Krankheit beim Affen unter besonderer Berücksichtigung der encephalitischen Reaktion. *Verh. Dtsch. Ges. Pathol.*, **65**, 208-212.

34. Chen C.-H., Chiu Y.-L., Wei F.-C., Koong F.-J., Liu H.-C., Shaw C.-K., Hwu H.-G. & Hsiao K.-J. (1999). – High seroprevalence of Borna virus infection in schizophrenic patients, family members and mental health workers in Taiwan. *Molec. Psychiatry*, **4** (1), 33-38.

35. Chen C.-H., Chiu Y.-L., Shaw C.-K., Tsai M.T., Hwang A.L. & Hsiao K.-J. (1999). – Detection of Borna disease virus RNA from peripheral blood cells in schizophrenic patients and mental health workers. *Molec. Psychiatry*, **4** (6), 566-571.

36. Cubitt B. & de la Torre J.C. (1994). – Borna disease virus (BDV), a nonsegmented RNA virus, replicates in the nuclei of infected cells where infectious BDV ribonucleoproteins are present. *J. Virol.*, **68** (3), 1371-1381.

37. Cubitt B., Oldstone C. & de la Torre J.C. (1994). – Sequence and genome organization of Borna disease virus. *J. Virol.*, **68** (3), 1382-1396.

38. Cubitt B. & de la Torre J.C. (1997). – Amantadine does not have antiviral activity against Borna disease virus. *Arch. Virol.*, **142** (10), 2035-2042.
39. Czech-Schmidt G. (1993). – Borna-Virus Infektionen im Tiermodell: serologische, virologische und molekularbiologische Untersuchungen, PhD Thesis. Free University, Berlin, 158 pp.
40. Danner K. (1982). – Borna-Virus und Borna-Infektionen: vom Miasma zum Modell. Enke, Stuttgart, 303 pp.
41. Danner K., Heubeck D. & Mayr A. (1978). – *In vitro* studies on Borna virus. I. The use of cell cultures for the demonstration, titration and production of Borna virus. *Arch. Virol.*, **57** (1), 63-75.
42. Danner K., Lüthgen K., Herlyn M. & Mayr A. (1978). – Vergleichende Untersuchungen über Nachweis und Bildung von Serumantikörpern gegen das Borna-Virus. *Zentralbl. Veterinärmed., B*, **25** (5), 345-355.
43. Danner K. & Mayr A. (1979). – *In vitro* studies on Borna virus. II. Properties of the virus. *Arch. Virol.*, **61** (4), 261-271.
44. De la Torre J.C. (1994). – Molecular biology of Borna disease virus: prototype of a new group of animal viruses. *J. Virol.*, **68** (12), 7669-7675.
45. De la Torre J.C., Bode L., Dürrwald R., Cubitt B. & Ludwig H. (1996). – Sequence characterization of human Borna disease virus. *Virus Res.*, **44** (1), 33-44.
46. De la Torre J.C., Gonzalez-Dunia D., Cubitt B., Mallory M., Mueller-Lantzsch N., Grässer F.A., Hansen L.A. & Masliah E. (1996). – Detection of Borna disease virus antigen and RNA in human autopsy brain samples from neuropsychiatric patients. *Virology*, **223** (2), 272-282.
47. Deuschle M., Bode L., Heuser I., Schmider J. & Ludwig H. (1998). – Borna disease virus proteins in cerebrospinal fluid of

patients with recurrent depression and multiple sclerosis. *Lancet*, **352**, 1828-1829.

48. Dietrich D.E., Schedlowski M., Bode L., Ludwig H. & Emrich H.M. (1998). – A viro-psycho-immunological disease-model of a subtype affective disorder. *Pharmacopsychiatry*, **31** (3), 77-82.

49. Dimmock N.J. (1993). – Neutralization of animal viruses. Viral carbohydrates, proteins and neutralization. *Curr. Top. Microbiol. Immunol.*, **183**, 1-149.

50. Dittrich W., Bode L., Ludwig H., Kao M. & Schneider K. (1989). – Learning deficiencies in Borna disease virus-infected but clinically healthy rats. *Biol. Psychiatry*, **26** (8), 818-828.

51. Dürrwald R. (1993). – Die natürliche Borna-Virus-Infektion der Einhufer und Schafe. Untersuchungen zur Epidemiologie, zu neueren diagnostischen Methoden (ELISA, PCR) und zur Antikörperkinetik bei Pferden nach Vakzination mit Lebendimpfstoff, DVM Thesis. Free University, Berlin, 156 pp.

52. Dürrwald R., Caramelli M., Zimmermann W., Muluneh A., Ludwig H. & Bode L. (1996). – Borna disease virus infection in sheep in and outside classical endemic areas in Europe. In Vaccines and control of infectious diseases: the way forward. Proc. 14th International Symposium of the World Association of Veterinary Microbiologists, Immunologists and Specialists in Infectious Diseases (WAVMI), 3-5 July, Edinburgh. Moredun Institute, Edinburgh, 61.

53. Dürrwald R. & Ludwig H. (1997). – Borna disease virus (BDV), a (zoonotic?) worldwide pathogen. A review of the history of the disease and the virus infection with comprehensive bibliography. *Zentralbl. Veterinärmed., B*, **44** (3), 147-184.

54. Ernst W. & Hahn H. (1926). – Bestehen Aussichten, gegen die seuchenhafte Gehirn-Rückenmarksentzündung der Pferde (Borna'sche Krankheit) zu immunisieren? *Berl. Münch. tierärztl. Wochenschr.*, **77**, 477-478.

55. Evengard B., Briese T., Lindh G., Lee S. & Lipkin W.I. (1999). – Absence of evidence of Borna disease virus infection in Swedish patients with Chronic Fatigue Syndrome. *J. Neurovirol.*, **5** (5), 495-499.
56. Fankhauser R. (1961). – Sporadische Meningo-Encephalomyelitis beim Rind. *Schweizer Arch. Tierheilkd.*, **103**, 225-235.
57. Ferszt R., Severus E., Bode L., Brehm M., Kühl K.-P., Berzewski H. & Ludwig H. (1999). – Activated Borna disease virus in affective disorders. *Pharmacopsychiatry*, **32** (3), 93-98.
58. Ferszt R., Kühl K.-P., Bode L., Severus E.W., Winzer B., Berghöfer A., Beelitz G., Brodhun B., Müller-Örtinghausen B. & Ludwig H. (1999). – Amantadine revisited: an open trial of amantadine sulfate treatment in chronically depressed patients with Borna disease virus infection. *Pharmacopsychiatry*, **32** (4), 142-147.
59. Fu Z.F., Amsterdam J.D., Kao M., Shankar V., Koprowski H. & Dietzschold B. (1993). – Detection of Borna disease virus-reactive antibodies from patients with affective disorders by Western immunoblot technique. *J. affect. Disord.*, **27**, 61-68.
60. Galiberti J.B. (1660). – Neugebahnter Tümmelplatz, und eröffnete Reitschul. Samt beygefügter Gestütt Ordnung und gründlicher Einzäumung wie auch der Pferde Cur, und Artzney. (Übersetzt vom Italienisch ins Teutsch und mit denen darzu gehörigen Figuren geziert durch Mathaeum Drummern von Pabenbach). Verlegung Michael Riegers Buchhandlers, Vienna.
61. Gonzalez-Dunia D., Cubitt B., Grässer F.A. & de la Torre J.C. (1997). – Characterization of Borna disease virus p56 protein, a surface glycoprotein involved in virus entry. *J. Virol.*, **71**, 3208-3218.
62. Goodwin F.K. & Jamison K.R. (1990). – Manic-depressive illness. Oxford University Press, New York, 960 pp.

63. Gosztanyi G. & Ludwig H. (1984). – Borna disease of horses. An immunohistological and virological study of naturally infected animals. *Acta neuropathol. (Berl.)*, **64** (3), 213-221.
64. Gosztanyi G. & Ludwig H. (1984). – Neurotransmitter receptors and viral neurotropism. *Neuropsychiat. Clin.*, **3**, 107-114.
65. Gosztanyi G. & Ludwig H. (1995). – Borna disease – neuropathology and pathogenesis. In Borna disease (H. Koprowski & W.I. Lipkin, eds). *Curr. Top. Microbiol. Immunol.*, **190**, 39-73.
66. Haga S., Yoshimura M., Motoi Y., Arima K., Aizawa T., Ikuta K., Tashiro M. & Ikeda K. (1997). – Detection of Borna disease virus genome in normal human brain tissue. *Brain Res.*, **770**, 307-309.
67. Hagiwara K., Nakaya T., Nakamura Y., Asahi S., Takahashi H., Ishihara C. & Ikuta K. (1996). – Borna disease virus RNA in peripheral blood mononuclear cells obtained from healthy dairy cattle. *Med. Microbiol. Immunol.*, **185**, 145-151.
68. Hagiwara K., Kawamoto S., Takahashi H., Nakamura Y., Nakaya T., Hiramune T., Ishihara C. & Ikuta K. (1997). – High prevalence of Borna disease virus infection in healthy sheep in Japan. *Clin. diagn. Lab. Immunol.*, **4** (3), 339-344.
69. Hallensleben W., Zocher M. & Staeheli P. (1997). – Borna disease virus is not sensitive to amantadine. *Arch. Virol.*, **142**, 2043-2048.
70. Hatalski C.G., Kliche S., Stitz L. & Lipkin W.I. (1995). – Neutralizing antibodies in Borna disease virus-infected rats. *J. Virol.*, **69**, 741-747.
71. Heinig A. (1969). – Die Bornasche Krankheit der Pferde und Schafe. In Handbuch der Virusinfektionen bei Tieren, Vol. 4 (H. Röhrer, ed.). Fischer, Jena, 83-148.
72. Herzog S. & Rott R. (1980). – Replication of Borna disease virus in cell cultures. *Med. Microbiol. Immunol.*, **168**, 153-158.

73. Herzog S., Frese K., Richt J.A. & Rott R. (1994). – Ein Beitrag zur Epizootiologie der Bornaschen Krankheit beim Pferd. *Wien. tierärztl. Monatsschr.*, **81**, 374-379.
74. Hiepe T. (1958/1959). – Die Bornasche Krankheit. Klinisch-diagnostische Untersuchungen an Pferden und Schafen mit besonderer Berücksichtigung des Liquor cerebrospinalis. Habilitationsschrift, Karl-Marx-Universität, Leipzig. *Math. Nat. R.*, **8**, 263-338.
75. Hirano N., Kao M. & Ludwig H. (1983). – Persistent, tolerant or subacute infection in Borna disease virus-infected rats. *J. gen. Virol.*, **64**, 1521-1530.
76. Hübner J. (1999). – Die Borna Disease Virus-Infektion der Katze in Deutschland und ihr Vergleich mit anderen feline Virusinfektionen, DVM Thesis. Free University, Berlin, 208 pp.
77. Igata-yi R., Yamaguchi K., Yoshiki K., Takemoto S., Yamasaki H., Matsuoka M. & Miakawa T. (1996). – Borna disease virus and the consumption of raw horse meat. *Nature Med.*, **2**, 948-949.
78. Ihlenburg H. (1966). – Experimentelle Prüfung der Empfänglichkeit der Katze für das Virus der Bornaschen Krankheit. *Arch. experim. VetMed.*, **20**, 859-864.
79. Ihlenburg H. & Brehmer H. (1964). – Beitrag zur latenten Borna-Erkrankung des Pferdes. *Monatsschr. VetMed.*, **19**, 463-465.
80. Iwahashi K., Watanabe M., Nakamura K., Suwaki H., Nakaya T., Nakamura Y., Takahashi H. & Ikuta K. (1997). – Clinical investigation of the relationship between Borna disease virus (BDV) infection and schizophrenia in 67 patients in Japan. *Acta psychiatr. scand.*, **96**, 412-415.
81. Iwata Y., Takahashi K., Peng X., Fukuda K., Ohno K., Ogawa T., Gonda K., Mori N., Niwa S.-I. & Shigeta S. (1998). – Detection and sequence analysis of Borna disease virus p24 RNA from peripheral blood mononuclear cells of patients with mood disorders or schizophrenia and of blood donors. *J. Virol.*, **72**, 10044-10049.

82. Jamison K.R. (1993). – Touched with fire. Manic-depressive illness and the artistic temperament. Free Press, New York, 370 pp.
83. Joest E. (1926). – Vergleichend-anatomische Betrachtungen über Encephalitis. *Klin. Wochenschr.*, **5**, 209-211.
84. Joest E. & Degen K. (1911). – Untersuchungen über die pathologische Histologie, Pathogenese und postmortale Diagnose der seuchenhaften Gehirn-Rückenmarksentzündung (Bornaschen Krankheit) des Pferdes. *Zeitschr. Infektionskr. parasit. Krank Hyg. Haustiere*, **9**, 1-98.
85. Jordan I., Briese T., Averett D.R. & Lipkin I.W. (1999). – Inhibition of Borna disease virus replication by ribavirin. *J. Virol.*, **73**, 7903-7906.
86. Kao M., Ludwig H. & Gosztonyi G. (1984). – Adaptation of Borna disease virus to the mouse. *J. gen. Virol.*, **65**, 1845-1849.
87. Kao M., Bode L., Gosztonyi G. & Ludwig H. (1990). – Escape from lethal disease in rats after Borna disease virus infection: survival with obesity syndrome (Abstract). *In Proc. 8th International Congress of Virology*, 26-31 August, Berlin. International Union of Microbiological Societies, Berlin, 108.
88. Kao M., Hamir A.N., Rupprecht C.E., Fu Z.F., Shankar V., Koprowski H. & Dietzschold B. (1993). – Detection of antibodies against Borna disease virus in sera and cerebrospinal fluid of horses in the USA. *Vet. Rec.*, **132**, 241-244.
89. Kishi M., Nakaya T., Nakamura Y., Zhong Q., Ikeda K., Senjo M., Kakinuma M., Kato S. & Ikuta K. (1995). – Demonstration of human Borna disease virus RNA in human peripheral blood mononuclear cells. *FEBS Lett.*, **364**, 293-297.
90. Kishi M., Nakaya T., Nakamura Y., Kakinuma M., Takahashi T.A., Sekiguchi S., Uchikawa M., Tadokoro K., Ikeda K. & Ikuta K. (1995). – Prevalence of Borna disease virus RNA in peripheral blood

mononuclear cells from blood donors. *Med. Microbiol. Immunol.*, **184**, 135-138.

91. Kitani T., Kuratsune H., Fuke I., Nakamura Y., Nakaya T., Asahi S., Tobiume M., Yamaguti K., Machii T., Inagi R., Yamanishi K. & Ikuta K. (1996). – Possible correlation between Borna disease virus infection and Japanese patients with chronic fatigue syndrome. *Microbiol. Immunol.*, **40**, 459-462.

92. Kiupel H. & Wehr J. (1980). – Zum Vorkommen der sporadischen bovinen Enzephalomyelitis (SBE) im Norden der DDR. *Wiss. Z. Humboldt-Universität Berlin. Math. Nat. R.*, **29**, 57-59.

93. Kliche S., Briese T., Henschen A.H., Stitz L. & Lipkin W.I. (1994). – Characterization of a Borna disease virus glycoprotein, gp18. *J. Virol.*, **68**, 6918-6923.

94. Kliche S., Stitz L., Mangalam H., Shi L., Binz T., Niemann H., Briese T. & Lipkin W.I. (1996). – Characterization of the Borna disease virus phosphoprotein, p23. *J. Virol.*, **70**, 8133-8137.

95. Kohno T., Goto T., Takasaki T., Morita C., Nakaya T., Ikuta K., Kurane I., Sano K. & Nakai M. (1999). – Fine structure and morphogenesis of Borna disease virus. *J. Virol.*, **73**, 760-766.

96. Koprowski H. & Lipkin W.I. (eds) (1995). – Borna disease. *Curr. Top. Microbiol. Immunol.*, **190**, 1-134.

97. Krey H.F., Ludwig H. & Boschek C.B. (1979). – Multifocal retinopathy in Borna disease virus infected rabbits. *Am. J. Ophthalmol.*, **87**, 157-164.

98. Krey H.F., Ludwig H. & Rott R. (1979). – Spread of infectious virus along the optic nerve into the retina in Borna disease virus-infected rabbits. *Arch. Virol.*, **61**, 283-288.

99. Krey H.F., Stitz L. & Ludwig H. (1982). – Virus-induced pigment epithelitis in rhesus monkeys. Clinical and histological findings. *Ophthalmologica*, **185**, 205-213.

100. Kronevi T., Nordström M., Moreno W. & Nilsson P.O. (1974). – Feline ataxia due to nonsuppurative meningoencephalomyelitis of unknown aetiology. *Norsk Vet.*, **26**, 720-725.
101. Lange H., Herzog S., Herbst W. & Schliesser T. (1987). – Seroepidemiologische Untersuchungen zur Bornaschen Krankheit (Ansteckende Gehirn-Rückenmarkentzündung) der Pferde. *Tierärztl. Umsch.*, **42**, 938-946.
102. Lieb K., Hallensleben W., Czygan M., Stitz L. & Staeheli P. (1997). – No Borna disease virus-specific RNA detected in blood from psychiatric patients in different regions of Germany. The Bornavirus Study Group. *Lancet*, **350** (9083), 1002.
103. Lipkin W.I., Carbone K.M., Wilson M.C., Duchala C.S., Narayan O. & Oldstone M.B.A. (1988). – Neurotransmitter abnormalities in Borna disease. *Brain Res.*, **475**, 366-370.
104. Lipkin W.I., Travis G.H., Carbone K.M. & Wilson M.C. (1990). – Isolation and characterization of Borna disease agent cDNA clones. *Proc. natl Acad. Sci. USA*, **87**, 4184-4188.
105. Ludwig H., Becht H. & Groh L. (1973). – Borna disease (BD), a slow virus infection. Biological properties of the virus. *Med. Microbiol. Immunol.*, **158**, 275-289.
106. Ludwig H. & Becht H. (1977). – Borna disease – a summary of our present knowledge. In *Slow virus infections of the central nervous system: investigational approaches to etiology and pathogenesis of these diseases* (V. Ter Meulen & M. Katz, eds). Springer, New York, 75-83.
107. Ludwig H. & Thein P. (1977). – Demonstration of specific antibodies in the central nervous system of horses naturally infected with Borna disease virus. *Med. Microbiol. Immunol.*, **163**, 215-226.
108. Ludwig H., Koester V., Pauli G. & Rott R. (1977). – The cerebrospinal fluid of rabbits infected with Borna disease virus. *Arch. Virol.*, **55**, 209-223.

109. Ludwig H., Kraft W., Kao M., Gosztonyi G., Dahme E. & Krey H. (1985). – Borna-Virus Infektion (Borna-Krankheit) bei natürlich und experimentell infizierten Tieren: Ihre Bedeutung für Forschung und Praxis. *Tierärztl. Praxis*, **13**, 421-453.
110. Ludwig H., Bode L. & Gosztonyi G. (1988). – Borna disease: a persistent virus infection of the central nervous system. *Prog. med. Virol.*, **35**, 107-151.
111. Ludwig H. & Kao M. (1990). – Borna disease in sheep. *In Virus infections of ruminants* (Z. Dinter & B. Morein, eds). Elsevier, Amsterdam, 529-538.
112. Ludwig H., Furuya K., Bode L., Klein N., Dürrwald R. & Lee D.S. (1993). – Biology and neurobiology of Borna disease viruses (BDV), defined by antibodies, neutralizability and their pathogenic potential. *Arch. Virol. Suppl.*, **7**, 111-133.
113. Ludwig H., Bode L., Lundgren A.-L. & Dürrwald R. (1994). – Borna disease, the expression of a persistent reactivated virus infection in nerve- and blood cells of horses, cattle, and cats. *In Proc. 13th International Symposium of the World Association of Veterinary Microbiologists, Immunologists and Specialists in Infectious Diseases (WAVMI)* (G. Castrucci & B.E. Osburn, eds), 2-7 October, Perugia-Mantova. Società Editrice Esculapio, Bologna, 157-165.
114. Ludwig H. & Bode L. (1997). – The neuropathogenesis of Borna disease virus infections. *Intervirology*, **40**, 185-197.
115. Ludwig H., Bode L., Schedlowski M., Emrich H.M. & Dietrich D.E. (1998). – Stress and human Borna virus infection. *In Stress and the nervous system* (C.L. Bolis & J. Licinio, eds). WHO/RPS/98.2. World Health Organization, Geneva, 119-128.
116. Lundgren A.-L. (1992). – Feline non-suppurative meningoencephalomyelitis. A clinical and pathological study. *J. comp. Pathol.*, **107**, 411-425.

117. Lundgren A.-L., Czech G., Bode L. & Ludwig H. (1993). – Natural Borna disease in domestic animals other than horses and sheep. *J. vet. Med., B*, **40**, 298-303.
118. Lundgren A.-L., Zimmermann W., Bode L., Czech G., Gosztonyi G., Lindberg R. & Ludwig H. (1995). – Staggering disease in cats: isolation and characterization of the feline Borna disease virus. *J. gen. Virol.*, **76**, 2215-2222.
119. Lundgren A.-L., Johannisson A., Zimmermann W., Bode L., Rozell B., Muluneh A., Lindberg R. & Ludwig H. (1997). – Neurological disease and encephalitis in cats experimentally infected with Borna disease virus. *Acta neuropathol. (Berl.)*, **93**, 391-401.
120. Lüscho D. (1999). – Borna-Disease-Virus (BDV)-Infektionen und Erkrankungen bei Equiden: serologische und molekularepidemiologische Untersuchungen unter besonderer Berücksichtigung der phylogenetischen Sequenzanalyse, DVM Thesis. Free University, Berlin, 132 pp.
121. Malik T.H., Kobayashi T., Ghosh M., Kishi M. & Lai P.K. (1999). – Nuclear localization of the protein from the open reading frame x1 of the Borna disease virus was through interactions with the viral nucleoprotein. *Virology*, **258**, 65-72.
122. Malkinson M., Weisman Y., Ashash E., Bode L. & Ludwig H. (1993). – Borna disease in ostriches. *Vet. Rec.*, **133**, 304.
123. Malkinson M., Weisman Y., Perl S. & Ashash E. (1995). – A Borna-like disease of ostriches in Israel. In Borna disease (H. Koprowski & W.I. Lipkin, eds). *Curr. Top. Microbiol. Immunol.*, **190**, 31-38.
124. Malkinson M., Weisman Y., Perl S., Ashash E., Teplitsky V. & Pitlik S. (1999). – Borna disease of livestock in Israel (Abstract). *In Proc. 11th International Congress of Virology, 9-13 August, Sydney. International Union of Microbiological Societies, Sydney, 71.*

125. Matthias D. (1954). – Der Nachweis von latent infizierten Pferden, Schafen und Rindern und deren Bedeutung als Virusreservoir bei der Bornaschen Krankheit. *Arch. experim. VetMed.*, **8**, 506-511.
126. Matthias D. (1955). – Neue Forschungsergebnisse bei der Bornaschen Krankheit der Pferde. *Monatsschr. VetMed.*, **10**, 123-126.
127. Mayr A. & Danner K. (1978). – Borna – a slow virus disease. *Comp. Immunol. Microbiol. infect. Dis.*, **1**, 3-14.
128. Metzler A., Ehrensperger F. & Wyler R. (1978). – Natürliche Bornavirusinfektion beim Kaninchen. *Zentralbl. Veterinärmed.*, **B, 25**, 161-164.
129. Metzler A., Ehrensperger F. & Danner K. (1979). – Bornavirus-Infektion bei Schafen: Verlaufsuntersuchungen nach spontaner Infektion, unter besonderer Berücksichtigung der Antikörperkinetik im Serum und Liquor cerebrospinalis. *Schweizer Arch. Tierheilkd.*, **121**, 37-48.
130. Mizutani T., Inagaki H., Araki K., Kariwa H., Arikawa J. & Takashima I. (1998). – Inhibition of Borna disease virus replication by ribavirin in persistently infected cells. *Arch. Virol.*, **143**, 2039-2044.
131. Möhlmann H. & Maas A. (1960). – Wertigkeitsprüfung des Borna-Trockenimpfstoffes 'Dessau' bei Pferden unter den Verhältnissen der Praxis. *Arch. experim. VetMed.*, **14**, 1267-1280.
132. Moussu R. & Marchand L. (1924). – L'encéphalite enzootique du cheval (maladie de Borna). *Rec. Méd. vét.*, **100**, 5-44, 65-90.
133. Müller L.F. & Dorn H.-J. (1951). – Blutbild und Blutsenkung bei der Bornaschen Krankheit der Pferde. *Experim. Veterinärmed.*, **3**, 61-64.
134. Nakamura Y. (1998). – Isolation of Borna disease virus from autopsy brain of a schizophrenia patient. *Hokkaido Igaku Zasshi*, **73**, 287-297.

135. Nakamura Y., Kishi M., Nakaya T., Asahi S., Tanaka H., Sentsui H., Ikeda K. & Ikuta K. (1995). – Demonstration of Borna disease virus RNA in peripheral blood mononuclear cells from healthy horses in Japan. *Vaccine*, **13**, 1076-1079.
136. Nakamura Y., Asahi S., Nakaya T., Bahmani M.K., Saitoh S., Yasui K., Mayama H., Hagiwara K., Ishihara C. & Ikuta K. (1996). – Demonstration of Borna disease virus RNA in peripheral blood mononuclear cells derived from domestic cats in Japan. *J. clin. Microbiol.*, **34**, 188-191.
137. Nakamura Y., Watanabe M., Kamitani W., Taniyama H., Nakaya T., Nishimura Y., Tsujimoto H., Machida S. & Ikuta K. (1999). – High prevalence of Borna disease virus in domestic cats with neurological disorders in Japan. *Vet. Microbiol.*, **70**, 153-169.
138. Nakaya T., Tada M., Takahashi H., Fujiwara S., Sakuma S., Sawamura Y., Abe H. & Ikuta K. (1996). – Expression of Borna disease virus messages in clinical samples from patients with brain malignant tumors. *Proc. Jpn Acad.*, **72**, 157-162.
139. Nakaya T., Takahashi H., Nakamura Y., Asahi S., Tobiume M., Kuratsune H., Kitani T., Yamanishi K. & Ikuta K. (1996). – Demonstration of Borna disease virus RNA in peripheral blood mononuclear cells derived from Japanese patients with chronic fatigue syndrome. *FEBS Lett.*, **378**, 145-149.
140. Narayan O., Herzog S., Frese K., Scheefers H. & Rott R. (1983). – Pathogenesis of Borna disease in rats: immune-mediated viral ophthalmoencephalopathy causing blindness and behavioral abnormalities. *J. infect. Dis.*, **148**, 305-315.
141. Narayan O., Herzog S., Frese K., Scheefers H. & Rott R. (1983). – Behavioral disease in rats caused by immunopathological responses to persistent Borna virus in the brain. *Science*, **220** (4604), 1401-1403.
142. Nicolau S. & Galloway I.A. (1928). – Borna disease and enzootic encephalomyelitis of sheep and cattle. Medical Research

Council Special Reports Series, Vol. 121. His Majesty's Stationery Office, London, 183 pp.

143. Nicolau S., Dimancesco-Nicolau O. & Galloway I.A. (1929). – Etude sur les septinévrites à ultravirus neurotropes. *Ann. Inst. Pasteur*, **43**, 1-88.

144. Nishino Y., Funaba M., Fukushima R., Mizutani T., Kimura T., Iizuka R., Hiram H. & Hara M. (1999). – Borna disease virus infection in domestic cats: evaluation by RNA and antibody detection. *J. vet. med. Sci.*, **61**, 1167-1170.

145. Nitzschke E. (1963). – Untersuchungen über die experimentelle Bornavirus-Infektion bei der Ratte. *Zentralbl. Veterinärmed., B*, **10**, 470-527.

146. Nitzschke E. & Rott R. (1957). – Züchtung des Virus der Bornaschen Krankheit im bebrüteten Hühnerei. *Berl. Münch. tierärztl. Wochenschr.*, **70**, 101-102.

147. Nowotny N. & Weissenböck H. (1995). – Description of feline nonsuppurative meningoencephalomyelitis ('staggering disease') and studies of its etiology. *J. clin. Microbiol.*, **33**, 1668-1669.

148. Pette H. (1931). – Tierexperimentelle Studien zur Frage der Viruswanderung im Zentralnervensystem. *Dtsch. Zeitschr. Nervenheilkd.*, **121**, 113-164.

149. Pette H. & Környey S. (1935). – Über die Pathogenese und die Histologie der Bornaschen Krankheit im Tierexperiment. *Dtsch. Zeitschr. Nervenheilkd.*, **136**, 20-65.

150. Planz O., Rentzsch C., Batra A., Rziha H.-J. & Stitz L. (1998). – Persistence of Borna disease virus-specific nucleic acid in blood of a psychiatric patient. *Lancet*, **352**, 623.

151. Planz O., Rentzsch C., Batra A., Batra A., Winkler T., Büttner M., Rziha H.-J. & Stitz L. (1999). – Pathogenesis of Borna disease

virus: granulocyte fractions of psychiatric patients harbor infectious virus in the absence of antiviral antibodies. *J. Virol.*, **73**, 6251-6256.

152. Plata-Salaman C.R., Ilysin S.E., Gayle D., Romanovitch A. & Carbone K.M. (1999). – Persistent Borna disease virus infection of neonatal rats causes brain regional changes of mRNAs for cytokines, cytokine receptor components and neuropeptides. *Brain Res. Bull.*, **49**, 441-451.

153. Pletnikov M.V., Rubin S.A., Vasudevan K., Moran T.H. & Carbone K.M. (1999). – Developmental brain injury associated with abnormal play behaviour in neonatally Borna disease virus-infected Lewis rats: a model of autism. *Behav. Brain Res.*, **100**, 43-50.

154. Pringle C.R. (1997). – The order Mononegavirales – current status. *Arch. Virol.*, **142**, 2321-2326.

155. Pyper J.M. & Gartner A.E. (1997). – Molecular basis for the differential subcellular localization of the 38- and 39-kilodalton structural proteins of Borna disease virus. *J. Virol.*, **71**, 5133-5139.

156. Rantam F.A. (1997). – Bornaviren und Zellkulturen. Isolierung infektiöser animaler und humaner Bornaviren und ihre biologische Charakterisierung, DVM Thesis. Free University, Berlin, 183 pp.

157. Reeves N.A., Helps C.R., Gunn-Moore D.A., Blundell C., Finnemore P.L., Pearson G.R. & Harbour D.A. (1998). – Natural Borna disease virus infection in cats in the United Kingdom. *Vet. Rec.*, **143**, 523-526.

158. Richt J.A., Alexander R.C., Herzog S., Hooper D.C., Kean R., Spitsin S., Bechter K., Schüttler R., Feldmann H., Heiske A., Fu Z.F., Dietzschold B., Rott R. & Koprowski H. (1997). – Failure to detect Borna disease virus infection in peripheral blood leukocytes from humans with psychiatric disorders. *J. Neurovirol.*, **3**, 174-178.

159. Roggendorf W., Sasaki S. & Ludwig H. (1983). – Light microscope and immunohistological investigations on the brain of

Borna disease virus-infected rabbits. *Neuropathol. appl. Neurobiol.*, **9**, 287-296.

160. Rott R., Herzog S., Fleischer B., Winokur A., Amsterdam J., Dyson W. & Koprowski H. (1985). – Detection of serum antibodies to Borna disease virus in patients with psychiatric disorders. *Science*, **228**, 755-756.

161. Salvatore M., Morzunov S., Schwemmler M. & Lipkin W.I. (1997). – Borna disease virus in brains of North American and European people with schizophrenia and bipolar disorders. Bornavirus Study Group. *Lancet*, **349** (9068), 1813-1814.

162. Sauder C., Müller A., Cubitt B., Mayer J., Steinmetz J., Trabert W., Ziegler B., Wanke K., Mueller-Lantzsch N., de la Torre J.C. & Grässer F.A. (1996). – Detection of Borna disease virus (BDV) antibodies and BDV RNA in psychiatric patients: evidence for high sequence conservation of human blood-derived BDV RNA. *J. Virol.*, **70**, 7713-7724.

163. Sauder C. & de la Torre J.C. (1998). – Sensitivity and reproducibility of RT-PCR to detect Borna disease virus (BDV) RNA in blood: implications for BDV epidemiology. *J. virol. Meth.*, **71**, 229-245.

164. Schädler R., Diringler H. & Ludwig H. (1985). – Isolation and characterization of a 14500 molecular weight protein from brains and tissue cultures persistently infected with Borna disease virus. *J. gen. Virol.*, **66**, 2479-2484.

165. Schmidt J. (1912). – Untersuchungen über das klinische Verhalten der seuchenhaften Gehirnrückenmarksentzündung (Bornaschen Krankheit) des Pferdes nebst Angaben über diesbezügliche therapeutische Versuche. *Berl. Münch. tierärztl. Wochenschr.*, **28**, 581-586, 597-603.

166. Schmidt J. (1952). – Die Bornakrankheit des Pferdes. 55 Jahre Forschung und Lehre. *Arch. experim. VetMed.*, **6**, 177-187.

167. Schneemann A., Schneider P.A., Lamb R.A. & Lipkin W.I. (1995). – The remarkable coding strategy of Borna disease virus: a new member of the nonsegmented negative strand RNA viruses. *Virology*, **210**, 1-8.
168. Schneider P.A., Briese T., Zimmermann W., Ludwig H. & Lipkin W.I. (1994). – Sequence conservation in field and experimental isolates of Borna disease virus. *J. Virol.*, **68**, 63-68.
169. Schneider P.A., Hatalski C.G., Lewis A.J. & Lipkin W.I. (1997). – Biochemical and functional analysis of the Borna disease virus G protein. *J. Virol.*, **71**, 331-336.
170. Schulze W. (1960). – Bornasche Krankheit. In Leitfaden der Ziegenkrankheiten für Tierärzte und Studierende der Tierheilkunde. Hirzel, Leipzig, 42 pp.
171. Schüppel K.-F., Kinne J. & Reinacher M. (1994). – Bornavirus-Antigennachweis bei Alpakas (*Lama pakos*) sowie bei einem Faultier (*Choloepus didactylus*) und einem Zwergflußpferd (*Choeropsis liberiensis*). In Verh.bericht XXXVI Int. Symp. Erkrankg Zootiere (R.R. Hofmann & R. Ippen, eds). 11-15 May, Kristiansund, Norway. Institut für Zoo- und Wildtierforschung, Berlin, 189-194.
172. Schwemmle M., Jehle C., Formella S. & Staeheli P. (1999). – Sequence similarities between human bornavirus isolates and laboratory strains question human origin. *Lancet*, **354**, 1973-1974.
173. Seifried O. & Spatz H. (1930). – Die Ausbreitung der enzephalitischen Reaktion bei der Bornaschen Krankheit der Pferde und deren Beziehung zu der Enzephalitis epidemica, der Heine-Medinschen Krankheit und der Lyssa des Menschen. Eine vergleichend-pathologische Studie. *Z. ges. Neurol. Psychiat.*, **124**, 317-383.
174. Shankar V., Kao M., Hamir A.N., Sheng H., Koprowski H. & Dietzschold B. (1992). – Kinetics of virus spread and changes in levels of several cytokine mRNAs in the brain after intranasal infection of rats with Borna disease virus. *J. Virol.*, **66**, 992-998.

175. Sierra-Honigmann A.M., Rubin S.A., Estafanous M.G., Yolken R.H. & Carbone K.M. (1993). – Borna disease virus in peripheral blood mononuclear and bone marrow cells of neonatally and chronically infected rats. *J. Neuroimmunol.*, **45**, 31-36.
176. Sigurdsson B. (1954). – Rida: a chronic encephalitis of sheep with general remarks on infections which develop slowly and some of their special characteristics. *Br. vet. J.*, **110**, 341-354.
177. Snyder S.H. (1986). – Drugs and the brain. Scientific American Books, New York, 224 pp.
178. Solbrig M.V., Fallon J.H. & Lipkin W.I. (1995). – Behavioral disturbances and pharmacology of Borna disease. *In Borna disease* (H. Koprowski & W.I. Lipkin, eds). *Curr. Top. Microbiol. Immunol.*, **190**, 93-99.
179. Sprankel H. & Richarz K. (1976). – Nicht-reproduktives Verhalten von *Tupaia glis* Diard im raum-zeitlichen Bezug. Eine quantitative Analyse. *Z. Säugetierkd.*, **41**, 77-101.
180. Sprankel H., Richarz K., Ludwig H. & Rott R. (1978). – Behavior alterations in tree shrews (*Tupaia glis*, Diard 1820) induced by Borna disease virus. *Med. Microbiol. Immunol.*, **165**, 1-18.
181. Steinbach F. (1994). – Isolierung und Charakterisierung equiner peripherer Blutmonozyten und ihre Bedeutung für Herpes-(EHV-1-) und Borna-(BDV-) Virusinfektionen, DVM Thesis. Free University, Berlin, 189 pp.
182. Stitz L., Krey H. & Ludwig H. (1980). – Borna disease in rhesus monkeys as a model for uveo-cerebral symptoms. *J. med. Virol.*, **6**, 333-340.
185. Stitz L., Dietzschold B. & Carbone K.M. (1995). – Immunopathogenesis of Borna disease. *In Borna disease* (H. Koprowski & W.I. Lipkin, eds). *Curr. Top. Microbiol. Immunol.*, **190**, 75-92.

186. Stitz L., Planz O. & Bilzer T. (1998). – Lack of antiviral effect of amantadine in Borna disease virus infection. *Med. Microbiol. Immunol.*, **186**, 195-200.
187. Stitz L., Noske K., Planz O., Furrer E., Lipkin W.I. & Bilzer T. (1998). – A functional role for neutralizing antibodies in Borna disease: influence on virus tropism outside the central nervous system. *J. Virol.*, **72** (11), 8884-8892.
188. Stoyloff R. (1996). – Borna-Virus-Infektion und Glykoproteine: biochemische und biologische Untersuchungen zu Struktur und Funktion von GP17, PhD Thesis. Free University, Berlin. Cuvillier, Göttingen, 234 pp.
189. Stoyloff R., Briese T., Borchers K., Zimmermann W. & Ludwig H. (1994). – N-glycosylated protein(s) are important for the infectivity of Borna disease virus (BDV). *Arch. Virol.*, **137**, 405-409.
190. Stoyloff R., Bode L., Wendt H., Mulzer J. & Ludwig H. (1996). – The hydrophobic mannose derivative 1B6TM efficiently inhibits Borna disease virus *in vitro*. *Antiviral Chem. Chemother.*, **7**, 197-202.
191. Stoyloff R., Strecker A., Bode L., Franke P., Ludwig H. & Hucho F. (1997). – The glycosylated matrix protein of Borna disease virus is a tetrameric, membrane-bound viral component essential for infection. *Eur. J. Biochem.*, **246**, 252-257.
192. Stoyloff R., Bode L., Borchers K. & Ludwig H. (1998). – Neutralization of Borna disease virus depends upon terminal carbohydrate residues (α -D-Man, β -D-GlcNAc) of glycoproteins gp17 and gp94. *Intervirology*, **41**, 135-140.
193. Takahashi H., Nakaya T., Nakamura Y., Asahi S., Onishi Y., Ikebuchi K., Takahashi T.A., Katoh T., Sekiguchi S., Takazawa M., Tanaka H. & Ikuta K. (1997). – Higher prevalence of Borna disease virus infection in blood donors living near thoroughbred horse farms. *J. med. Virol.*, **52**, 330-335.

194. Traub E. (1939). – Choriomeningitis der Mäuse. *In* Handbuch der Viruskrankheiten, Vol. 2 (E. Gildenmeister, E. Haagen & O. Waldmann, eds). Fischer-Verlag, Jena, 355-364.
195. Trichtern V. (1716). – Pferd-Anatomie, oder Neu auserlesen vollkommen verbessert und ergänztes Roß Artzney Buch. *In* Verlegung des Autors. Gedruckt zu finden in Frankfurt und Leipzig bey A.J. Felßenecker, 192-203.
196. Tsukamoto T. & Ludwig H. (1983). – Long term cultures of neural retina and pigment epithelium from newborn rabbits. *Zeitschr. Naturforsch., C (Biosci.)*, **38**, 141-145.
197. Vahlenkamp T.W., Enbergs H.K. & Müller H. (1999). – Presence of Borna disease virus (BDV) RNA in cells of the peripheral blood (Abstract). *In* Proc. 11th International Congress of Virology, 9-13 August, Sydney. International Union of Microbiological Societies, Sydney, 272.
198. Vandevelde M. & Braund K.G. (1979). – Polioencephalomyelitis in cats. *Vet. Pathol.*, **16** (4), 420-427.
199. Van de Woude S., Richt J.A., Zink M.C., Rott R., Narayan O. & Clements J.E. (1990). – A Borna virus cDNA encoding a protein recognized by antibodies in humans with behavioral diseases. *Science*, **250** (4985), 1278-1281.
200. Veith J.H. (1822). – Handbuch der Veterinär Kunde in besonderer Beziehung auf die Seuchen der nutzbarsten Haussäugethiere für Physiker, Kreis Chirurgen, Thierärzte und Oekonomen. Verlag der Geistinger'schen Buchhandlung auf dem Kohlmarkte, Vienna, 394-396.
201. Von Ostertag R. (1924). – Neue Aussichten der Bekämpfung der ansteckenden Gehirnrückenmarksentzündung (Kopfkrankheit, Bornaschen Krankheit) der Pferde. Erfolgreiche Behandlung mit Hexamethylentetramin (Urotropin). *Berl. Münch. tierärztl. Wochenschr.*, **40**, 705-711.

202. Von Sind J.B. (1767/1781). – Der im Feld und auf der Reise geschwind heilende Pferdearzt, welcher einen gründlichen Unterricht von den gewöhnlichsten Krankheiten der Pferde im Feld und auf der Reise wie auch einen auserlesenen Vorrath der nützlichsten und durch die Erfahrung bewährtesten Heilungsmitteln eröffnet, 2nd and 3rd Ed. Brönner, Frankfurt.
203. Von Sprockhoff H. (1954). – Untersuchungen über die Komplementbindungsreaktion bei der Borna'schen Krankheit. *Zentralbl. Veterinärmed.*, **1**, 494-503.
204. Von Sprockhoff H. (1956). – Zur biologischen Charakterisierung des Borna-s-Antigens. *Zeitschr. Immunitätsforsch.*, **113**, 379-385.
205. Wagner K., Ludwig H. & Paulsen J. (1968). – Fluoreszenzserologischer Nachweis von Borna-Virus Antigen. *Berl. Münch. tierärztl. Wochenschr.*, **81**, 395-396.
206. Walther F. (1899). – Gehirn-Rückenmarksentzündung bei Pferden und Schafen in der Amtshauptmannschaft Borna. In Mitteilungen aus den Berichten der Bezirksthierärzte auf das Jahr 1899. *Ber. Veterinärwesen Königr. Sachsen*, **44**, 80.
207. Waltrip II R.W., Buchanan R.W., Summerfelt A., Breier A., Carpenter W.T. Jr, Bryant N.L., Rubin S.A. & Carbone K.M. (1995). – Borna disease virus and schizophrenia. *Psychiatry Res.*, **56**, 33-44.
208. Weisman Y., Malkinson M., Ashash E. & Nir A. (1993). – Serum therapy of a parietic syndrome of ostriches. *Vet. Rec.*, **133**, 172.
209. Weissenböck H., Nowotny N. & Zoher J. (1994). – Feline Meningoencephalomyelitis ('staggering disease') in Österreich. *Wien. tierärztl. Monatsschr.*, **81**, 195-201.
210. Weissenböck H., Nowotny N., Caplazi P., Kolodziejek J. & Ehrensperger F. (1998). – Borna disease in a dog with lethal meningoencephalitis. *J. clin. Microbiol.*, **36**, 2127-2130.

211. Weissenböck H., Suchy A., Caplazi P., Herzog S. & Nowotny N. (1998). – Borna disease in Austrian horses. *Vet. Rec.*, **143**, 21-22.
212. Yamaguchi K., Sawada T., Naraki T., Igata-Yi R., Shiraki H., Horii Y., Ishii T., Ikeda K., Asuo N., Okabe H., Mochizuki M., Takahaschi K., Yamada S., Kubo K., Yashiki S., Waltrip II R.W. & Carbone K. (1999). – Detection of Borna disease virus-reactive antibodies from patients with psychiatric disorders and from horses by electrochemiluminescence immunoassay. *Clin. diagn. Lab. Immunol.*, **6**, 696-700.
213. Zimmermann W., Dürrwald R. & Ludwig H. (1994). – Detection of Borna disease virus RNA in naturally infected animals by a nested polymerase chain reaction. *J. virol. Meth.*, **46**, 133-143.
214. Zimmermann W., Breter H., Rudolph M. & Ludwig H. (1994). – Borna disease virus: immunoelectron microscopic characterization of cell-free virus and further information about the genome. *J. Virol.*, **68**, 6755-6758.
215. Zwick W. (1939). – Bornasche Krankheit und Enzephalomyelitis der Tiere. *In* Handbuch der Viruskrankheiten, Vol. 2 (E. Gildenmeister, E. Haagen & O. Waldmann, eds). Fischer-Verlag, Jena, 254-354.
216. Zwick W. & Seifried O. (1925). – Übertragbarkeit der seuchenhaften Gehirn- und Rückenmarksentzündung des Pferdes (Borna'schen Krankheit) auf kleine Versuchstiere (Kaninchen). *Berl. Münch. tierärztl. Wochenschr.*, **41**, 129-132.
217. Zwick W. & Witte J. (1932). – Zur Frage der Schutzimpfung und der Inkubationsfrist bei der Bornaschen Krankheit. *Arch. Tierheilkd.*, **64**, 116-124.
-

Fig. 1

Borna, the city which gave its name to the disease (near Leipzig in Saxony, Germany)

Lithograph by C.W. Arldt from a painting by G. Taubert (18th Century)

Fig. 2

Pioneers in Borna disease virus research

- a) E. Joest (1873-1926), Leipzig, Germany
- b) W. Zwick (1871-1941), Giessen, Germany
- c) S.S. Nicolau (1896-1967), Paris, France
- d) I.A. Galloway (1900-1969), London, United Kingdom

Fig. 3

The head of a horse showing early signs of Borna disease

Typical position of the head held low, sleepy appearance and depression

- 1a) Stabled horses, free of Borna disease (Germany, 1995)
- 1b) Stabled horses, some of which showed signs of Borna disease (endemic area, Bavaria, Germany, 1994)
- 2) Presence of clinical symptoms suggestive of Borna disease (endemic area, Bavaria, Germany, 1993-1994)
- 3a) Clinical and histological evidence of Borna disease (endemic area, Bavaria, Germany, 1993-1994)
- 3b) Clinical and histological evidence of Borna disease (endemic area, Saxony, Germany, 1992); only serum available

Fig. 4

Borna disease virus infection in horses with and without Borna disease

Fig. 5

A sheep showing an advanced stage of Borna disease: staggering movements and severe ataxia

Fig. 6

A heifer in the stage preceding the final phase of Borna disease

Presentation of neurological disease, diarrhoea and recumbency

- | | |
|--------------------------------------|----------------------------------|
| 1. Horses (Germany) | 6. Sheep (north-eastern Germany) |
| 2. Horses (United States of America) | 7. Sheep (northern Italy) |
| 3. Horses (France) | 8. Cats (Sweden) |
| 4. Horses (Iceland) | 9. Cats (eastern Germany) |
| 5. Cattle (north-eastern Germany) | 10. Cats (western Germany) |
| | 11. Dogs (southern Germany) |

Groups 1, 2, 3, 5 and 7	Healthy animals (5 and 7 from herds with previous records of Borna disease)
Group 4	Horses with disease of unknown aetiology
Group 8	Cats with Borna disease (staggering disease)
Groups 9 and 11	Animals with diseases other than Borna disease kept isolated
Group 10	Mainly healthy animals, kept in homes

Fig. 7

Seroprevalence in horses, cattle, sheep, cats and dogs infected with Borna disease virus (1993-1998)

1. German Red Cross, 1998
2. Benjamin Franklin Hospital, Free University of Berlin, 1993-1994 and 1997-1998
3. Medical School of Hanover, 1997-1998
4. Benjamin Franklin Hospital, Free University of Berlin, 1993-1994 and 1997-1998
5. Medical School of Hanover, 1997-1998

Fig. 8

Borna disease virus infection in patients with affective disorders

Acute depressive episode (point prevalence)

Fig. 9

Borna disease virus specific antigen

- a) Primary rabbit brain cells 'stained' with antiserum of an infected rabbit
- b) Persistently infected green monkey kidney cells (cell line G26) 'stained' with the same rabbit antiserum or with cerebrospinal fluid of an infected horse

C	: cerebellum	MO	: medulla oblongata
EC	: entorhinal cortex	OB	: olfactory bulb
Hd	: hippocampus dorsalis	OT	: olfactory tract
Hm	: hippocampus medialis	PC	: pons cerebri
Hv	: hippocampus ventralis	TH	: thalamus

Fig. 10

Predilection sites for Borna virus and specific antigen in the limbic areas of the brain of an intra-cerebrally infected rabbit

Sites are shown as shaded, dotted or striped areas

Courtesy: Masato Furuya

Fig. 11

Borna disease virus infection in rats (litter maids)

The animal on the left was infected intra-nasally one day after birth and remained infected but showed no symptoms besides learning deficiencies. The two remaining rats were infected at two months of age and acquired either typical symptoms with fatal disease or an obesity syndrome

Fig. 12

Infected bipolar neuron of rabbit retina culture

The Borna virus-specific s-antigen serves as a neuronal marker
(196)

Fig. 13

Current testing systems for the detection of Borna disease virus infection

CSF: cerebrospinal fluid

KDa: kiloDaltons

Fig. 14

The use of Western blotting to demonstrate Borna disease virus antigen in the cerebrospinal fluid of different patients with mood disorders (patient numbers: 36, 86, 137 and 163) and two patients with multiple sclerosis (patient numbers 27 and 62)

Borna disease virus-specific rabbit antiserum was used to trace specific antigens (horizontal arrows) (47)