

## REGULAR PAPER

A.-L. Lundgren · R. Lindberg · H. Ludwig · G. Gosztonyi

## Immunoreactivity of the central nervous system in cats with a Borna disease-like meningoencephalomyelitis (staggering disease)

Received: 28 November 1994 / Revised: 13 January 1995 / Revised, accepted: 1 March 1995

**Abstract** The inflammatory cell composition and the expression of major histocompatibility complex (MHC) antigens in the central nervous system (CNS) of 13 cats with a spontaneous, Borna disease-like meningoencephalomyelitis (staggering disease) was investigated by immunohistochemistry with a panel of monoclonal and polyclonal antibodies. T lymphocytes were the predominating inflammatory cells within the adventitial space. CD4<sup>+</sup> T cells were more abundant than CD8<sup>+</sup> T cells. Scattered IgG<sup>-</sup>, IgA<sup>-</sup> and IgM<sup>-</sup>-containing cells were found in the adventitial space and surrounding neuropil, often adjacent to neurons. There was a markedly increased MHC class II expression in cells morphologically resembling microglia. In several cats, Borna disease virus specific antigen was detected, but only in a few cells, mainly of macrophage character. Our findings indicate a long-standing inflammatory reaction in the CNS of cats with staggering disease, possibly triggered and sustained by a persistent viral infection.

**Key words** Borna disease virus · Cat diseases · Encephalomyelitis · Lymphocyte subsets

### Introduction

In domestic cats and large Felidae, non-suppurative meningoencephalomyelitis of unknown origin has been reported from different parts of the world [14, 20, 42, 43]. Viral infection is the presumed cause of this disease complex, although several attempts to isolate a virus have failed [42]. In Sweden, a feline neurological disorder commonly referred to as “staggering disease” has been observed since the early 1970s [24]. Clinical manifestations include motor disturbances and behavioral changes. The underlying lesion is a non-suppurative meningoencephalomyelitis, with predilection for the brain stem [28]. Although the histopathology as well as the clinical and laboratory findings strongly suggest a viral infection, evidence indicates that none of the known feline viruses capable of causing lesions in the central nervous system (CNS) of cats are involved [28]. Recently, a feline neurological disorder apparently identical to staggering disease was reported in Austria [44]. Despite extensive virological investigations, no virus could be isolated from the diseased cats.

Borna disease virus (BDV) is a highly neurotropic, negative s-stranded RNA virus of approximately 8900 bases [6, 8], which causes encephalomyelitis in horses, sheep, ostriches and cattle [4, 26, 30]. The findings of BDV-specific antibodies and antigen in human psychiatric patients indicate that BDV may be involved in the etiology of some mental disorders in man [3, 5, 36]. Recently it has been suggested that BDV may also be the cause of feline staggering disease, since more than 40% of cats with this disease in one study had serum antibodies to BDV [29]. The finding of BDV-specific antibodies prompted further investigations, which led to the detection and partial characterization of a feline BDV isolate from cats affected with staggering disease [9]. However, the importance of this newly isolated cat virus for the etiopathogenesis of the disease remains to be clarified.

A striking morphological feature of staggering disease is the presence of broad adventitial cuffs (ACs), containing tightly packed lymphocytes, monocytes, and plasma

A.-L. Lundgren (✉) · R. Lindberg  
Department of Pathology, Box 7028,  
Faculty of Veterinary Medicine,  
Swedish University of Agricultural Sciences,  
S-75007 Uppsala, Sweden  
Fax: 46-18-67-35-32

H. Ludwig  
Institute of Virology, Free University of Berlin, Nordufer 20,  
D-13353 Berlin, Germany

G. Gosztonyi  
Institute of Neuropathology, Free University of Berlin,  
Hindenburgdamm 30, D-12200 Berlin, Germany

cells [28]. Since this indicates a marked and long-standing immune reaction in the neural tissue, we decided to investigate the immune phenomena in the CNS of cats with this disease. The humoral and cell-mediated immune responses and the presence of BDV antigen in neural tissues were examined, using immunohistochemical methods. The results indicated that the adventitial inflammatory infiltrates of this meningoencephalomyelitis are T cell-dominated immune reactions, associated with marked immune activation of macrophages/microglial cells in the neural parenchyma. The present study also provided evidence suggesting that the immune response is triggered by a chronic infection. Furthermore, our results give credence to the previous notion that BDV or a BDV-like agent is involved in staggering disease.

## Materials and methods

### Animals

Thirteen cats with a clinical diagnosis of staggering disease were chosen for the study. These cats were referred for euthanasia and necropsy from the University Clinics, Faculty of Veterinary Medicine, Uppsala, or from private veterinary practitioners in Sweden, during 1991–1993. All cats were received alive by one of the authors (A.-L.L.) for re-assessment of the clinical diagnosis. The cats were between 1.5 and 7 years old (mean age 4 years) at the time of death. Eleven were domestic shorthairs or longhairs and

one an Abyssinian. Seven were neutered males and six were females. Major signs of the disease were hindleg incoordination, fluctuating fever, and mental changes. The duration of the disease varied between 2 weeks and 1.5 years (mean 3 months). To assess the effect of clinical duration upon the immunoreactivity, the cats were allocated to one of two groups: early disease (1–8 weeks, mean 4 weeks,  $n = 11$ ) and late disease (10 and 18 months respectively,  $n = 2$ ). Serum samples from all cats were negative for antibodies to feline coronavirus using an enzyme-linked immunosorbent assay (ELISA) (Svanova Biotech, Uppsala, Sweden), and also negative for antibodies to feline immunodeficiency virus and the presence of feline leukemia virus antigen (Cite-Combo-Test, Idexx Corp., Portland, Me.). Serum was also analyzed for BDV antibodies by indirect immunofluorescence as previously described [3]. Four cats (31%) had BDV-specific antibodies, with titers ranging between 1:20 and 1:80. All cats were killed by a pentobarbital overdose and then necropsied immediately. Nine cats 1–5 years old were used as controls for the immunohistochemical studies. Five of these, including two that were clinically healthy and devoid of lesions at necropsy, and three with lesions unrelated to the nervous system (pulmonary edema, traumatic injury, pyothorax), were killed and examined post mortem in the same way as the staggering disease cases. The remaining four cats were barrier-bred, clinically healthy animals obtained from Iffa-Credo, l'Arbresle, France. Heparinized blood from these cats was analyzed by flow cytometry to assess the reactivity to peripheral blood cells of the monoclonal antibodies used in the study.

### Tissue processing

The brain and spinal cord were removed intact from all cats at necropsy. In the cases of staggering disease, the brain was divided

**Table 1** Proportions of cell populations in peripheral blood lymphocytes (PBL), in various lymph node compartments and in brains from control cats reactive with monoclonal antibodies used to detect leukocyte and histocompatibility antigens (ND not done,

NP not present, BDV Borna disease virus, *mono* monocyte, *macro* macrophage, *gran* granulocytes, *MHC* major histocompatibility complex, + occasional cells, ++ scattered cells, +++ frequent occurrence of cells, ++++ predominant occurrence of cells)

Antibody <sup>a</sup>	Dilution used <sup>b</sup>	Source	Reference	PBL <sup>c</sup>	Lymph node				Brain
					Lymphoid follicles	Paracortex	Medullary cords	Medullary sinus	
CF54A (CD3-like)	1:800 (1:50)	VMRD, Pullman, Wa., USA		57.2%	++	+++ /++++	++ /+++	+	ND
CAT30A (CD4)	1:50 (1:50)	VMRD	[41]	31.1%	++	+++	++	+	ND
FT2 (CD8)	1:50 (1:25)	Southern Biotechnology, Birmingham, Ala., USA	[23]	14.6%	NP/+	+++	++	NP	ND
F46A (CD21-like)	1:800 (1:50)	VMRD		25.4%	+++ /++++	+ /++	++	+	ND
DH59B (mono/macro/gran)	1:100	VMRD		ND	+ /++	++	+++	+	ND
H58A (MHC I)	1:100	VMRD		ND	++ /+++	++	+++	+	Diffuse staining of vessel walls
CAT82A (MHC II)	1:200	VMRD		ND	+++ /++++	++ /+++	++ /+++	+	Endothelial cells
Kfu2 (25-kDa antigen of BDV)	1:10	H. Ludwig, Institute of Virology, FU, Berlin	[27]	ND	NP	NP	NP	NP	NP

<sup>a</sup> Raised against antigen specified within parentheses

<sup>b</sup> Dilution used for PBL/flow cytometry stated within parentheses

<sup>c</sup> Percentage given as mean values of four control cats (samples taken on nine different occasions)

into two halves by a longitudinal incision through the corpus callosum and the brain stem. Small pieces were cut from one of the brain halves and snap frozen in liquid nitrogen for virological investigations. The other half was cut into transverse sections, whereafter small pieces (approximately 5 × 3 mm) of the cerebral cortex, thalamus, mesencephalon and spinal cord were snap frozen in liquid nitrogen for immunohistochemistry. Frozen material was stored at -70°C prior to examination. Specimens from the above-mentioned brain regions and also from the basal ganglia, hippocampus, caudal colliculus, cerebellum, various segments of the spinal cord, and internal organs were fixed in buffered 10% formalin, embedded in paraffin, and cut into 4-µm-thick sections which were stained with hematoxylin and eosin (H&E) for light microscopy. Selected sections were stained according to the Gallyas impregnation technique [15] for demonstration of microglial cells. Some paraffin sections were also used for immunohistochemistry. From control cats, pieces of the brain, spleen, and retropharyngeal lymph nodes were snap frozen in liquid nitrogen and stored at -70°C for immunohistochemistry. Specimens were also taken from the brain, spinal cord, and various internal organs for light microscopy.

#### Immunohistochemical staining of tissues

##### *Leukocyte antigens*

The primary antibodies used were murine monoclonal antibodies and these are listed and specified in Table 1. An irrelevant mouse monoclonal IgG1 antibody (X931, Dakopatts, Glostrup, Denmark) served as a negative control substitute for primary antibody. All antibodies were applied on frozen sections of lymph nodes and spleen from control cats and appropriate dilutions were established. In addition, antibodies reactive with feline major histocompatibility complex (MHC) class I and II antigens were applied on frozen sections of brain tissue from a control cat. The reactivity of monoclonal antibodies with feline peripheral blood lymphocytes was analyzed by flow cytometry, using a FACStar PLUS (Becton Dickinson Immunocytometry Systems, San José, Calif.) with standard optical equipment. From each cat with staggering disease, except one in which only the cerebral cortex was available, two blocks from different parts of the brain and one from the spinal cord were chosen for immunohistochemical staining (Table 2). From two of the control cats, the cerebral cortex, spleen, and retropharyngeal lymph nodes were investigated.

After embedment in O.C.T. compound (Miles, Elkhart, Ind.), serial 4-µm cryostat sections were cut onto chromium-gelatin-coated glass slides, air dried for 12 h and stored at -70°C. Prior to staining, sections were further air dried for 1 h and fixed in acetone for 10 min. Nonspecific protein binding was minimized by incubation for 30 min with 5% normal rabbit serum in TRIS-buffered saline, pH 7.6 (TBS). For the monoclonal antibodies CAT82A and DH59B, 10% and 20% normal rabbit sera were used, respectively, for this procedure. The alkaline phosphatase-anti-alkaline phosphatase (APAAP) method [7] was used for immunostaining, with a rabbit anti-mouse antibody (Dakopatts) as the secondary antibody, Fast Red as the chromogen, and hematoxylin as the counterstain.

##### *Immunoglobulins*

The primary antibodies were goat polyclonal antibodies reactive with Fc fragments of feline IgG (Jackson ImmunoResearch, West Grove, Pa.), feline IgM (Kirkegaard & Perry, Gaithersburg, Md.), and feline IgA (Nordic Immunological Laboratories, Capistrano Beach, Calif.). All polyclonal antibodies to feline immunoglobulins were tested on sections from the spleen and small intestine, and optimal dilutions were established according to the intensity and selectivity of the staining. Normal goat serum was used as a negative control substitute for primary antibody.

Formalin-fixed, paraffin-embedded sections from the brain and spinal cord of cats with staggering disease were stained with H&E

and evaluated by light microscopy for the presence of plasma cells. If plasma cells were observed, further sections of the tissue were immunostained for immunoglobulins (Table 3). Sections were deparaffinized and endogenous peroxidase activity was quenched by incubation in 3% H<sub>2</sub>O<sub>2</sub> for 5 min. Nonspecific protein binding was minimized by incubation for 30 min with 20% normal rabbit serum in TBS. The peroxidase-antiperoxidase (PAP) method [38] was used with a rabbit anti-goat antibody (Dakopatts) as the secondary antibody, 3-amino-9-ethylcarbazole (AEC) as the chromogen, and hematoxylin as the counterstain.

##### *BDV antigen*

For detection of BDV, the murine monoclonal antibody Kfu2 [27], specific for the 25-kDa antigen of BDV, was used on paraffin sections of the CNS from all the diseased cats; it was diluted 1:10 in 0.05 M TRIS and replaced by TRIS or an irrelevant mouse monoclonal antibody (X931, Dakopatts) as a negative control. The tissue areas examined were essentially the same as those used for staining of immunoglobulins (Table 3). Sections were deparaffinized and then treated with 0.1% proteinase K (Boehringer Mannheim, Germany) in 0.05 M TRIS for 10 min at 37°C. The avidin-biotin immunoperoxidase (ABC) method [21] was used with a biotinylated horse anti-mouse antibody (Vector laboratories, Burlingame, Calif.) as the secondary antibody, AEC as the chromogen, and hematoxylin as the counterstain. Nonspecific protein binding was minimized by incubation for 20 min in normal horse serum diluted 1:50 in TRIS. Endogenous peroxidase activity was quenched with 0.3% H<sub>2</sub>O<sub>2</sub> for 20 min. This was done after application of the anti-BDV antibody and the biotinylated secondary antibody to minimize undue influence on antigen. Brain sections from a horse with confirmed Borna disease were used as positive controls. Brain sections from normal control cats were also examined for comparison.

In addition, two different BDV-specific hyperimmune rabbit sera (LL2 and BP11) [27], were applied on selected CNS sections from some of the diseased cats. The protocol used for immunostaining was similar to the one used for Kfu2, with the following modifications: BDV-specific rabbit serum was diluted 1:100, a biotinylated goat anti-rabbit antibody (Vector laboratories) served as secondary antibody and normal goat serum, diluted 1:5, was used to minimize nonspecific protein binding.

#### Interpretation

##### *Leukocyte antigens*

For each antibody used, 2 to 12 adventitial cuffs (ACs) in every CNS section were examined for immunoreactivity, and the occurrence of positive cells in each AC was scored from not present (NP) to +++++, where + = occasional, ++ = scattered, +++ = frequent and +++++ = predominant. The presence of antigen-expressing cells in the neural parenchyma was graded in a similar fashion. Whenever possible, individual ACs were followed in serial sections and examined for reactivity to every antibody used. For each antibody, stained ACs were divided into two groups based on the occurrence of positive cells (+ to ++, and +++ to +++++), and the frequency of each group was calculated (Table 2).

##### *Immunoglobulins*

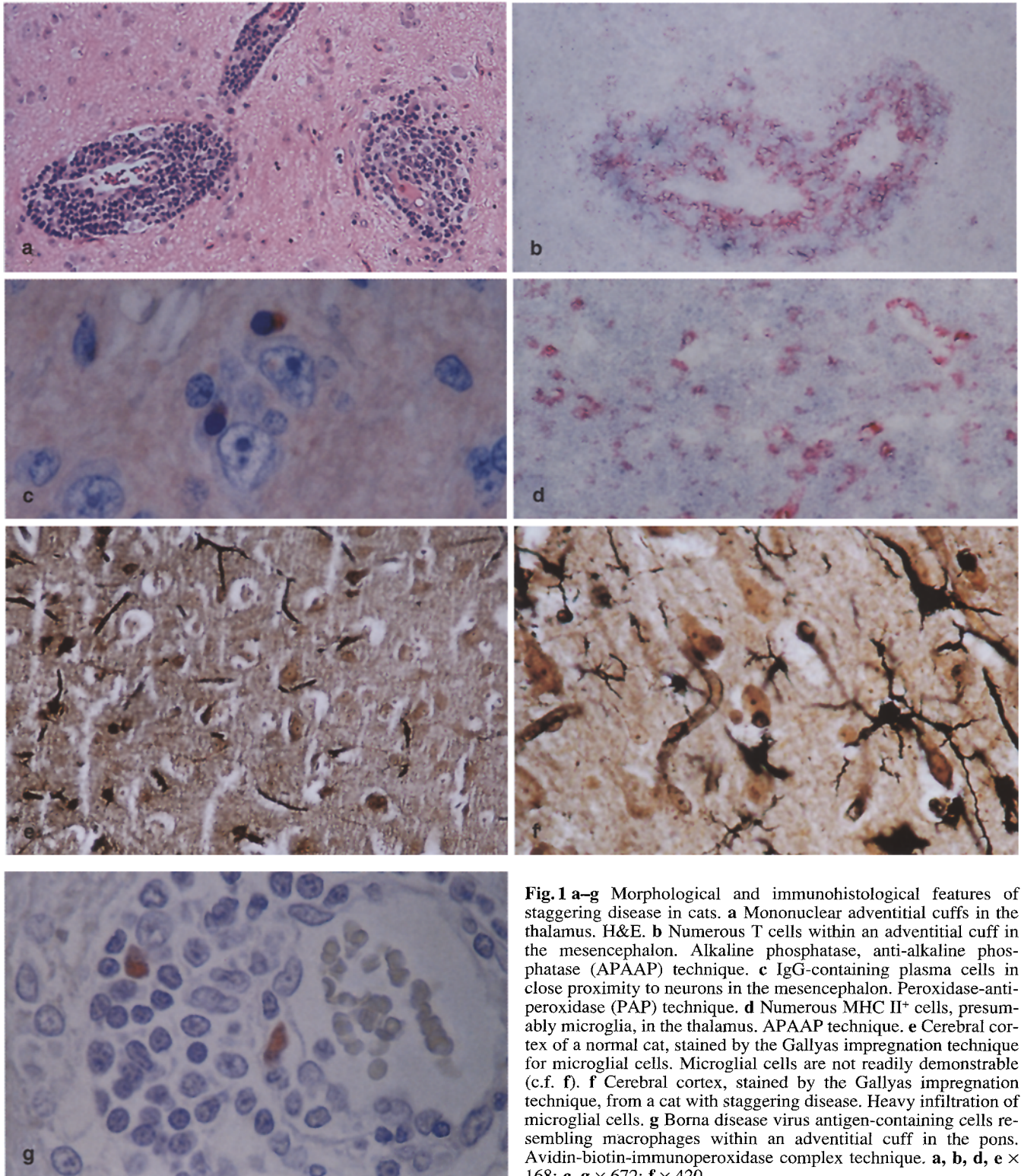
In each section, 2–30 ACs were examined for immunoreactivity to each antibody applied. The presence of specific cells in ACs and neural parenchyma was graded as described above. The frequency of positively stained ACs divided into two groups (+ to ++, and +++ to +++++) was calculated for each antibody (Table 3).

## Results

### Histopathology

Throughout the brain and spinal cord, but with predilection for the basal ganglia, thalamus and mesencephalon, a marked inflammatory reaction with mononuclear adventi-

tial cuffing, neuronophagia and scattered microglial nodules was present in every cat with staggering disease (Fig. 1 a). Axonal degeneration and fragmentation were commonly observed in the crura cerebri and in the ventrolateral regions of the spinal cord. The lesions were consistent with those previously described in this disease [28]. In the two long-standing cases, the inflammatory reaction



**Fig. 1 a–g** Morphological and immunohistological features of staggering disease in cats. **a** Mononuclear adventitial cuffs in the thalamus. H&E. **b** Numerous T cells within an adventitial cuff in the mesencephalon. Alkaline phosphatase, anti-alkaline phosphatase (APAAP) technique. **c** IgG-containing plasma cells in close proximity to neurons in the mesencephalon. Peroxidase-anti-peroxidase (PAP) technique. **d** Numerous MHC II<sup>+</sup> cells, presumably microglia, in the thalamus. APAAP technique. **e** Cerebral cortex of a normal cat, stained by the Gallyas impregnation technique for microglial cells. Microglial cells are not readily demonstrable (c.f. **f**). **f** Cerebral cortex, stained by the Gallyas impregnation technique, from a cat with staggering disease. Heavy infiltration of microglial cells. **g** Borna disease virus antigen-containing cells resembling macrophages within an adventitial cuff in the pons. Avidin-biotin-immunoperoxidase complex technique. **a, b, d, e**  $\times 420$ ; **c, g**  $\times 672$ ; **f**  $\times 420$



**Table 2** Percentage of adventitial cuffs (ACs) with positive staining by monoclonal antibodies against leukocyte antigens (APAAP method). (+/++ occasional to scattered cells, +++/++++ frequent to predominant occurrence of cells)

Case no.	Duration of clinical signs	Areas of CNS examined	Total no. of ACs examined	F46A (CD21-like)		CF54A (CD3-like)		CAT30A (CD4)		FT2 (CD8)		DH59B (Mono/macro)	
				+/++	+++/++++	+/++	+++/++++	+/++	+++/++++	+/++	+++/++++	+/++	+++/++++
1	4 weeks	Cerebral cortex	65	61.5	0	100	0	100	0	57.1	0	33.3	0
2	10 months	Cerebral cortex Spinal cord	22	100	0	23.0	77.0	85.7	14.3	37.5	0	42.9	0
3	4-5 weeks	Cerebral cortex Spinal cord	26	88.9	0	36.4	63.6	80.0	0	72.7	0	33.3	0
4	2 weeks	Cerebral cortex Thalamus Spinal cord	57	90.5	0	27.8	72.2	73.7	0	40.9	0	27.3	0
5	6 weeks	Mesencephalon Thalamus Spinal cord	45	50.0	50.0	42.1	57.9	86.6	6.7	61.5	0	84.2	0
6	3 weeks	Mesencephalon Thalamus Spinal cord	47	63.6	0	31.6	68.4	86.7	0	72.2	0	60.0	0
7	3 weeks	Mesencephalon Thalamus Spinal cord	46	19.0	81.0	29.4	70.6	79.0	0	88.4	7.7	75.0	0
8	18 months	Mesencephalon Thalamus Spinal cord	24	83.3	16.7	33.3	66.7	83.3	16.7	0	0	50.0	0
9	4 weeks	Mesencephalon Thalamus Spinal cord	27	42.8	0	23.1	69.2	91.7	0	50.0	0	66.7	0
10	2-3 weeks	Mesencephalon Thalamus Spinal cord	38	60.0	0	20.0	80.0	83.3	11.1	58.8	0	77.0	0
11	8 weeks	Mesencephalon Thalamus Spinal cord	25	57.2	28.6	17.7	82.3	70.0	30.0	12.5	0	85.7	0
12	5 weeks	Mesencephalon Thalamus Spinal cord	37	66.7	33.3	13.0	87.0	66.7	33.3	71.5	0	95.0	0
13	4 weeks	Mesencephalon Medulla oblongata Spinal cord	31	55.0	40.0	42.9	57.1	90.5	4.8	35.0	0	68.4	0
Mean ± SD				64.5 ± 22.0	19.2 ± 25.9	26.2 ± 12.0	73.2 ± 12.0	82.9 ± 9.1	9.0 ± 11.7	50.6 ± 25.1	0.6 ± 2.1	61.4 ± 22.3	0 ± 0

was substantially reduced. Occasional additional findings included lymphocytic chorioiditis and optic neuritis. There were no significant lesions outside the CNS in any of the cats with staggering disease.

## Cellular phenotypes

### *Adventitial infiltrates*

The majority of the inflammatory cells expressed pan-T cell antigen (CD3-like). On average, CD3<sup>+</sup> cells (Fig. 1 b) occurred frequently to predominantly in 73% of the ACs, while the corresponding figure for cells expressing B cell antigen (CD21-like) was 19% (Table 2). However, infiltrates with numerous B cells were just as common as those with numerous T cells in individual cases. Although the pan-T cell marker stained the majority of cells, in

most cases only occasional to scattered cells expressed CD4 or CD8 antigens. In three cases, CD4<sup>+</sup> and CD8<sup>+</sup> cells were present in similar amounts, while in the remaining ten cases CD4<sup>+</sup> cells were more abundant. Frequent to predominant occurrence of CD4<sup>+</sup> cells was found in a minority of the ACs (mean 9%), and the corresponding figure for CD8<sup>+</sup> cells was only 0.6%. This paucity of CD8<sup>+</sup> cells led us to apply another monoclonal antibody, OKT8 (VMRD, Pullman, Wash.), reactive with human CD8, but the results were similar. The antibody DH59B, which detects a molecule expressed on monocytes and macrophages, but presumably not on microglial cells (W Davis, personal communication), was reactive with occasional to scattered cells in many ACs (mean 61% of the cuffs). In no case did DH59B<sup>+</sup> cells constitute the majority in any adventitial infiltrate.

Occasional to scattered immunoglobulin-bearing cells were found within ACs in all cases (Table 3). By their

**Table 3** Percentage of adventitial cuffs (ACs) with positive staining by polyclonal antibodies against feline immunoglobulins (PAP method). (+/++ occasional to scattered cells, +++/++++ frequent to predominant occurrence of cells, *ND* not done, *obl.* oblongata)

Case no.	Duration of clinical signs	Areas of CNS examined	Total no. of ACs examined	IgM		IgG		IgA	
				+/++	+++/++++	+/++	+++/++++	+/++	+++/++++
1	4 weeks	Thalamus Obex Spinal cord	30	50.0	0	90.0	0	61.1	0
2	10 months	Mesencephalon Obex Spinal cord	27	59.3	0	77.3	4.5	75.0	0
3	4-5 weeks	Cerebral cortex Mesencephalon Spinal cord	26	42.3	3.9	75.0	0	65.2	0
4	2 weeks	Cerebral cortex Cerebellum	17	29.4	0	61.1	0	56.3	0
5	6 weeks	Cerebral cortex Thalamus Obex	28	46.4	0	78.6	0	53.6	0
6	3 weeks	Cerebral cortex Mesencephalon Spinal cord	25	48.0	0	76.0	0	48.0	0
7	3 weeks	Cerebral cortex Cerebellum Spinal cord	30	60.0	0	72.0	0	67.9	0
8	18 months	Thalamus Spinal cord	6	33.3	0	100	0	50.0	0
9	4 weeks	Cerebral cortex Spinal cord	8	75.0	0	62.5	0	100	0
10	2-3 weeks	Cerebral cortex Basal ganglia	20	40.0	0	75.0	0	35.0	0
11	8 weeks	Cerebral cortex Basal ganglia	16	37.5	0	ND	ND	60.0	0
12	5 weeks	Cerebral cortex Medulla obl.	20	70.0	10.0	80.0	0	85.0	0
13	4 weeks	Cerebral cortex Medulla obl.	10	70.0	0	50.0	0	35.0	0
Mean ± SD				50.9 ± 14.8	1.1 ± 2.9	74.8 ± 13.1	0.4 ± 1.3	60.9 ± 18.4	0 ± 0

morphological appearance, most of them were plasma cells. Some, however, were consistent with macrophages or large lymphoid cells. IgG<sup>+</sup> cells predominated, although IgA<sup>-</sup> and IgM-bearing immunocytes also occurred, even in the two chronic cases. The immunoglobulin-bearing cells were mainly located in the periphery of the ACs, from where they seemed to be migrating into the surrounding neuropil.

The cell composition of the ACs was similar in different sections of the particular brain areas examined. A comparison between ACs from animals with a short (early) and those with a long (late) duration of clinical signs showed only slight differences, despite the fact that the signs had persisted for at least 10 months in the latter group. Cells expressing CD8 were notably sparse in late disease, whereas no significant difference in the percentage of CD3<sup>+</sup> or CD4<sup>+</sup> cells was observed between early and late disease.

The majority of ACs showed frequent to predominant occurrence of cells expressing MHC class I and of those expressing MHC class II antigen.

#### *Neural parenchyma*

In 11 of the 13 cats examined, scattered CD3<sup>+</sup> cells were found in the neural parenchyma in at least one CNS section. The majority of these cells were in regions close to ACs. CD4<sup>+</sup> cells were found in the neural parenchyma in 2 cases and CD8<sup>+</sup> cells in 2 other cases. Only in 1 case did the B cell marker (F46A) stain cells in the neural parenchyma. Immunoglobulin-bearing cells (mostly plasma cells and also cells compatible with macrophages or large lymphoid cells) were found in areas adjacent to adventitial infiltrates and also in other regions, often close to neurons (Fig. 1c). Some of these neurons were degenerate or dead, but the majority appeared intact. In most cases, intermingled IgM<sup>+</sup>, IgG<sup>+</sup> and IgA<sup>+</sup> cells were present. No DH59B<sup>+</sup> cells were detected in the parenchyma.

In 7 cases, MHC class II<sup>+</sup> cells occurred frequently in the neural parenchyma, while the remaining 6 cases all showed occasional to scattered class II<sup>+</sup> cells in at least one CNS section. To judge from the staining reactions in serial sections, some of these cells were undoubtedly lymphoid cells. However, a proportion of the remaining cells could not be classified. These cells were rather small and rounded, resembled microglia, and were mostly confined to the gray matter (Fig. 1d). Although class II<sup>+</sup> cell processes were sometimes seen surrounding neurons, no staining of the neuronal cell bodies was observed.

In 7 of the 13 examined cats, occasional to scattered MHC class I<sup>+</sup> cells were found in the neural parenchyma in some CNS regions. As in the case of the class II<sup>+</sup> cells, some class I<sup>+</sup> cells appeared to be consistent with lymphoid cells, while others resembled microglia. The extent of labelling with the anti-class I antibody was much less pronounced than that observed with the anti-class II antibody. No staining of neurons was detected. In sections impregnated by the Gallyas stain, numerous microglial cells

were observed in the gray matter of cats with staggering disease, indicating that the majority of MHC II<sup>+</sup> cells were probably identical with these. Microglial cells were much more abundant in cats with this disease than in control cats (Fig. 1e, f).

#### *Detection of BDV antigen*

In 7 cats, the BDV-specific antibody Kfu2 stained a few mononuclear cells resembling macrophages within or close to the Virchow-Robin space (Fig. 1g). Kfu2 also stained a small number of cells in the neural parenchyma. Some of these cells were small, rounded and lymphoid in appearance, while others were larger and bore resemblance to glial cells. In one case, faint staining of a neuron was also observed. No specific localization pattern could be observed: some of the positive cells were found in different parts of the cerebral cortex, others in the pons and medulla oblongata. The staining results obtained with the hyperimmune rabbit sera LL2 and BP11 was similar to the results achieved with Kfu2.

---

## **Discussion**

The purpose of this study was to elucidate the immunological reactions of the CNS in a feline non-suppurative meningoencephalomyelitis referred to as staggering disease [28]. As BDV is regarded as a putative etiological agent in this disorder [9, 29], it was of interest to investigate CNS sections for the presence of BDV antigen and to compare the distribution of antigen with the localization of inflammatory reactions.

Several methods for estimating frequencies of inflammatory cells in brain tissue sections have been described, including counting of all cells in individual ACs and calculating the percentage of positively stained cells [10]. Apart from being extremely time-consuming, this procedure is unsuitable for cryostat sections with their rather obscure cellular details, making it sometimes difficult to establish the exact number of cells in an infiltrate. We decided instead to use a semiquantitative method which allows characterization of a large number of ACs (as many as 65 in an individual cat) and should provide a good indication of the frequencies of different cell populations.

Clearly, T lymphocytes were the predominating inflammatory cells within the adventitial space. The prevalence of CD4<sup>+</sup> and CD8<sup>+</sup> T cells was, however, lower than would be expected from the marked abundance of cells positively stained by the pan-T cell marker. It should be noted that the pan-T cell marker used in this study (CF54A) has not yet been fully characterized. When applied to peripheral blood lymphocytes from control cats, using flow cytometry, this marker reacted with a population of cells (mean value 11.5%) which was neither CD4<sup>+</sup> nor CD8<sup>+</sup> (Table 1). Recent flow cytometric studies indicate that some of these cells actually belong to the monocyte population (data not shown). Furthermore, it is possi-

ble that a minor CD3<sup>+</sup> CD4<sup>-</sup> CD8<sup>-</sup> T cell population might exist in cats, as it does in humans, where such cells express the CD7 phenotype and constitute a small population in the peripheral blood and the small intestine [31, 37]. An element of subjectivity in the interpretation of the results may also partly explain the discrepancy. A more accurate quantification of different sets of lymphocytes may be obtained by flow cytometry after extraction of mononuclear blood cells from the brain [22]. Such studies in cats with staggering disease are in progress.

CD4<sup>+</sup> T cells were more abundant than CD8<sup>+</sup> T cells. Unlike human and rat macrophages, feline macrophages do not express detectable amounts of CD4 antigen [1]. The predominance of CD4<sup>+</sup> cells cannot, therefore, be ascribed to labelling of macrophages. Within the adventitial spaces, CD4<sup>+</sup> T cells would have the opportunity to interact with MHC II<sup>+</sup> antigen-presenting macrophages, thereby triggering important immunological reactions [12]. It should be noted that normal cats seem to have an unusually high degree of MHC II expression not only on B cells, but also on resting T cells [32, 34]. The strong MHC II expression within ACs in our cases could, therefore, be a phenomenon merely related to the presence of numerous lymphocytes. In the neural parenchyma, however, increased MHC II expression on cells resembling microglia was clearly observed in the diseased cats. This enhanced MHC II expression may be related to direct effects of virus replication or stimuli from lymphokines secreted by T cells [19].

In several viral encephalitides, including experimental Borna disease in Wistar rats, the inflammatory cells in the neuropil consist almost exclusively of monocytes, macrophages and microglial cells, and only exceptionally of a few T cells [16, 33]. In the present feline encephalitis, both the paucity of T cells in the neural parenchyma and the marked infiltration of microglial cells seem to indicate a similar pattern. However, the macrophage marker used (DH59B) failed to stain cells in the neuropil. It is possible that this antibody only labels a limited population of monocytes/macrophages and that the actual number of blood-derived macrophages could be considerably higher. As mentioned above, there is also a possibility that some of the CD3<sup>+</sup> cells in the neural parenchyma belong to the monocyte population.

A large number of plasma cells and other immunoglobulin-bearing cells were found scattered in the parenchyma, often adjacent to degenerate or morphologically intact neurons. The finding of plasma cells in such close proximity to neurons suggests that antibodies may be involved in the mechanism of virus clearance from infected cells. Such antibody-mediated clearance of virus has been described in other encephalitides, e.g., rabies [11], and Sindbis virus encephalitis in mice [19]. In the case of rabies, however, the antibody involved is a neutralizing and protective antibody which causes complete clearance of virus from the cells [11]. If a similar mechanism occurs in cats with staggering disease, it must be assumed that the antibody involved fails to clear the virus completely, at least allowing some persistence of viral RNA to account for the chronic nature of the disease.

Interestingly, there were considerable numbers of IgM- and IgA-bearing immunocytes intermingled with IgG immunocytes, even in the two long-standing cases. Similar findings in dogs and humans with chronic inflammatory diseases of the CNS suggest that this phenomenon may be associated with persistent viral infection with continuous or intermittent expression of antigen [13, 40].

The pattern of inflammation in the cats is very similar to that of a subacute viral encephalitis, such as natural or experimentally induced Borna disease [17, 25, 26, 35]. A mixed composition of cells in the adventitial space, strong expression of MHC class II antigen on macrophages/microglial cells and the presence of scattered plasma cells and B lymphocytes in the neural parenchyma, as found in the present cat encephalitis, are also features of experimental Borna disease in adult Lewis and Wistar rats [10, 33]. The paucity of BDV-specific antigen in the cat encephalitis argues against BDV as the causative agent. On the other hand, such antigen has recently been demonstrated in several cats with staggering disease in an ELISA using homogenized brain tissue (Bode and Lundgren, unpublished observation). It seems, however, that the amount of BDV antigen in cats, as shown in such antigen assays, is much smaller than in other animal species. Furthermore, preliminary studies have shown feline BDV to have a cell tropism in culture different from that of other BDV isolates [9, 27]. It is possible that cats with staggering disease carry BDV with an antigenic configuration that is altered by its adaptation to the cat, making the antibodies employed in our study insufficient for feline BDV detection. This notion is supported by the observation that BDV antigen is undetectable by immunohistochemistry in newborn Wistar rats, intracerebrally inoculated with homogenized brain suspensions from cats with staggering disease (Lundgren et al., submitted for publication). In contrast, after inoculation of brain suspensions from such infected newborn rats into adult rats, full-blown Borna disease with strong expression of BDV antigen occurs.

The pathogenesis of feline staggering disease is obscure. Clearly, a strong immune reaction is present in the form of heavy mononuclear cell infiltrates in the adventitial space. Neuronal degeneration and necrosis obviously occur [28], but the mechanisms underlying this remain to be elucidated. In the present study, no morphological sign of cytotoxic T cell activity against neurons could be found. This is in accordance with the sparsity of CD8<sup>+</sup> T cells and the fact that no expression of MHC class I on neurons was observed in the cat brains. In contrast, neuronal lesions in BDV-infected Lewis rats are claimed to be caused by a virus-induced, cell-mediated immunopathological reaction mainly involving CD8<sup>+</sup> T cells, rather than by the virus itself [2, 39]. This notion is supported by the facts that BDV lacks a cytolytic effect in tissue cultures and that in the acute phase of the disease, the lytic effect *in vivo* is not strongly expressed [39]. It has been shown, however, that at a later stage of Borna disease severe widespread cytopathic changes occur in the neurons [18]. Since the role of BDV in feline staggering disease is



not clear, it is not possible at this stage to determine the importance of these mechanisms for the neuronal lesions in the cat encephalitis.

In conclusion, the results of the present study provide evidence for a strong, immunological reaction in the CNS of cats with non-suppurative meningoencephalomyelitis (staggering disease). Our findings indicate a long-standing inflammatory process, possibly triggered and sustained by a persistent viral infection.

It is of vital importance to establish the role of BDV in the etiology and pathogenesis of feline staggering disease. Such studies are currently in progress.

**Acknowledgements** The authors wish to thank Dr. Anders Johansson for performing the flow cytometry analysis and Mrs. Åsa Gessbo and Miss Christina Nilsson for technical assistance. This work was supported by a grant from the AGRIA insurance company and a contract of the EU (no: BMH 1-CT 94-1791).

## References

- Ackley CD, Hoover EY, Cooper MD (1990) Identification of a CD4 homologue in the cat. *Tissue Antigens* 35: 92–98
- Bilzer T, Stütz L (1994) Immune-mediated brain atrophy. CD8<sup>+</sup> T cells contribute to tissue destruction during Borna Disease. *J Immunol* 153: 818–823
- Bode L, Riegel S, Lange W, Ludwig H (1992) Human infections with Borna disease virus: seroprevalence in patients with chronic disease and healthy individuals. *J Med Virol* 36: 309–315
- Bode L, Dürrwald R, Ludwig H (1994) Borna virus infections in cattle associated with fatal neurological disease. *Vet Rec* 135: 283–284
- Bode L, Steinbach F, Ludwig H (1994) A novel marker for Borna disease virus infection. *Lancet* 343: 297–298
- Briese T, Schneemann A, Lewis AJ, Park YS, Kim S, Ludwig H, Lipkin WI (1994) Genomic organization of Borna disease virus. *Proc Natl Acad Sci USA* 91: 4362–4367
- Cordell JL, Falini B, Erber WN, Ghosh AK, Abdulaziz Z, MacDonald S, Pulford KAF, Stein H, Mason DY (1984) Immunoenzymatic labeling of monoclonal antibodies using immune complexes of alkaline phosphatase and monoclonal anti-alkaline phosphatase (APAAP complexes). *J Histochem Cytochem* 32: 219–229
- Cubitt B, Oldstone C, Torre JC de la (1994) Sequence and genome organization of Borna disease virus. *J Virol* 68: 1382–1396
- Czech-Schmidt G (1993) Borna-Virus Infektionen im Tiermodell: serologische, virologische und molekularbiologische Untersuchungen. Inaugural-Dissertation, Freie Universität, Berlin, Germany
- Deschl U, Stütz L, Herzog S, Frese K, Rott R (1990) Determination of immune cells and expression of major histocompatibility complex class II antigen in encephalitic lesions of experimental Borna disease. *Acta Neuropathol* 81: 41–50
- Dietzschold B, Kao M, Zheng YM, Chen XY, Maul G, Fu ZF, Rupprecht CE, Koprowski H (1992) Delineation of putative mechanisms involved in antibody-mediated clearance of rabies virus from the central nervous system. *Proc Natl Acad Sci USA* 89: 7252–7256
- Esiri MM, Gay D (1990) Immunological and neuropathological significance of the Virchow-Robin space. *J Neurol Sci* 100: 3–8
- Felgenhauer K (1982) Differentiation of the humoral immune response in inflammatory diseases of the central nervous system. *J Neurol* 228: 223–237
- Flir K (1973) Encephalomyelitis bei Grosskatzen. *DTW Dtsch Tierärztl Wochenschr* 80: 401–404
- Gallyas F (1970) Silver staining of micro- and oligodendroglia by means of physical development. *Acta Neuropathol (Berl)* 16: 35–38
- Gosztanyi G (1992) Acute viral encephalitis: a monocyte/macrophage induced disease? *Clin Neuropathol* 11: 261–262
- Gosztanyi G, Ludwig H (1984) Borna disease of horses: an immunohistological and virological study of naturally infected animals. *Acta Neuropathol (Berl)* 64: 213–221
- Gosztanyi G, Ludwig H (1995) Borna disease: neuropathology and pathogenesis. *Curr Top Microbiol Immunol* 190: 40–72
- Griffin DE, Levine B, Tyor W, Irani DN (1992) The immune response in viral encephalitis. *Semin Immunol* 4: 111–119
- Hoff EJ, Vandeveld M (1981) Non-suppurative encephalomyelitis in cats suggestive of a viral origin. *Vet Pathol* 18: 170–180
- Hsu SM, Raine L, Fanger H (1981) The use of avidin-biotin-peroxidase complex (ABC) in immunoperoxidase techniques: a comparison between ABC and unlabeled antibody (PAP) procedures. *J Histochem Cytochem* 29: 577–580
- Irani DN, Griffin DE (1991) Isolation of brain parenchymal lymphocytes for flow cytometric analysis. *J Immunol Methods* 139: 223–231
- Klotz FW, Cooper MD (1986) A feline thymocyte antigen defined by a monoclonal antibody (FT2) identifies a subpopulation of non-helper cells capable of specific cytotoxicity. *J Immunol* 136: 2510–2514
- Kronevi T, Nordström M, Moreno W, Nilsson PO (1973) Feline ataxia due to nonsuppurative meningoencephalomyelitis of unknown aetiology. *Nord Veterinaemed* 26: 720–725
- Ludwig H, Kraft W, Kao M, Gosztanyi G, Dahme E, Krey H (1985) Borna-Virus-Infektion (Borna-Krankheit) bei natürlich und experimentell infizierten Tieren: ihre Bedeutung für Forschung und Praxis. *Tierärztl Prax* 13: 421–453
- Ludwig H, Bode L, Gosztanyi G (1988) Borna disease: a persistent virus infection of the central nervous system. *Prog Med Virol* 35: 107–151
- Ludwig H, Furuya K, Bode L, Klein N, Dürrwald R, Lee DS (1993) Biology and neurobiology of Borna disease virus (BDV), defined by antibodies, neutralizability and their pathogenic potential. *Arch Virol [Suppl]* 7: 111–133
- Lundgren AL (1992) Feline non-suppurative meningoencephalomyelitis. A clinical and pathological study. *J Comp Pathol* 107: 411–425
- Lundgren AL, Ludwig H (1993) Clinically diseased cats with non-suppurative meningoencephalomyelitis have Borna disease virus-specific antibodies. *Acta Vet Scand* 34: 101–103
- Malkinson M, Weisman Y, Bode L, Ludwig H (1993) Borna disease in ostriches. *Vet Rec* 133: 304
- Moretta L, Pende D, Bottino C et al. (1987) Human CD3<sup>+</sup>, 4<sup>-</sup>, 8<sup>-</sup> WT31 T lymphocyte populations expressing the putative cell receptor  $\gamma$  gene product. A limiting dilution and clonal analysis. *Eur J Immunol* 17: 1229–1234
- Neefjes JJ, Hensen EJ, Kroon TIP de, Ploegh HL (1986) A biochemical characterization of feline MHC products: unusually high expression of class II antigens on peripheral blood lymphocytes. *Immunogenetics* 23: 341–347
- Petrov S (1993) Analyse der entzündlichen Reaktion bei der experimentellen Borna-Encephalitis der Ratte. Inaugural-Dissertation, Freie Universität, Berlin, Germany
- Rideout BA, Moore PF, Pedersen NC (1990) Distribution of MHC class II antigens in feline tissues and peripheral blood. *Tissue Antigens* 36: 221–227
- Roggendorf W, Sasaki S, Ludwig H (1983) Light microscopic and immunohistologic investigations on the brain of Borna Disease virus infected rabbits. *Neuropathol Appl Neurobiol* 9: 287–296
- Rott R, Herzog S, Fleischer B, Winokur H, Amsterdam JD, Dyson W, Koprowski H (1985) Detection of serum antibodies to Borna disease virus in patients with psychiatric disorders. *Science* 228: 755–756

37. Spencer JO, MacDonald TT, Diss TC, Walker-Smith JA, Ciclitira PJ, Isaacson PG (1989) Changes in intraepithelial lymphocyte subpopulations in coeliac disease and enteropathy associated T cell lymphoma (malignant histiocytosis of the intestine). *Gut* 30:339–346
38. Sternberger LA (1979) *Immunocytochemistry*, 2nd edn. Wiley, New York
39. Stitz L, Bilzer T, Richt JA, Rott R (1993) Pathogenesis of Borna disease. *Arch Virol [Suppl]* 7:135–151
40. Tipold A, Pfister H, Zurbriggen A, Vandevelde M (1994) Intrathecal synthesis of major immunoglobulin classes in inflammatory diseases of the canine CNS. *Vet Immunol Immunopathol* 42:149–159
41. Tompkins MB, Gebhard DH, Bingham HR, Hamilton MJ, Davis WC, Tompkins WAF (1990) Characterization of monoclonal antibodies to feline T lymphocytes and their use in the analysis of lymphocyte tissue distribution in the cat. *Vet Immunol Immunopathol* 26:305–317
42. Truyen U, Stockhofe-Zurwieden N, Kaaden OR, Pohlenz J (1990) A case report: encephalitis in lions. Pathological and virological findings. (*DTW Dtsch Tierärztl Wochenschr* 97:89–91)
43. Vandevelde M, Braund KG (1979) Polioencephalomyelitis in cats. *Vet Pathol* 16:420–427
44. Weissenböck H, Nowotny N, Zoher J (1994) Feline meningoencephalomyelitis (“Staggering disease”) in Österreich. *Wien Tierärztl Monatsschr* 81:195–201