Vet. Res. 33 (2002) 127–138
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DOI: 10.1051/vetres:2002002

Review article

Borna disease: current knowledge and virus detection in France

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(Received 29 June 2001; accepted 30 October 2001)

Abstract – For over two centuries, Borna disease (BD) has been described as a sporadically occurring infectious meningoencephalomyelitis affecting horses and sheep in Central Europe. Over the last decade, the BD epidemiology has been discussed. Firstly, its geographical distribution seems larger than what was previously thought. Secondly, the disease can affect a large number of warm-blooded animal species, including humans. The aetiological agent is the Borna disease virus (BDV), an enveloped, nonsegmented negative-stranded RNA virus classified in the new virus family *Bornaviridae* (*Mononegavirales* order). It can induce severe clinical signs of encephalitis with striking behavioural disturbances and may cause death. BDV genome has recently been detected in France in the blood and brain of several animal species (horses, bovines, foxes).

meningoencephalomyelitis / Borna disease virus / epidemiology / diagnosis

Résumé – La maladie de Borna : connaissances actuelles et détection du virus en France. La maladie de Borna est connue depuis plus de deux siècles en Europe centrale comme une méningoencéphalomyélite d'origine infectieuse affectant les chevaux et les moutons. Depuis ces dix dernières années, l'épidémiologie de la maladie a été révisée puisque la répartition géographique de la maladie de Borna s'avère plus large que rapportée jusqu'alors. En outre, elle peut affecter un grand nombre d'espèces animales à sang chaud, y compris l'homme. L'agent étiologique est le BDV (virus de la maladie de Borna), virus enveloppé, à ARN simple brin, non segmenté et de polarité négative, récemment classifié dans la nouvelle famille des *Bornaviridae*, (ordre des *Mononegavirales)*. Le BDV peut induire des signes cliniques sévères d'encéphalite virale avec des troubles comportementaux importants et il peut entraîner la mort de l'animal. Récemment, le génome du BDV a été mis en évidence en France dans le sang et le cerveau de plusieurs espèces animales (chevaux, bovins, renards).

méningoencéphalomyélite / virus de la maladie de Borna / épidémiologie / diagnostic

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Table of contents

1. INTRODUCTION

Borna disease was first described at the end of the 18th century in southern Germany where it occurred sporadically as an infectious disease of the central nervous system (CNS). It owes its name to the town Borna in Saxony, where a large number of horses including a cavalry regiment died during an epidemic in 1895 [19]. The viral aetiology was described at the beginning of the 20th century [68]. Borna disease was then known as a nonpurulent meningoencephalomyelitis, often fatal in horses and sheep bred in the endemic region: Germany and in the upper Rhine valley between Switzerland, Austria and the Principality of Liechtenstein [62, 67]. The Borna disease virus (BDV) has now been isolated in several other countries and in many warm-blooded animal species, including humans. In humans, BDV could be responsible for psychiatric disorders such as schizophrenia, autism, chronic fatigue syndrome or chronic depression. However, the zoonotic aspect of the Borna disease virus infections is still largely controversial.

BDV can also be experimentally transmitted to a broad range of animal species, from chickens to nonhuman primates. Two animal models have been selected: the adult Lewis rat, which is an excellent model for the study of the BDV infection immunopathogenesis; and the neonatally BDV-infected rat, which has become a good model for the study of the troubles of neurological development of viral aetiology [10, 22].

This paper describes the recently discovered aetiology of the Borna disease, the main characteristics of Borna disease itself and the difficulty of its diagnosis. Then BDV detection in France is presented.

2. THE RECENTLY DISCOVERED AETIOLOGY OF THE BORNA DISEASE

2.1. Classification of BDV

The knowledge of BDV has improved thanks to the establishment of persistently infected cell lines [30, 41]. The first reference strain (strain V) isolated in 1929, was obtained from a brain homogenate of a diseased horse, after a series of passages on the rabbit [19]. The aetiological agent of Borna disease was recently characterized: BDV is an enveloped, negative, non-segmented, single-stranded (NNS) RNA virus. Two isolates (reference strains V and He/80) were recently completely sequenced. BDV was classified into the *Mononegavirales* order and it is the only prototype of the new *Bornaviridae* family within this order [9, 15].

The *Mononegavirales* order also includes *Filoviridae* (Marburg and Ebola viruses), *Paramyxoviridae* (measle and mumps viruses) and *Rhabdoviridae* (rabies and vesicular stomatitis viruses). The BDV genome is very compact (8.9 kb) as compared to the other RNA NNS viruses (11 to 15 kb), but its genomic organisation is similar. Its genome encodes six open reading frames (ORFs) (I, II, III, IV, V, x1) divided into three transcription units: the first transcription unit encodes the nucleoprotein N (p40), the second one encodes the phosphoprotein P (p24) (cofactor of polymerase) and the protein X (p10) in overlapping ORFs, and the third transcription unit encodes the matrix protein M (p16), the membrane protein GP (p56) and the RNA-polymerase L (p180 or p190) (Fig. 1).

2.2. BDV uniqueness

This new *Bornaviridae* family was created because BDV biology has unusual aspects. First, BDV is the only NNS RNA animal virus with a nuclear site of replication and transcription [8]. Secondly, its genome compaction is overcome by the overlapping of ORF and transcription units [57, 59] and by post-transcriptional RNA splicing [15, 38]. BDV is also characterized by strict neurotropism, noncytolytic and low rate replication and persistence in the central nervous system [49, 58].

Finally, unlike the majority of other RNA viruses, the BDV genome sequence is extremely stable over time (the first isolate,

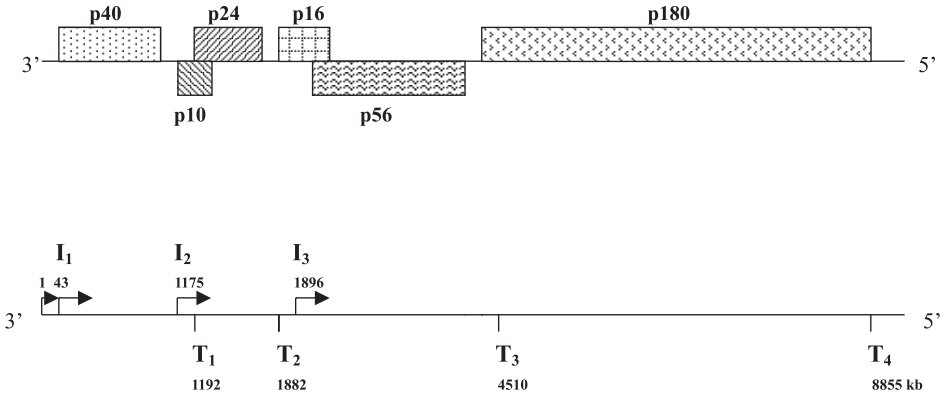

Figure 1. BDV genomic organization. I_1-I_3: initiation sites of transcription. T_1-T_4 : termination sites of transcription. Note that the first and second units overlap in the intergenic region. Note also that p40 transcripts can be initiated at two different sites.

strain V, is from 1929), geographic location and particularly animal species [5, 32, 51, 58, 62]. The genome of persistent BDV in cell culture is also very stable, even after hundreds of cell generations [62]. The complete nucleotide sequences of the two reference strains (strains V and He/80) are 95% homologous, despite very different origins and histories of in vivo and in vitro passages. Most of the strains are genomically very close and the nucleotide changes are frequently located on the third codon and do not alter the amino acid sequence.

A new genotype (strain No/98) isolated from an Austrian horse (located out of the endemic region) shows 15% genetic variability with all other BDV strains. However the amino acid sequence is very stable compared to the other BDV strains (93 to 98% similarity for all proteins except for protein X which has 81% similarity) [46]. A phylogenetic tree (Fig. 2) based on 273 nucleotides of the p24 gene shows the high genetic similarity between BDV strains isolat- ed from various animal species and the new No/98 strain. The discovery of this

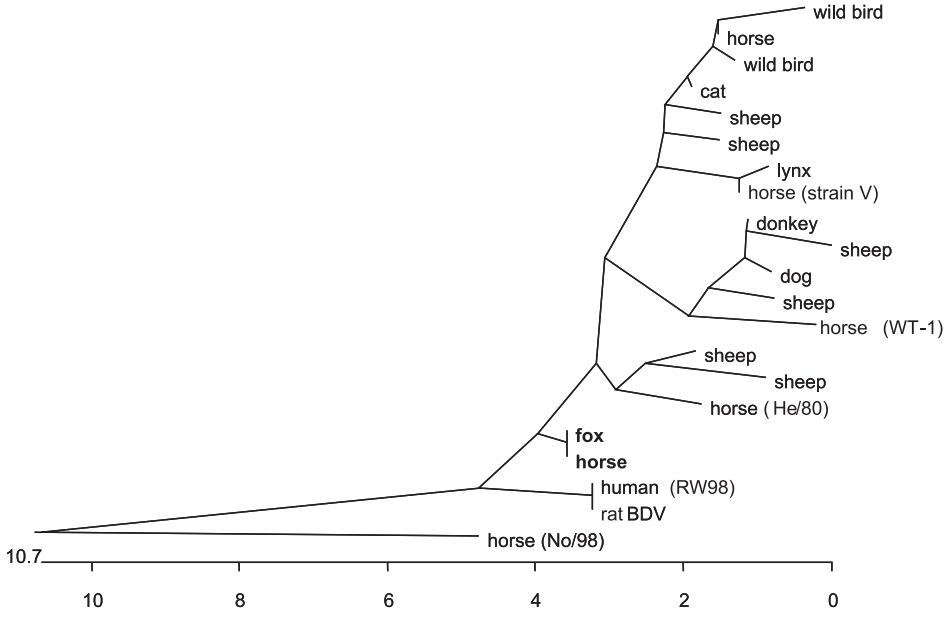

Figure 2. Phylogenetic tree of BDV isolates. This tree is based on nucleotide sequences (1482-1744) of the BDV-p24 protein. Sequence information was from EMBL/Gen Bank accession numbers AF136236, AF158630, AF201073, AF232700, AF232701, AF374596, AF374798, AJ250178, AJ277119, AJ277120, L27077, S67507, U04608, U94864, U94868, U94872, U94876, U94880, U94883, U94884, U94885 and (Staeheli et al., 2000) [62], completed by recent lynx, fox, horse and wild bird sequences respectively published by Degiorgis et al. [17], Dauphin et al. [16] and Berg et al. [4]. The sequences were aligned using the clustal alignment algorithm of the software MegAlign (DNASTAR, USA). The length of each branch represents the distance between the sequences, while the scale at the bottom of the tree indicates the number of substitution events. The BDV sequences determined from French animals are in bold letters.

new genotype raises fundamental questions [62]. Other BDV variants might be present outside the endemic region. However, BDV strains identified outside the endemic region (USA, Japan, United Kingdom…) are almost identical to laboratory strains derived from the European isolates. These BDV detections in animals and humans living outside of the endemic region could then merely represent artefacts, caused by accidental sample contamination by classical laboratory strains. Staeheli et al. [62] also suggest that a great number of strains may exist worldwide and the detection methods currently available only detect part of them.

3. BORNA DISEASE

3.1. Host range and geographic distribution

Recent epidemiological data suggest that host spectra and geographic distribution are wider than previously evaluated. The first reports of Borna disease only concerned horses and sheep, but BDV infection has been found in other warm-blooded animal species, such as cattle [11, 26], goats, rabbits [42], dogs [66], cats [2, 39, 47], and a wide variety of other species such as zoo animals: ostriches [40], deers, monkeys, alpacas, llamas [51, 53], or wild animals such as lynx [17], foxes [16] and wild birds [4]. The experimental disease is possible for almost all of these species, including primates. Moreover, in the last ten years, specific markers for BDV infection have been detected in humans, in particular psychiatric patients, but the zoonotic aspect of the disease is still highly controversial [48, 60, 62].

Borna disease geographic distribution is still not fully determined. It seemed for a long time to be restricted to an endemic region (central Europe) [51], but infections have recently been reported in northern Europe, in the USA, Japan, Iran and Israel [32, 35, 44]. However, clinical cases of horses and sheep have very rarely been reported outside the endemic region [4], except for one case described by Nowotny et al. (2000) [46]. This case was an Austrian horse infected with a new BDV genotype (No/98).

Sero-epizootiological studies have shown that BDV is geographically more widely distributed and is also present at higher rates in animals than previously thought. It is difficult to know whether this is due to a larger BDV dissemination or merely to a higher interest for the virus, associated with an improvement in the diagnostic methods [34, 48]. Moreover the reason for the restricted BDV geographic distribution despite all animal trades around the world, is still unknown.

3.2. Clinical signs

Clinical signs in naturally or clinically infected animals depend on the infected animal species and on the viral strain. The incubation period is variable, between two weeks and a few months.

3.2.1. Natural infection

Clinical signs are mostly observed in horses and sheep. BDV infections in horses are often clinically inapparent. In the horse, Borna disease results in simultaneous or consecutive disorders in behaviour, sensitivity and motility [19]. During the initial phase, nonspecific signs such as hyperthermia, anorexia, alternance of colic and constipation are observed. During the acute phase, neurological signs result from meningoencephalitis: abnormal posture, ataxia, proprioceptive deficit, repetitive movements (bruxism, circular ambulation, trismus, nystagmus, strabismus, myosis). These signs can be associated with abnormal reactions to external stimuli such as hyperexcitability, aggressiveness, lethargy, somnolence and stupor. In

the final phase, paralysis can appear, followed by convulsions often associated with head pressing (result of a high cerebrospinal fluid pressure caused by the inflammation reaction in the CNS) and decubitus. Death usually occurs after one to three weeks and the death rate in horses is above 80% (50% in sheep) [19, 24, 52]. In animals that have survived the acute phase of the disease, recurrent episodes can appear for the rest of the animals life (chronic infection) with depression, apathy, somnolence, fearfulness, in particular after a stress [19, 48]. Viral persistence for the whole life without apparent disease has also been described in naturally infected animals of various species [34]. The Borna disease in less described in sheep. In sheep flocks, clinical Borna disease can affect a large proportion of animals [51], and the death rate is around 50% for diseased animals.

3.2.2. Experimental infection

A lot of laboratory animal species are susceptible to BDV infection. The incubation period, mortality and severity of the disease considerably depend on the infected animal species, viral variant and host immune status. In adult immunocompetent animals, the infection causes the same meningoencephalitis as in horses and sheep. On the contrary, immunodepressed animals or animals with an immature immune system show more discrete clinical signs.

Results of BDV infection in the rat depend on the inbred rat strain and the virus isolate. Lewis rats are highly susceptible to the infection and represent an excellent model to study Borna disease pathogenesis. They develop a biphasic disease; the first phase is characterized by abnormal movements, aggressiveness and hyperactivity, comparable to the one described in horses. This hyperactive phase is followed by apathic behaviour and somnolence, sometimes associated with the obesity syndrome (5 to 10% of the rats), or fertility troubles [21, 45, 53]. The hyperactive phase corresponds to a massive CNS inflammation, completed by important neuronal destruction. Then, the inflammatory response strongly decreases during the chronic phase despite the virus being present at very important levels in the CNS. This animal model has allowed to determine that Borna disease pathogenesis results from the cell mediated immune response [63].

Rat infection is also a good tool to study BDV spread in animals [13, 43]. On this point, BDV biology is very close to rabies virus biology. BDV replicates in neural processes located at the inoculation site (in the olfactive nerve or nerve endings in the oropharyngeal and intestinal regions). Then BDV migrates intra-axonally and centripetally as ribonucleoparticules (RNP), along the axons to the brain. The delay of clinical disease onset depends on the distance between the inoculation site and the CNS. BDV replicates in glial cells and neurons, preferentially in the limbic system (particularly the hippocampus), then it disseminates progressively throughout the whole CNS, and later, throughout the autonomic and peripheral nervous systems [23, 43].

The newborn rat infection (infected 24 hours following birth) is a good model for "persistent tolerant infection of the newborn". Even though the viral load in the CNS is high and the virus spreads in the whole body, no cellular antiviral response appears. These rats display neurobehavioural and learning disorders associated with postnatal CNS development abnormalities [1, 14, 21]. BDV might alter neuronal differentiation and migration, and even communication between neurons [21].

3.3. Transmission

We still have much to learn about the route of transmission [54, 61, 62]. BDV is probably shed in nasal, salivary and

conjunctival secretions since BDV-RNA has been detected in these secretions [31, 50]. An olfactory route of transmission from horse to horse has been proposed (via the olfactive nerve), either by direct contact with these secretions, or through contaminated food or water. The presence of BDV-RNA and proteins in peripheral blood mononuclear cells (PBMC) suggests possible transmission by blood. However, direct transmission of BDV from horses to horses or sheep to sheep has never been shown [62]. One should note that vertical transmission has been reported in horses [16, 27].

Horses and sheep natural infections are rare and sporadic. Nevertheless BD is the most predominant viral CNS disease in horses in Germany [48]. The disease has seasonal influence: the incidence increases during the spring and in the early summer [19, 51]. Moreover, genome mutations identified in BDV strains isolated from horses, sheep and other domestic animals are not specific to animal species. This fact suggests a common viral source [48, 62]. The natural source of infection is still unknown, but rodents are a potential reservoir and vector, even though their role in the Borna epidemiology has not yet been demonstrated. The only study concerning wild rodents has shown no natural infection [64]. Other wild species could be implicated in the BDV epidemiological cycle, such as wild birds [4].

A lot of clinically healthy or subclinically infected seropositive animals can also represent potential infection sources for other animals, and probably humans [51]. The infection is not systematically diagnosed, mainly because it often remains clinically unapparent. German studies have shown that the average seroprevalence of BDV-specific antibodies is 11.5% in the clinically healthy German horses [31], while this seroprevalence reaches 22.5% in the endemic region [48].

3.4. Differential diagnosis

It is important to make the distinction between Borna disease and other infections like rabies, equine Herpesviruses infection, tick-borne encephalitis, botulism, bacterial meningitis, viral (West Nile) and parasitic encephalomyelitis [48].

Besides other affections in cattle and sheep (listeriosis, botulism, intoxications…), the Borna disease clinical diagnosis should be distinguished from the Bovine Spongiform Encephalopathy and ovine scrapie, although Borna disease takes its course more rapidly.

3.5. BDV human infection?

Firstly, numerous serological studies have shown the presence of BDV-specific antibodies in sera of psychiatric and neurological patients in Germany, Japan and the USA [51]. These BDV-specific (or specific to another close virus) antibodies are also detected in healthy patients, but at lower rates and proportions as compared to psychiatric patients. However serological results are still contradictory between laboratories.

Secondly, BDV-RNA was first detected by RT-nested-PCR in the peripheral blood of several psychiatric patients by Bode et al. in 1995 [6]. Many studies are still aimed at confirming or not the presence of BDV-RNA in humans. Some laboratories do not detect any BDV-RNA in human blood; others detect BDV-RNA both in the blood of psychiatric patients and healthy people. But, some amplified BVD strains have the same sequences as laboratory strains, suggesting laboratory contaminations [60, 62]. Other studies have allowed the detection of BDV and/or RNA viral antigens in psychiatric patients' brains [18, 25, 55]. But, even though certain groups have detected BDV in humans, no one has definitely demonstrated that it causes human psychiatric diseases. Considering the current knowledge about Borna, we have to remain very cautious

with the possibility of human disease. To date, BDV has not been identified as a clear etiology of human disease. However, considering the knowledge on Borna disease in non human species, it is tempting to speculate about the clinical outcomes of human BDV infection [12].

4. BORNA DISEASE DIAGNOSIS

The diagnosis of Borna disease is difficult, mainly because of low viral replication and excretion rates. Although a wide variety of tools have been developed for BDV diagnosis, standards have yet not been established and large differences and contradictions have been observed between the published results (particularly serological surveys) [28, 48, 62].

Borna disease can be diagnosed by serology, viral isolation, antigen detection, RT-nested-PCR, but none of these methods is yet sensitive and specific enough to be used alone for a sure diagnosis.

4.1. Clinical diagnosis

Animals exhibit a variety of clinical nonspecific symptoms. Therefore, clinical diagnosis alone cannot be sufficient to diagnose the disease. Moreover, meningoencephalitis induces a slight alteration of protein concentration and cerebrospinal fluid (CSF) amounts during the acute phase. These indicators are, however, nonspecific of Borna disease [48, 62].

4.2. Serological diagnosis

The serological diagnosis can be applied to living animals by antibody detection in blood and/or CSF. Western blot [31], ELISA [19] and immunofluorescence assay (IFA) [30] can be used. IFA is acknowledged to be the most reliable method for the detection of BDV-specific antibodies, even though comparative interassays have shown very variable results, partly due to variable sensitivity linked to the use of different cell systems [62]. Serological tests are mostly based on antibody detection directed against the most immunogenic BDV proteins, p24 and p40.

Antibody titers are usually very low in BDV-infected animals and their detection requires particularly sensitive techniques [36, 62]. Antibodies are detectable in 100% cases during the acute phase of the disease but they are hardly detectable in a subacute or chronic disease.

4.3. Histopathological diagnosis

Various degrees of encephalitis are observed, in particular with lymphocyte infiltrations in the perivascular and parenchyma regions. Joest-Degen inclusion bodies located in nuclei of infected neurons have been used as BDV-specific markers, but they are not systematically observed [23].

Sensitivity of virus detection can be enhanced by immunohistochemistry which permits to visualize the major BDV antigens: p40 (nucleoprotein) and p24 (phosphoprotein) using monoclonal or polyclonal antibodies [51]. However BDV-infected cells are not uniformly distributed in the brain tissue; sometimes Borna disease cases can escape detection [11]. Finally, in situ hybridization can also complete histology, by additional RNA detection [29].

4.4. Virological diagnosis

4.4.1. Viral isolation

Classical viral isolation methods from brain tissues are heavy and poorly sensitive, due to the low number of infectious particles [28, 62]. BDV can be easily cultivated on Vero cells (monkey kidney cells) and MDCK (dog kidney cells). BDV persistently infects cells without cytopathic effects.

4.4.2. RT-PCR or RT-nested-PCR [37]

This technique can be used for brain or blood samples. However this extremely sensitive technique is prone to cross-contamination between samples as well as laboratory contamination. Moreover, this technique (using BDV standard primers) is not able to detect variant strains that have altered sequences in the target gene. This was the case with the new genotype identified by Nowotny et al. [46].

RT-nested-PCR detects sequences encoding the two major BDV-proteins p24 and p40 [56]. The amplified BDV-products after the two successive PCR are 528 bp for the p40 gene and 391 bp for the p24 gene. In order to control RT-nested-PCR in each reaction sample and to avoid the use of a BDV-positive control reaction that could induce contamination, internal RNA standard molecules (named "mimics") have been produced [37]. These standards can be easily discriminated from the Borna fragments by agarose gel electrophoresis.

5. BDV INFECTIONS IN FRANCE

Apart from recent serological surveys that have revealed the presence of seropositive horses in France [7, 20], only one recent study has searched for the BDV genome in France [37]. Even though no enzootic BDV has ever been reported in France, BDV is likely present in France, as it now is in several other countries.

The RT-nested-PCR described in 4.4.2 [37] was used to detect the viral genome in 171 animal (horse, fox, bovine, dog and sheep) brains and 25 horse blood samples. Some of the animals tested showed nervous disorders. BDV-RNA was detected in three horse brains, one cow brain and six fox brains, and also in fourteen blood samples [16]. Among these 14 horse blood samples that were infected with BDV, four represented interesting cases from an epidemiological point of view. They were living in the same stable and the BDV genome was detected twice in two of them, which presented nervous disorders and were sampled twice at a 6-month-interval. Another horse had already been detected BDV-seropositive in a previous serological survey [20]. It should be noted that this horse did not show any clinical signs, which is still compatible with BDV infection since asymptomatic infections are often reported. The BDV genome was also detected in a mare and her foal: BDV has been transmitted either by horizontal or by vertical transmission. Vertical transmission of the BDV infection has already been described by Hagiwara et al. [27], but for a pregnant mare. A preliminary study achieved with an ELISA which is currently developed by the same authors and with an ELISA developed by Berg et al. [3], showed the presence of anti-BDV antibodies in most sera of these PCR-positive horses (unpublished results).

One sequence of BDV originating from a fox brain showed more than 5% divergence with all the strains manipulated in the laboratory. Therefore, it is possible to consider that this case is not a laboratory contamination. The degree of genetic divergence (0–6.5%) between these BDV PCR products and the reference strains (strains V and He/80) is in accordance with those described in previous studies [5, 33, 59, 62, 65]. Furthermore, the comparison of BDV sequences from animals of the same or different species shows the same level of divergence whether in France or elsewhere [62]. The p24 sequences of BDV from one horse and one French fox written in bold on the phylogenetic tree appeared identical (Fig. 2).

This first description of the BDV genome in foxes can be of importance concerning Borna disease epidemiology since foxes could play a role as a reservoir and/or a vector of the virus. This question is all the more important since no reservoir has yet been identified. It is necessary to focus our

future studies on perfecting serological tools, with the aim of standardization of the diagnostic methods and estimating the prevalence of infection in different animal species in France.

6. CONCLUSION

Borna disease has an increasing importance in the scientific community because of its wide host spectrum, its large geographical distribution, its possible character of emerging zoonosis, and its possible confusion with important or actual diseases such as BSE, scrapie, rabies and West Nile. However, a lot of questions are still open concerning the epidemiology of the disease. One example is why the geographical location is still limited to central Europe despite the very important animal trades. Progress is also necessary in terms of serological as well as viral diagnosis. There is a particular need for standardization of the techniques in order to evaluate the real importance of this disease in humans and animals and outside the endemic region.

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