

# Staggering Disease in a Cat: *The First Case of Borna Disease Virus Infection in a Belgian Cat*

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## ABSTRACT

A 1-year-old female domestic cat showing progressive locomotor and neurological problems for 4 weeks was euthanized and examined. On autopsy, the brain was macroscopically normal. Test results for rabies, feline spongiform encephalopathy, feline infectious peritonitis, feline immunodeficiency virus, feline leukemia virus, and feline herpes virus (type I) were negative. Histologic examination of the brain revealed a nonsuppurative meningoencephalitis. Frozen brain material tested positive for the presence of Borna disease virus specific antigens. The present case appears to be the first description of a Borna disease virus infection in a cat in Belgium.

## INTRODUCTION

Borna disease, named after the city Borna in

Saxony (Germany) where severe outbreaks of the disease with neurologic signs had occurred in the horse population, is usually accompanied by progressive encephalomyelitis.<sup>1</sup> However, Borna disease virus (BDV) infections in animals and humans recently has been recognized as a more complex disease, leading to a strict differentiation of virus infections with and without clinical signs.<sup>2,3</sup> The infection is spread in many species, including a variety of ungulates, birds, cats, and dogs.<sup>2,4-10</sup>

Progress regarding the pathogenic effects of this virus has been made, particularly for experimental infections, but conclusions obtained under experimental conditions must be applied cautiously to natural infections.<sup>2,3,11-13</sup> It has generally been accepted that BDV causes a persistent infection of the central nervous system (CNS), which might be expressed as mild behavioral changes or severe neurological disease.<sup>12,14-16</sup> Under experimental conditions, clinical manifestation can range from dra-

matic to subtle or nearly imperceptible signs.<sup>2</sup> An even broader range of signs can be observed in natural infections, depending on the host's vulnerability. BDV has been studied intensively with regard to its morphology, biological properties, and its single and negative-stranded RNA. It has been grouped to the order of Mononegavirales with the new family Bornaviridae.<sup>17</sup>

In cats, a spontaneous nonsuppurative meningoencephalomyelitis had long been known without an etiopathogenic link to BDV.<sup>18-21</sup> Collaborative studies between groups in Uppsala and Berlin on diseased cats with ataxia and other neurological symptoms showed that BDV infections were associated with clinical symptoms of staggering disease and suggested a mutual influence of BDV infection and feline immunodeficiency virus infection as well as demonstrating that a similar, albeit less severe, clinical disease could be demonstrated in experimentally infected cats.<sup>7,22-26</sup>

Spontaneous nonsuppurative meningoencephalitis in cats accompanied by ataxia and behavioral abnormalities and monitored by antibodies or the neuropathologic picture of encephalomyelitis was also reported from other countries in Europe and Asia.<sup>8,9,12,27-30</sup> This report presents the first known case of Borna disease in a cat in Belgium.

## CASE REPORT

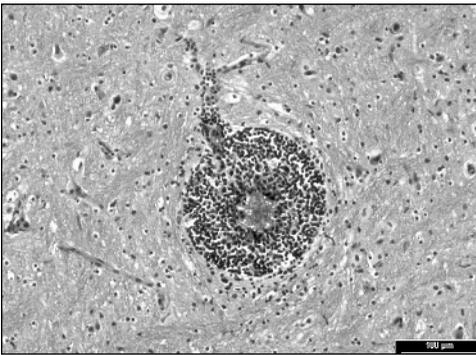
A 1-year-old female domestic cat exhibited locomotor problems, loss of balance, and incoordination with staggering movements that had increased over a 4-week period. These signs worsened during the week following the examination, and the cat eventually became unable to sit or lower its head. The cat's appetite remained unchanged, but there was increased vocalization and the cat could not stop walking in circles. The cat exhibited hypermetria, hyperesthesia, pruritus, and finally became totally manic, leading to the necessity to administer an anesthetic, and eventually, euthanasia.

Rabies or feline spongiform encephalopathy were initially suspected, and a thor-

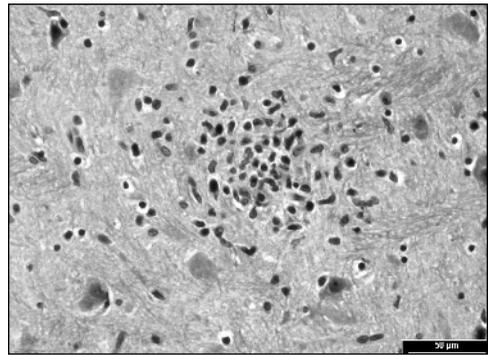
ough necropsy was not performed, precluding a neurological examination or evaluation of blood or cerebrospinal fluid. The skin on the left shoulder exhibited alopecia and erythema. The brain appeared macroscopically normal. Subsequently, examination for rabies using a direct immunofluorescence technique and virus isolation in a mouse neuroblastoma cell line was negative.

Direct immunofluorescence staining was negative for feline infectious peritonitis virus, feline leukemia virus, and feline herpes (type I) virus. A negative result for feline immunodeficiency virus was obtained using chromatography testing. Hematoxylin-eosin staining of tissue from the cerebrum, cerebellum, and brainstem revealed moderate to severe perivascular cuffing with lymphocytes, plasma cells, and some macrophages of the meningeal blood vessels of the cerebellum and cerebrum (Figure 1). Widespread lymphoplasmacytic cuffing, multifocal astrocytosis, gliosis, and neuronophagia were present in the cerebrum and brainstem (Figure 2). A diagnosis of nonsuppurative meningoencephalitis was made. Feline spongiform encephalopathy could be excluded histologically and immunohistochemically using the monoclonal antiprion protein antibody (clone 3F4, DAKO; Gentbrugge, Belgium).

Frozen brain material was tested for the presence of BDV-specific N (p40) and P (p24)-protein antigens (major structural proteins of the virus core) using a highly specific antigen ELISA (cAg) recently described in full detail.<sup>31</sup> Prior to ELISA testing, brain tissue was suspended in sterile 20% phosphate-buffered saline (PBS) and ultrasonicated. The brain suspension (diluted 1:4) tested weakly positive for BDV, with an extinction of 0.143 at 405 nm. Negative samples and PBS testing were significantly below the cut-off of 0.1 extinction. Likewise, the positive ELISA of the brain suspension was titratable and became negative at higher dilutions (0.066 extinction at a dilution of 1:16). BDV-specific RNA was extracted from an aliquot of the brain sample, and nested reverse tran-



**Figure 1.** Microphotograph of lymphoplasmacytic cuffing (Hematoxylin–eosin stain).



**Figure 2.** Microphotograph of microgliosis and neuronophagia (Hematoxylin–eosin stain).

scriptase polymerase chain reactions (RT-PCRs) were run to amplify fragments of three genes encoding p40, p24, and p16 using the same primers and a slightly modified method previously described).<sup>31</sup> No specific amplicates could be obtained for all three BDV genes.

Based on clinical signs demonstrated by the cat, the classical neuropathologic picture, and the laboratory results, a diagnosis of Borna disease for this case of staggering disease was made.

## DISCUSSION

The neurologic signs described for the present case are very similar to those described by Lundgren and Ludwig<sup>2</sup> and Lundgren et al.<sup>7</sup> The macroscopic and microscopic findings in this case also corresponded very well with those reported in the literature. There were no significant gross lesions; microscopically the disorder is generally characterized by a nonsuppurative (meningo)encephalomyelitis dominated by perivascular cuffing, which is often reported to be very thick and spreading to the adjacent parenchyma.<sup>7,23,27,32</sup> It is also accompanied by neuronal degeneration.<sup>23,33,34</sup> Lesions are confined largely to the gray matter, although white matter may also be affected, and lesions are most severe in the midbrain, midbrain–diencephalon junction, and hypothalamus.<sup>23,27,34</sup> Inflammation of the meninges (present in this case) and spinal

cord is generally mild.<sup>33</sup> Small, round, or oval eosinophilic intranuclear inclusions (Joest–Degen bodies), which are pathognomonic for Borna disease in horses, may occur in neurons of the brain stem, hippocampus and cerebrospinal ganglia.<sup>33,35</sup> In the present case, however, no such viral inclusions were detectable.

In BDV-specific laboratory tests, a weak antigen presence could be measured, indicating that small amounts of viral proteins (p40, p24) were present. This is in agreement with previous observations in naturally and experimentally infected cats that demonstrated only a few antigen-positive neurons and occasionally antigen-positive glial cells.<sup>23,26</sup> In this respect, cat brains with Borna disease were clearly different from those of horses.<sup>36</sup>

Although viral RNA was not detected, this finding is not in conflict with BDV infection based on similar investigations using animal and human specimens.<sup>3</sup> It is known that viral RNA is much more fragile than the corresponding translation products. Furthermore, both negative RT-PCRs and the relatively low level of antigen in this sample from a cat may be explained by the fact that only a single specimen of an undefined region of the brain was available for examination.

Several reports from clinically diseased or inconspicuously infected cats describe BDV-specific circulating antibodies or even circulating immune complexes as well as

plasma antigen (Flower, Kamieh, and Bode, unpublished data).<sup>9,22,25,29</sup> In the present case, however, a blood sample that could have provided more information and evaluated in context with published laboratory data was not available.<sup>3</sup>

Although transmission of BDV is still not definitively described, it could occur via nasal and buccal secretions and orally. The virus infects nerve endings of the olfactory epithelium and migrates intra-axonally to the CNS where it induces the lesions described above.<sup>35,27</sup> After having reached the CNS, the infection spreads intra-axonally within the brain and to other nervous tissues and then centrifugally towards the peripheral nervous system, where it infects the nerves of different organs and can then be excreted by natural secretions.<sup>2,35,38</sup> However, vector(s) or reservoir(s) for this virus have not yet been identified. Likewise, the common pathways of intraspecific and interspecific transmission are unknown in both natural hosts and experimentally infected animals.<sup>2</sup>

In addition to neurons and glial cells, BDV infection of the CNS is characterized by infection of astrocytes and prominent astrocytosis.<sup>14,35,39,40</sup> Astrocytes play a major role in maintaining the appropriate microenvironment in the CNS required for normal neuronal activity.<sup>41</sup> One of the most important functions of astrocytes is to regulate the level of extracellular glutamate, a major excitatory neurotransmitter.<sup>42</sup> Glutamate accumulates as a consequence of neuronal activity, and excessive levels of extracellular glutamate often result in neuron toxicity and death.<sup>42</sup> Evidence indicates that glutamate-mediated neurotoxicity could play a major role in CNS disorders, including virus-induced diseases. Astrocytes are one of the target cells of BDV infection in the CNS and show severe and specific impairment in the ability of glutamate uptake.<sup>43</sup> The strong evidence of an interaction of BDV proteins with neurotransmitter receptors, such as glutamate and aspartate, first suggested by Gosztonyi and Ludwig,<sup>35</sup> might play a functional role in brain pathogenesis and has recently been

strengthened by investigating clinical features of transgenic uninfected mice, solely expressing P-protein in glial cells.<sup>3,44</sup>

In conclusion, BDV infection of cats, which could lead to clinical symptoms or asymptomatic cases, should carefully be followed in neurological diseases of this important pet species. As cats are one of human's closest companions and a zoonotic spreading of BDV is presently not known (although most unlikely, as based on our present knowledge) the infected cat should remain in the focus of comparative medicine.<sup>2,3,24,45</sup>

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