Staggering disease in cats: isolation and characterization of the feline Borna disease virus


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A Borna disease virus (BDV)-like agent was isolated from the central nervous system (CNS) of cats with a spontaneous non-suppurative encephalomyelitis ('staggering disease'). In contrast to the rabbit-adapted BDV strain V, which can be propagated in several primary and permanent cell cultures, the cat virus grew only in embryonic mink brain cells. Infection of adult Wistar rats with feline brain tissue material did not result in clinical disease during a period of 5 months, nor in growth of infectious virus in the brain. However, using the brain suspension of a newborn rat inoculated with feline brain tissue material, it was possible to induce typical Borna disease (BD) in four adult rats. This indicates a possible adaptation of the cat virus during passages in rats. By the use of an RT-PCR technique, BDV-specific RNA could be detected in a majority of brain samples from diseased cats. BDV-specific antigen was demonstrated in feline CNS samples both by immunohistochemistry and ELISA. However, the amount of BDV RNA and BDV antigen was less in the cats as compared to horses with BD, providing further support for the notion that a distinct feline BDV strain exists.

Introduction

A neurological disorder in cats, commonly referred to as 'staggering disease' (SD), has been observed in Sweden since the early 1970s (Kronевi et al., 1974). In a representative group of animals, SD was found to be associated with a non-suppurative meningoencephalomyelitis predominantly affecting the brain stem and the limbic system (Lundgren, 1992). Similar feline encephalomyelitides have been reported from other parts of the world, including Australia (Borland & McDonald, 1965) and the United States (Vandevenelde & Braund, 1979). Although the histopathology of SD suggests a viral aetiology, no correlation has been found with established feline viruses such as feline leukaemia, feline immunodeficiency and feline infectious peritonitis viruses (Lundgren, 1992; Ström et al., 1992).

Borna disease (BD) is a neurological syndrome that results from a persistent virus infection of the central nervous system (CNS) (Ludwig et al., 1988), previously reported in horses, sheep and a broad range of experimentally infected animals (Nicolaï & Galloway, 1928; Narayan et al., 1983; Ludwig et al., 1985).

Recently, BD has been found also in cattle (Bode et al., 1994b) and even in ostriches (Malkinson et al., 1994). The aetiologial agent of BD has been characterized as a negative-stranded, non-segmented enveloped RNA virus which replicates in the nucleus and from its genetic make-up belongs to the Mononegavirales (Briese et al., 1992, 1994; Cubitt & de la Torre, 1994; Zimmermann et al., 1994b). BDV infection of humans has been suspected from serological studies (Rott et al., 1985; Bode, 1995). The recent findings of viral antigen as well as BDV-specific nucleic acid in peripheral blood monocytes of psychiatric patients (Bode et al., 1994a, 1995) imply the existence of a human infection.

The preferential localization of the inflammatory reaction within the limbic system in SD, as well as the clinical manifestations including altered mentality of diseased cats, are suggestive of BD (Ludwig et al., 1988). We showed in initial studies that 40% of clinically affected cats had BDV-specific antibodies (Lundgren & Ludwig, 1993). Furthermore, the results of seroepidemiological investigations in Germany showed 13% seropositive cats in a population of animals with undefined neurological disorders (Lundgren et al., 1993). The recent findings of BDV antibodies in a group of Austrian cats with SD (Weissenböck et al., 1994) gives further...
support to the notion that BDV may be incriminated in this disease.

Evidence for BDV infection in the cat – one of man’s closest companions – has raised concern and interest, since the viral reservoir of human infections is still unknown. The present study was prompted by the suspicion of a link between BDV and feline SD, based upon the antibody findings in cats and the similarities between the two diseases. Different virological and molecular biological methods were used in order to identify and isolate a possible infectious agent from cats with SD.

This is the first demonstration of BDV-specific antigen and nucleic acid in cats, as well as of the isolation of a feline strain of BDV and some of its properties.

Methods

Animals and tissue samples. Eleven Swedish cats, clinically diagnosed as having SD, were studied (Table 1). Seven were neutered males (all domestic shorthairs), one a neutered female (Abyssinian), and three were intact females (domestic shorthairs and Persian). Ages ranged from 1.5–11 years (mean age 4.3 years). All cats were euthanized by pentobarbital overdose. Prior to euthanization, serum samples were taken and stored at –20 °C. The post-mortem interval was less than 45 min in all cases. At necropsy, the brain was cut transversally in coronal sections and tissue specimens from the cerebral cortex, basal ganglia, hippocampus, thalamus, midbrain and cerebellum were snap frozen in liquid nitrogen and stored at –70 °C for molecular biological and virological studies. Pieces of the spinal cord were taken from the cervical, thoracic and lumbar segments and treated likewise. Specimens from the same brain regions as well as the spinal cord and various internal organs were fixed in buffered 10% formalin, embedded in paraffin, cut 4 μm thick and stained with haematoxylin and eosin (HE) for light microscopy. Paraffin sections for immunohistochemistry were also prepared from six of the cats.

Indirect immunofluorescence test (IFT). Young rabbit brain (YRB) cells infected after one passage with 5 × 10^4 focus forming units (f.f.u.) of BDV per cell were seeded on coverslips. The cells were fixed 5 days later with cold acetone and stored at –20 °C before use. The IFT was carried out as described earlier (Bode et al., 1992), using the double-stain technique. In brief, the cells were incubated simultaneously with equal volumes of serum and the BDV-specific monoclonal antibody (MAb) Kfu3 (Ludwig et al., 1993), which recognizes the 40 kDa protein of BDV, diluted 1:100 in phosphate-buffered saline (PBS) with 1% fetal calf serum (FCS). After washing, the cells were incubated with FITC-labelled goat anti-cat or anti-species (corresponding to the sera used) IgG (Dianova) and TRITC-labelled F(ab')2, rat anti-mouse IgG (Dianova). The fluorescent staining of the cells was examined with a Zeiss ICM 405 microscope.

Animal experiments. A total of 78 Wistar rats were used for inoculation experiments.

Thirty-five newborn (1 day old) and eight adult (4 weeks old) rats were inoculated intracerebrally (i.c.) with 20 μl of tissue suspension of different parts of brain and spinal cord in PBS (10%, w/v) from cats with SD. The newborn rats were inoculated with material from cats no. 154, 249, 423 and 436; the adult rats with material from cats no. 249 and 436. The first newborn rat was sacrificed 2 weeks after infection and the remainder in weekly intervals up to 10 weeks after infection. The adult rats were observed daily for typical signs of BD. In the event of such signs, the animals were sacrificed immediately, otherwise 5 months after infection.

Twenty-four newborn and four adult rats were inoculated i.c. with brain suspensions from the newborn rats originally infected with cat tissue material. The newborn rats were sacrificed 2 and 3 weeks after infection, with the exception of eight rats which were kept for 7 months. The adult rats were observed daily for typical signs of BD. In the event of such signs, the animals were sacrificed immediately, otherwise 5 months after infection.

Seven newborn rats were inoculated i.c. with suspensions of embryonic mink brain (ENG) cells, infected with brain tissue material from cats no. 154 and 249 (third passage). The animals were sacrificed at intervals of 2 weeks, starting 6 weeks after infection.

Brain material from the 78 experimentally inoculated animals was titrated on YRB cells, and the sera were tested for BDV-specific antibodies using the IFT.

From each of the experimentally inoculated groups of rats, selected animals were perfused transcardially with the fixative of Somogyi & Takagi (1982). Alternatively, the animals were sacrificed without perfusion and half of the brain used for infectivity assays, the other fixed by immersion in a paraformaldehyde–glutaraldehyde–picric acid solution (Somogyi & Takagi, 1982). Coronal brain slices were embedded in paraffin and stained with HE for light microscopy or processed for immunohistochemistry.

Tissue culture, infectivity assay and neutralization. For propagation of virus from original cat tissue material (thalamus/hippocampus from cat 154; cerebral cortex and spinal cord from cat 249; cerebral cortex, thalamus and spinal cord from cat 423; cerebral cortex and spinal cord from cat 436) the following cell lines were used: YRB, Crandell feline kidney cells (CRFK), embryonic mink brain cells (ENG), a human oligodendroglia cell line (Oligo) and a primary cat brain cell line (K.G). The different cell lines were grown in Dulbecco’s modified Eagle’s medium (DMEM) (Flow Laboratories) supplemented with 5% FCS and 5% CO₂. The cells were propagated for at least six passages and after each passage an aliquot of cells were fixed with acetone and incubated for 1 h at 37 °C with sera from naturally infected horses, neutralizing antisera of infected rats (rat pool) and rabbits (BP-11), and the MAb Kfu2 (Ludwig et al., 1993; Zimmermann et al., 1994b). The cells were processed as described for IFT.

For infectivity studies, the second passage of YRB cells was seeded in 24-well tissue culture plates (Nunc), and the cells were infected with a tissue suspension in DMEM (10%, w/v) of rat brain samples which had been sonicated with a Branson sonifier (20 cycles/min, 40 mA) (Gosztonyi & Ludwig, 1984; Ludwig et al., 1993) for 2 min. Virus titrations followed our standard assay system, expressing the amount of infectivity as focus-forming units per ml (f.f.u./ml).

For neutralization experiments, the rabbit serum BP-11 (50% focus reduction at dilution 1:1000), rat pool serum (at dilution 1:200), species-specific BDV-negative control sera and MABs against BDV (Kfu2, W1) and herpes simplex virus were used (Ludwig et al., 1993).

Immunohistochemistry

(a) Cats. The murine MAb Kfu2, recognizing the 24 kDa protein of BDV, was used on paraffin sections of the CNS from six of the diseased cats (9, 211, 249, 293, 423 and 436), diluted 1:1000, rat pool serum (at dilution 1:200), species-specific BDV-negative control sera and MABs against BDV (Kfu2, W1) and herpes simplex virus were used (Ludwig et al., 1993).
Table 1. *BDV*-specific findings in Swedish cats with staggering disease

<table>
<thead>
<tr>
<th>Cat no.</th>
<th>Duration of illness (weeks)</th>
<th>BDV antibody titre in serum</th>
<th>CNS area</th>
<th>BDV RNA by n-PCR and hybridization</th>
<th>BDV antigens by ELISA†</th>
</tr>
</thead>
<tbody>
<tr>
<td>9</td>
<td>3</td>
<td>0</td>
<td>Cortex</td>
<td>+</td>
<td>(+)</td>
</tr>
<tr>
<td>154</td>
<td>6</td>
<td>0</td>
<td>Thalamus/hippocampus</td>
<td>0</td>
<td>NA</td>
</tr>
<tr>
<td>167</td>
<td>3</td>
<td>1:1280</td>
<td>Cortex</td>
<td>0</td>
<td>+/0</td>
</tr>
<tr>
<td>187</td>
<td>1</td>
<td>NA</td>
<td>Thalamus</td>
<td>NA</td>
<td>+/0</td>
</tr>
<tr>
<td>193</td>
<td>4</td>
<td>0</td>
<td>Basal ganglia</td>
<td>NA</td>
<td>+/0</td>
</tr>
<tr>
<td>211</td>
<td>4</td>
<td>0</td>
<td>Cortex</td>
<td>+</td>
<td>(+)</td>
</tr>
<tr>
<td>249</td>
<td>5</td>
<td>0</td>
<td>Spinal cord</td>
<td>0</td>
<td>+/0</td>
</tr>
<tr>
<td>293</td>
<td>4</td>
<td>0</td>
<td>Basal ganglia</td>
<td>0</td>
<td>NA</td>
</tr>
<tr>
<td>351</td>
<td>8</td>
<td>0</td>
<td>Cortex</td>
<td>+</td>
<td>(+)</td>
</tr>
<tr>
<td>423</td>
<td>2</td>
<td>0</td>
<td>Thalamus</td>
<td>0</td>
<td>(+)</td>
</tr>
<tr>
<td>436</td>
<td>4</td>
<td>1:20</td>
<td>Cortex</td>
<td>+</td>
<td>+/0</td>
</tr>
</tbody>
</table>

* NM, neutered male; NF, neutered female; F, female.
† Antigens by ELISA: 0, negative; +/0, questionably positive; (+) weakly positive; +, positive.
NA, Sample not available.

40 kDa proteins of BDV, as well as with polyclonal anti-BDV antibodies (Ludwig et al., 1993). Finally, CNS sections from cat 436 were pre-treated in an autoclave (Bankfalvi et al., 1994) prior to immunostaining with the same panel of antibodies.

(b) Rats. For the demonstration of BDV antigen in rat brains, the MAb Kfu1 (Ludwig et al., 1993), recognizing the 24 kDa protein, was used on paraffin sections, diluted 1:1000. As detection system, the alkaline phosphatase–anti-alkaline phosphatase (APAAP) technique (Cordell et al., 1984) was used, with a rabbit anti-mouse antibody (Dako) as the secondary antibody and New Fuchsin as the chromogen. For identification of monocytes/macrophages/microglial cells, the MAB ED1 (Serotec) was used, diluted 1:500, with the APAAP method. Astrocytes were identified using a polyclonal rabbit antibody against cow gliarial acidic protein (GFAP; Dako), diluted 1:500, with the ABC method (Hsu et al., 1981). For details of the procedures, see Petrov (1993).

ELISA for detection of *BDV*-specific antigens. In addition to immunohistochemistry, detection of BDV antigens in cats with SD was performed with an ELISA described previously (Bode et al., 1990). Briefly, 10% brain suspensions were prepared in PBS and aliquots were frozen at −20 °C until use. The assay system followed the double sandwich ELISA principle by binding of an equal mixture of monoclonal BDV-specific catching antibodies (Kfu2 and W1), recognizing the 24 kDa and 40 kDa proteins respectively (Ludwig et al., 1993), to a 96-well microtitre plate format 12 × 8 (Immuno Modules, Maxi Sorp F8, Nunc) with wells pre-coated with goat anti-mouse IgG (Dianova). The brain suspensions were added to the wells at serial twofold dilutions, starting with 1:4, and were assayed after reaction with polyclonal rabbit anti-BDV serum and alkaline phosphatase-conjugated goat anti-rabbit IgG (Dianova). The brain suspensions were added to the wells at serial twofold dilutions, starting with 1:4, and were assayed after reaction with polyclonal rabbit anti-BDV serum and alkaline phosphatase-conjugated goat anti-rabbit IgG (Dianova). Each incubation step was followed by a washing step (six times with 0.9% NaCl+0.05% Tween 20), using a manual ELISA washing unit (Behring). After substrate reaction with p-nitrophenyl phosphate tablets (Sigma) and 3 M-NaOH to stop the reaction, the plates were read in a microplate ELISA reader (Bio-Rad) at 450 nm.

RNA extraction. RNA was extracted from several CNS samples from the 11 diseased cats and three healthy animals which served as negative controls. RNA extraction was done according to Chomczynski & Sacchi (1987) with some modifications, as described previously (Zimmermann et al., 1994a).
Fig. 1. Histopathological and immunohistochemical findings in Wistar rats infected with feline BDV. (a) Hippocampus of a normal, uninfected newborn rat: HE, × 55 (bar, 200 μm). (b) Degeneration of the hippocampal dentate gyrus in a newborn rat inoculated with brain homogenate from a cat with SD: HE, × 55. (c) Hippocampus of the same rat as in (a). Immunohistochemical staining with a polyclonal antibody against GFAP, ABC technique, × 55. Astrocytes are not readily demonstrable; cf. (d). (d) Astrocytosis of the hippocampus in the same rat as in (b), stained with a polyclonal antibody against GFAP, ABC technique, × 55.

Selection of primers, probe and reagents. The oligonucleotides were designed according to published sequences from the cDNA clone pAB5 encoding the 40 kDa protein (Lipkin et al., 1990) using the computer program OLIGO, version 4.0. The primer sequences have been described previously (Zimmermann et al., 1994a). The oligonucleotides were also used to selectively prime either the viral mRNA or the genome in the reverse transcriptase reaction.

For Southern hybridization, an 800 bp EcoRV insert of the plasmid pAB5 (Lipkin et al., 1990) was used as the probe. The Moloney murine leukaemia virus (M-MLV) reverse transcriptase and the corresponding buffer system were obtained from Life Technologies. All PCR reagents including nucleotides, 10 x reaction buffer and Taq polymerase (Amplitaq) were obtained from Perkin-Elmer Cetus.

RT-PCR assay and nucleic acid hybridization. RT-PCR was carried out as described elsewhere (Zimmermann et al., 1994a). In brief, the RT reaction was performed in a final volume of 20 μl containing 2 μg of total RNA, 50 mM-Tris-HCl, pH 8.3, 40 mM-KCl, 6 mM-MgCl₂, 1 mM-DTT, 0.5 mM each dNTP, 100 pmol of the appropriate external primer, 1 unit RNasin, and 10 units of M-MLV reverse transcriptase at 37 °C for 90 min. PCR amplifications were carried out in 50 μl volumes containing 10 μl of the RT reaction. Reaction conditions consisted of 10 mM-Tris·HCl, pH 8.3, 50 mM-KCl, 1.5 mM-MgCl₂, 200 mM each dNTP, 1 mM of each primer, and 0.5 units of Taq polymerase. The samples were processed through 30 cycles of 2 min at 94 °C, 2 min at 54 °C, and 2.5 min at 72 °C, with a final extension of 10 min at 72 °C.

Reaction mixtures for nested PCR were made as above, although the samples were processed through 22 cycles only for the first PCR. Two microlitres of the first reaction were used for a second round of PCR with the nested primer pair for an additional 22 cycles.

One-fifth (10 μl) of each reaction volume was analysed in a 1% agarose gel in the presence of 2 mg/ml ethidium bromide. A further identification of the PCR products was made by nucleic acid hybridization. The insert of the plasmid pAB5 was labelled with [α-32P]dCTP (spec. act. 3000 Ci/mmol; Amersham) using a random labelling primers kit (Life Technologies). Southern blotting and hybridization were carried out using standard methods as described elsewhere (Sambrook et al., 1989).

The quality of each RNA sample was controlled by amplification of β-actin RNA (Zimmermann et al., 1994a).

Results

Histopathology and serum antibodies of cats

Histopathological examination revealed a non-suppurative meningoencephalomyelitis, in character and localization pattern consistent with SD lesions, as
described previously (Lundgren, 1992; Lundgren et al., 1995). Lesions were especially pronounced in the brain stem and in the limbic system. In two cats (167 and 436), serum antibodies against BDV could be detected, with a titre of 1:1280 and 1:20, respectively (Table 1). The positive serum samples recognized nuclear antigen in BDV-infected YRB cells. The simultaneous use of the MAb Kfu3 gave identical antigen patterns, with dominant nuclear and additional faint cytoplasmic staining.

Tissue cultures and animal experiments

The embryonic mink brain (ENG) cell line was the only one which supported virus growth, and only with material from one cat (spinal cord from cat 249). In the third passage on ENG cells, virus propagation could be demonstrated by IFT with all the sera used and with Kfu3. However, the virus disappeared in the next passage of the cells. This result was reproduced in four independent experiments. In the seven newborn rats inoculated with the third passage of ENG cells infected with spinal cord from cat 249 or thalamus/hippocampus from cat 154, neither infectious virus nor BDV-specific antibodies could be demonstrated. Histopathological examination revealed slight astrocitosis and prominent cytopathic changes in the form of chromatolysis of neurons in all seven rats. In one case, degeneration of the dentate gyrus of the hippocampal formation was observed. BDV antigen was not detectable by immunohistochemistry.

Intracerebral inoculation of 35 newborn rats with samples from the CNS of diseased cats resulted in detectable virus growth in the rat brain with material from only one case (midbrain of cat 436). After 69 days, a titre of $6 \times 10^5$ f.f.u./ml on YRB cells, but no serum antibodies, could be detected in one of the rats inoculated with this particular cat sample. Brain material from this rat was resuspended and sonicated. Calibrated samples (100 f.f.u.) of the virus-containing tissue suspension could be neutralized to 0 f.f.u. with rabbit serum BP11 (dilution 1:500) and rat pool serum (dilution 1:50), whereas species-specific control sera and MAbs against BDV (Kfu2, W1) as well as herpes simplex virus had no neutralizing effect.

The same rat brain suspension was used for the infection of four adult rats. Within 30 days of infection, all four rats showed typical clinical signs of BD. In the brain tissue of the adult rats, BDV titres between $10^5$ and $10^6$ f.f.u./ml ($\leq 2 \times 10^8$ f.f.u./ml) were demonstrated, simultaneously with an antibody titre of 1:80. Histopathological examination of one animal showed a moderate non-suppurative encephalitis with astrocytosis. BDV-specific antigen could be demonstrated within neurons by immunohistochemistry.

Neither infectious virus nor antibodies against BDV could be detected in the remainder of the newborn rats directly inoculated with feline CNS samples. Histopathological examination of the brain, however, revealed cytopathic changes of neurons (chromatolysis) and slight to moderate astrocitosis in 17 out of 18 rats examined. In seven rats, there was a lytic degeneration of the dentate gyrus of the hippocampal formation, accompanied by a moderate reactive astrocitosis (Fig. 1 a–d). No BDV-specific antigen could be detected by immunohistochemistry in any of the rats examined.

In contrast, histopathological examination of newborn rats inoculated with rat brain suspensions infected with cat material revealed only slight cytopathic changes and astrocitosis, but strong expression of BDV-specific antigen within neurons (Fig. 2), in 10 out of 15 rats examined. A slight microgliosis, as detected with the ED1 antibody, was also present. Two rats which survived for 7 months showed a slight to severe degeneration of the hippocampal dentate gyrus as well as intranuclear Joest–Degen inclusion bodies in large neurons. Direct infection of eight adult rats with different samples from the CNS of diseased cats did not result in clinical disease during a period of 5 months. Neither infectious virus nor antibodies against BDV could be demonstrated in these rats. Histopathological evaluation was not performed.

Finally, a litter of two newborn rats infected with spinal cord material from cat 249 was eaten by its mother 3 days after inoculation. This mother rat died 36 days later with typical signs of BD. A virus titre of $10^5$ f.f.u./ml was found in the brain material of the mother rat. Tests for antibodies could not be performed.
Fig. 3. Detection of viral mRNA in CNS samples from three cats with SD. (a) RT-PCR and nested PCR with primers specific for the p40 gene of BDV, resulting in the amplification of a 750 bp and a 444 bp fragment, respectively. (b) Southern blot of the gel in (a) hybridized with pAB5. Lanes: 1-5, cat 436, different brain regions (1/cortex; 2/mesencephalon; 3/cerebellum; 4/thalamus; 5/spinal cord); 6-9, cat 423, different brain regions (6/cortex; 7/mesencephalon; 8/thalamus; 9/spinal cord); 10, cat 249, cerebral cortex; 11, cat 249, spinal cord; 12, healthy cat brain (negative control); 13, horse brain infected with BDV; 14, rat brain infected with BDV; 15, buffer control; 16, molecular DNA marker, 123 bp ladder.

Detection of BDV antigen and nucleic acid

In two of the six cats examined immunohistochemically (249 and 436), the BDV-specific antibody Kfu2 stained a few cells in the neural parenchyma. These cells were rather small, rounded or bean-shaped and bore a resemblance to glial cells. In addition, mononuclear cells consistent with macrophages were stained within or close to the Virchow–Robin space. Despite efforts to demask antigen by microwave or autoclave treatment, it was not possible to demonstrate BDV-specific antigen in neurons with any of the BDV-antibodies used.

Using the ELISA, BDV-specific antigen could be demonstrated in different, but not all, CNS areas in samples available from eight out of eleven cats (Table 1). Further experiments (four cats with SD, two samples each), using fourfold higher concentrated brain suspensions (20%, diluted from 1:2), revealed the presence of BDV-specific antigen in each brain area (data not shown). However, as compared to horses with BD, the amount of antigen in cats was considerably lower.

Nine out of the eleven cats with SD were positive for viral RNA (genomic and mRNA), whereas in the healthy control cats no specific amplification products were obtained (Table 1). Clear differences in the amount of amplified sequences could be shown in the tissue samples of the positive cats (Fig. 3a). Whereas in the cerebral cortex and cerebellum of cat 436 and in the thalamus of cat 423 a 750 bp band was visible (Fig. 3a), no specific amplification products were detectable in other samples after the first round of PCR. For comparison, the amplification products using RNA preparations from brains of a horse with BD and an experimentally infected adult rat are shown (Fig. 3a). In order to exclude degradation of RNA samples, the amplification of a 248 bp β-actin mRNA served as an internal control (data not shown). A second round of PCR using the nested primer pair revealed the amplification of the expected 444 bp BDV-specific band in several cat samples which had been negative in the first round. The specificity of the PCR was examined by Southern blotting and hybridization of the amplified DNA to the 32P-labelled 800 bp insert of the plasmid pAB5. As shown in Fig. 3(b) and Table 1, this step increased the sensitivity of the technique, enabling the detection of viral RNA in a majority of tissue samples from the diseased cats.

Discussion

The aim of this study was to isolate the agent most probably involved in the aetiology of a feline neurological disorder ("staggering disease"/SD). Clinical signs and histopathology as well as the finding of BDV-specific antibodies in cats with SD indicated that BDV or a related agent was associated with this disease (Lundgren
The seasonal incidence as well as the confinement to certain areas in Sweden (Lundgren & Ludwig, 1993) and Austria (Weissenböck et al., 1994), where the disease seemed to be endemic, supported this assumption.

The low frequency of antibody-positive cats conforms with the characteristic antibody prevalence in different animal species naturally infected with BDV (Ludwig et al., 1988), and so far there is no indication of a virus variant in the cats. On the other hand, the biological properties of the feline isolate described here clearly show differences from the rabbit-adapted BDV strain V. The latter has a broad host spectrum in tissue culture and animal species after experimental infection (Ludwig et al., 1985, 1988, 1993), whereas the feline isolate propagated only on ENG cells.

Infectious virus could be demonstrated after three passages in ENG cells, but the infectivity was lost in the next passage. Technical problems as a cause of this phenomenon can be excluded, since identical results were achieved in four independent experiments. These results might be reminiscent of efforts to propagate a possible agent from a human patient on rabbit cells, which similarly got lost (Rott et al., 1991). This is quite different from our experience that brain material from horses and sheep with BD (Gosztonyi & Ludwig, 1984; Ludwig et al., 1993) establishes a persistent infection at passage one in rabbit brain cells.

In our experience, newborn rats are highly sensitive to BDV infection. Despite this, the feline virus propagated only in one intracerebrally inoculated rat. Several other rats (without infectivity) displayed cytopathic changes of neurons, astrocytosis and (in seven cases) degeneration of the hippocampal dentate gyrus, typical of BDV infection (Ludwig et al., 1988). However, in this first passage no antigen was detectable by standard techniques. Only from the next passage on were BDV antigens recognized, possibly as signs of better adaptation. Five rats neither expressed BDV antigens nor showed cytopathic changes. However, their survival time was only 2 weeks, which might be too short for detectable pathological changes to develop. A further sign of better adaptation in the second passage in rat brains is the reduction of cytopathic changes as compared to the first passage. It is intriguing that the feline virus, during the first steps of adaptation, causes neuronal degeneration without production of standard BDV antigens. As yet, we have no explanation for this phenomenon.

The accidental infection of a mother rat which consumed its progeny, infected with feline material, points to another interesting feature of BDV pathogenesis since it indicates that oral infection by the first passage of the feline virus is also possible.

In all diseased cats examined by the ELISA, BDV-specific antigen could be detected in at least one tissue sample from the CNS. This contradiction of the immunohistochemical data might be explained by destruction of epitopes due to formalin fixation and other handling procedures. Clearly, the amount of BDV antigen detected with our techniques is significantly smaller in naturally infected cats than in other natural hosts, like horses. Essentially the same observation has been made in naturally infected cattle (Bode et al., 1994b).

Important data from this study, verifying the existence of a BDV-like agent in cats, are the detection of BDV RNA in nine out of eleven cats with SD. The amount of BDV RNA may vary markedly between different brain areas and may show preferences for one of the hemispheres, as seen in horses (Zimmermann et al., 1994a). The reduced amount of amplified sequences in several cat tissue samples could reflect the low number of BDV RNA molecules present as compared with the situation in horses. A possible reduced primer binding in the RT–PCR reaction based on changes in the sequence should result in a more dramatic reduction of amplified products. This can be excluded for the part of the cat virus genome used in our experiments.

Some of the brain samples were antigen-positive but PCR-negative. Apart from regional differences in the tissue samples, a possible explanation for this discrepancy may be that while the ELISA detects both the 24 kDa and the 40 kDa proteins, the primers used in our study amplify only the p40 gene of BDV. A similar phenomenon was recently observed in antigen-positive blood samples from psychiatric patients, where amplification products could be obtained with p24 primers but not with p40 primers (Bode et al., 1995).

The results of this study show that a feline virus, in some biological features similar to known BDV strains, exists and is associated with feline SD. Difficulties in propagation of the agent might be due to problems of adaptation. The analysis of the cat BDV genome is in progress. Sequence information on the complete genome of reference strains and of distinct genes of wild-type BDV (Briese et al., 1994; Cubbit et al., 1994; Schneider et al., 1994) should allow a detailed comparison between the different strains.

This first isolation of the feline wild-type BDV and the recent discovery of ‘footprints’ of a human BDV expressed in psychiatric patients (Bode et al., 1995) might raise further questions on the epidemiology of this neurotropic RNA virus.

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References


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