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Tick-borne encephalitis in dogs: neuropathological findings and distribution of antigen

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Abstract Eight dogs originating from different regions of Austria [all of them known as tick-borne encephalitis (TBE) areas] with severe neurological signs were either euthanatized or died spontaneously. Tick-borne encephalitis virus (TBEV) antigen was detected in the brains of five of these dogs by immunohistology, but not in the others. All of the dogs, however, had identical neuropathological changes. There were moderate lymphohistiocytic meningitis, widespread neuronal necroses, karyorrhexis of glial cells, numerous neuronophagic nodules, and extensive microgliosis. In the cerebellum, loss of Purkinje cells and proliferation of microglial cells in the molecular layer were found. All brain regions showed numerous perivascular cuffs consisting of lymphocytes, macrophages, plasma cells and, occasionally, red blood cells. The blood-derived cells were not restricted to the perivascular spaces but diffusely infiltrated the neuropil. The most severe changes were localized in the neuroparenchyma surrounding the fourth ventricle. Lesions were less severe in basal ganglia, thalamus, mesencephalon, nuclei of pons and medulla oblongata. Moderate lesions were found in the gray matter of neocortex and allocortex, hippocampus and molecular and Purkinje cell layers of the cerebellum. White matter was slightly to moderately affected. The choroid plexus was free of inflammation. Due to rapid virus clearance mechanisms in this disease, antigen was not detectable in all cases. Neuropathological changes identical with those of immunohistologically proven cases justified the diagnosis TBE in these cases. In addition, the neuropathological diagnosis was supported by the origin of the affected dogs from endemic areas, the seasonal occurrence of the disease and a clinical history of a highly febrile neurological disease with short duration.

Key words Tick-borne encephalitis · Dog · Neuropathology · Antigen distribution

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Introduction

Tick-borne encephalitis virus (TBEV), a member of the genus flavivirus within the family of Flaviviridae, is a frequent cause of meningoencephalitis in man in the endemic areas of Europe and Asia. In Europe, the virus is mainly transmitted by *Ixodes ricinus*, in the Baltic states and the European part of Russia also by *I. persulcatus* [14]. However, a few cases of transmission via milk from ruminants have been described [5, 8, 14]. Due to a vaccination program which was introduced more than 15 years ago, the incidence of the disease and the number of cases with fatal outcome have significantly declined in Austria [11, 14, 17] in contrast to other countries, where the virus is endemic.

Unlike other closely related members of the TBE group, e.g., louping ill (LI) virus, Spanish sheep encephalitis virus and Turkish sheep encephalitis virus which cause disease in sheep, TBEV itself seems to be primarily pathogenic for humans [12, 14, 16, 23]. Studies from the early seventies as well as recent studies, however, present evidence that the virus is pathogenic for dogs and is able to cause fatal meningoencephalitis [3, 4, 15, 19, 21, 22]. Neuropathologically documented cases of TBE in dogs have only been reported from Switzerland and Austria, so far [19, 22]. There are no reports concerning the pathogenicity of TBEV for farm animals except a single case report of a horse with fatal TBE [20].

This study describes and illustrates the neuropathology of immunohistologically proved TBE cases in dogs and compares these findings with histologically and clinically suspicious cases for which TBEV could not be detected.

Table 1 Signalment, origin, date of death, and results of TBEV immunohistology of the dogs with TBE (*m* male, *f* female, *e* euthanatized, *d* spontaneous death, *IHC* immunohistochemistry for TBEV antigen)

No.	Breed	Sex	Age	Origin	Date of death		IHC
1	Terrier-mix	m	10 years	Linz[a]	3. 6. 1994	e	+
2	Husky	m	2 years	Vienna	20. 6. 1995	e	+
3	Husky	m	3 years	Seekirchen[b]	26. 7. 1995	e	+
4	Husky	m	2 years	Stoob[c]	14. 5. 1996	d	+
5	Rottweiler	f	1 year	Wiesen[c]	24. 5. 1997	d	+
6	Bastard	m	3 years	Vienna	6. 5. 1994	e	–
7	Pekingese	f	3 months	Vienna	24.10. 1994	e	–
8	Irish setter	m	5 months	Vienna	18. 6. 1996	e	–

[a] Upper Austria; [b] Salzburg; [c] Burgenland

Materials and methods

Histology

The material used for this study originates from eight dogs which were submitted for necropsy after euthanasia or spontaneous death because of severe neurological disease during the period January 1994–July 1997. The submissions were accompanied by a clinical anamnesis from the referring veterinarians, from which the data for the collective clinical signs of the affected dogs were taken. For four dogs the whole carcass was available for study; for three dogs only the head and for one dog only formalin-fixed brain samples were available. After necropsy the brain and in one case also the spinal cord was removed and fixed in 7% neutral buffered formalin for 48–72 h. Transversal sections of at least five different regions were consistently cut and embedded in paraffin wax. The examined regions were: (1) frontal cortex including basal ganglia; (2) temporal and parietal cortex with hippocampus and thalamus/hypothalamus; (3) mesencephalon (colliculus caudalis); (4) cerebellum including the pedunculi and adjacent pons/medulla oblongata; and (5) medulla oblongata (obex). In one case one section each from cervical, thoracal and lumbal portion of the spinal cord were additionally examined. The sections were stained with hematoxylin and eosin and cresyl echt violet.

The neuropathology of immunohistologically positive cases was evaluated. The quality, quantity and distribution of the lesions were largely identical in all 5 cases. Thus, these neuropathological changes were taken as standard and 14 hitherto etiologically undiagnosed cases of nonsuppurative encephalitis were reexamined to determine whether some of them were to meet these criteria. Three cases with consistent neuropathology, which additionally were clinically suspicious of TBE, originated from endemic areas and had contracted disease during the months May to October were identified and included in the study.

Immunohistology

Polyclonal rabbit antibodies to TBEV (strain “Hochosterwitz”; dilution 1 : 3000) were used for antigen detection. Antigen unmasking was performed on dewaxed sections using a 0.01% solution of protease type XIV (Sigma, St. Louis, Mo.). Endogenous peroxidase activity was blocked by incubation with 2% H_2O_2 in methanol. The sections were incubated with 10% normal goat serum (NGS) for 30 min to reduce background staining. Consecutively, the sections were incubated for 16 h at 4° C with primary antibodies diluted in phosphate-buffered saline solution containing 1% NGS. Subsequent steps were performed using the Vectastain® ABC-kit (Vector Laboratories, Burlingame, Calif.) according to the manufacturer’s instructions. Brain from a mouse with experimental TBE served as positive control, and a normal dog brain as negative control.

The quantity of antigen in the immunohistologically stained sections was assessed according to the following score system: +, only few positive cells, staining reaction predominantly weak; ++, a moderate number of positive cells (easily detectable in low-power fields) with a strong staining reaction in more than half of the positive cells; and +++, numerous positive cells, with a predominantly strong staining reaction. In addition, rabbit antibodies to suid herpesvirus 1 (Aujeszky’s disease virus, SHV-1; dilution 1 : 800; kindly provided by M. Pensaert, Gent), canine distemper virus (CDV; dilution 1 : 200; Behringwerke, Marburg) and hamster antibodies to rabies virus (RV; dilution 1 : 2000; kindly provided by J. Damoser, Mödling) were applied, using the technique described above, to exclude these viruses as the etiology of those cases in which TBEV could not be demonstrated.

Results

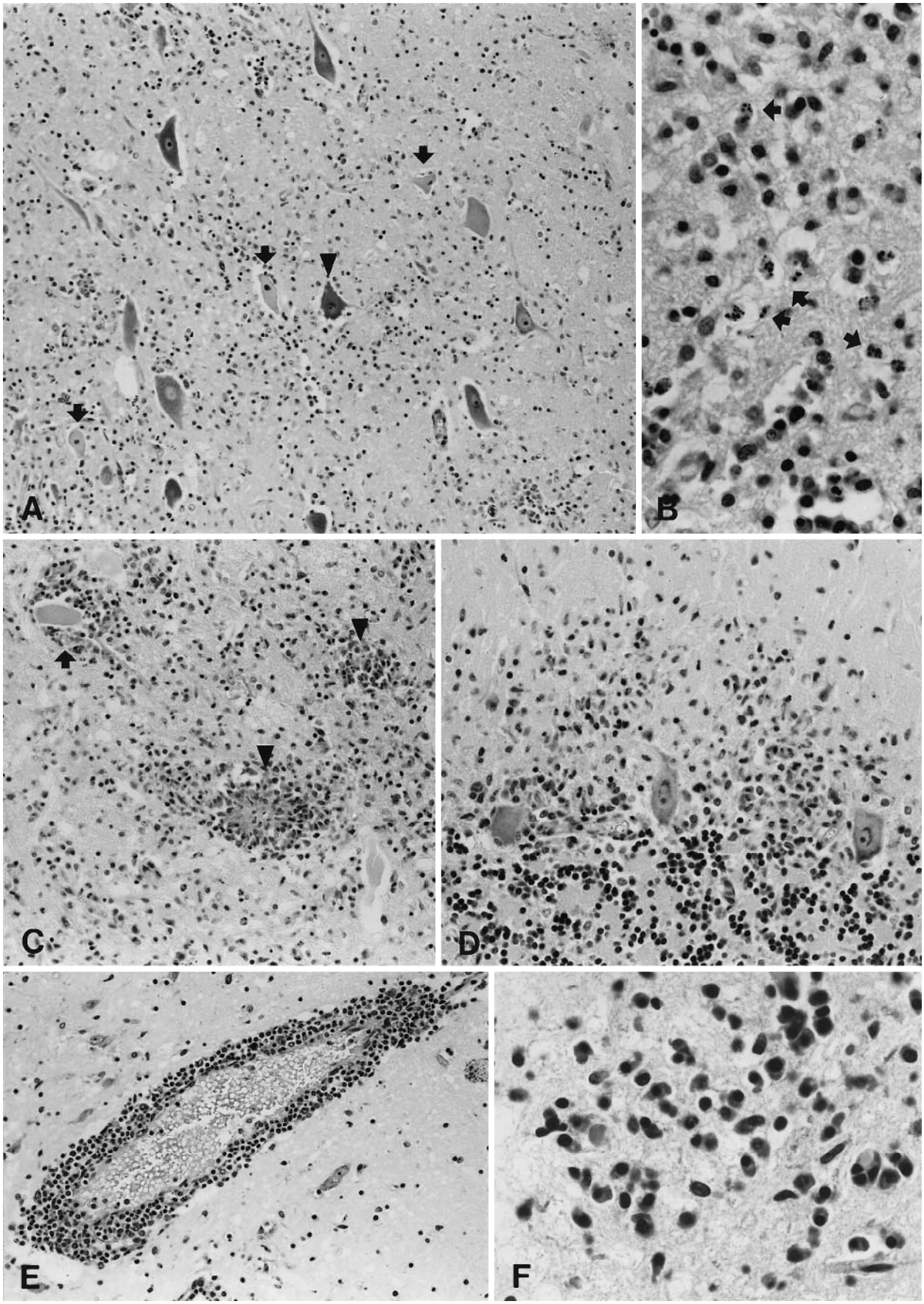
Epizootiological and clinical findings

The breed, sex, age, origin and date of death of the affected dogs are listed in Table 1. All dogs originated from known TBE endemic areas of different Austrian federal states (Vienna, Upper Austria, Salzburg, Burgenland). Disease duration from first signs to euthanasia or spontaneous death ranged from 3 days to 1 week. In two of the dogs prior to nerval signs apathia, anorexia or retention of urine were noticed. In six dogs body temperature was elevated (39.5–42.0° C). The following nerval signs were reported: convulsions (5 ×), tremor (2 ×), ataxia (2 ×), hyperesthesia (1 ×), hemiplegia (1 ×), tetraplegia (1 ×), recumbency (1 ×), opisthotonus (1 ×), epileptiform seizures (1 ×), anisocoria (1 ×), miosis (1 ×), and nystagmus (1 ×). In all animals the nerval signs were progressive, and euthanasia was requested in most cases.

Characteristics of the encephalitic lesions

There was moderate, diffuse lymphohistiocytic meningitis. A prominent feature in the neuroparenchyma was dif-

Fig. 1 A Numerous degenerate *(arrowhead)* or necrotic *(arrows)* neurons and diffuse proliferation of glial cells; nucleus tractus spinalis nervi trigemini, dog 4. **B** Karyorrhexis of numerous glial cells *(arrows)* in an area of diffuse gliosis; dog 2. **C** Necrotic neuron, surrounded by microglial cells *(arrow)*, two neuronophagic nodules *(arrowheads)* and diffuse gliosis; nucleus vestibularis lateralis; dog 3. **D** Focal microgliosis (glial shrubbery) in the molecular layer; cerebellum, dog 3. **E** Perivascular cuff, consisting of lymphocytes, monocytes and plasma cells; thalamus, dog 3. **F** Numerous plasma cells diffusely infiltrating the neuropil; thalamus, dog 3. **A–F** Cresyl echt violet; **A** × 120, **B** × 330, **C** × 165, **D** × 240, **E** × 175, **F** × 490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fuse neuronal necrosis (Fig. 1 A) and diffusely distributed karyorrhexis of glial cells (Fig. 1 B). In areas with severe neuronal necrosis, neuronophagic nodules, consisting predominantly of microglial cells and macrophages were found (Fig. 1 C). These nodules partially became coalescent with adjacent ones, thus leading to a diffuse pattern of neuropil infiltration. In all brain regions, a diffuse increase of microglial cells throughout the gray matter was noticed (Fig. 1 A). Very rare focal granulocytic infiltrations were present. In the cerebellum, some necrotic Purkinje cells were seen and Purkinje cells were lacking in certain areas. In areas with loss of Purkinje cells there was proliferation of microglial cells in the molecular layer, either diffusely distributed or arranged as a so-called glial shrubbery (Fig. 1 D). In gray and white matter there was diffusely distributed, moderate to severe perivascular cuffing which consisted of lymphocytes, macrophages, plasma cells and, occasionally, red blood cells (Fig. 1 E). In three cases, plasma cells were the predominating cell type. The blood-derived cells did not remain restricted to the perivascular spaces but diffusely infiltrated the neuropil, particularly in areas with neuronal damage and glial reaction (Fig. 1 F).

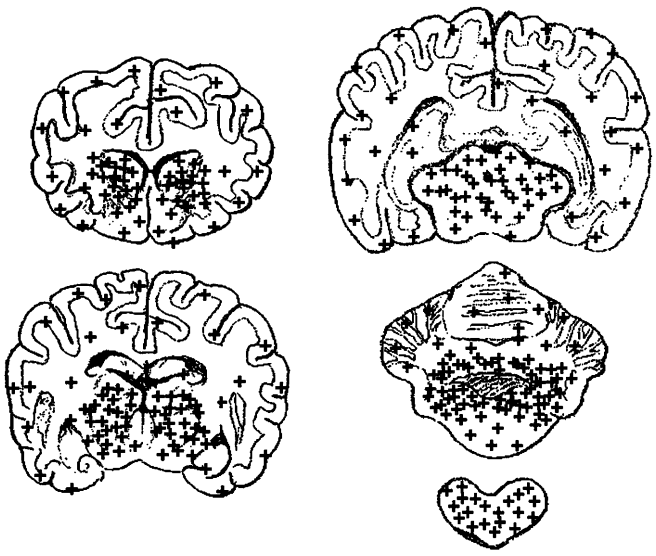

Fig. 2 Distribution pattern of histological lesions in transversal brain sections of dogs with TBE. The most prominent lesions are present in basal ganglia, thalamus *(left side),* mesencephalon, neuroparenchyma surrounding fourth ventricle, and medulla oblongata *(right side)*

Distribution of encephalitic lesions

In all animals the lesions were found throughout the central nervous system (CNS). Some areas, however, were consistently more severely affected than others. The distribution of lesions is schematically shown in Fig. 2. The most severe changes were localized in the neuroparenchyma surrounding the fourth ventricle, particularly nuclei vestibulares, nucleus tractus spinalis nervi trigemini and cerebellar roof nuclei. Less severe lesions were present in basal ganglia, thalamus, mesencephalon, nuclei of pons and medulla oblongata. Moderate changes were found in the gray matter of neocortex and allocortex, hippocampus and molecular and Purkinje cell layer of the cerebellum. White matter was slightly to moderately affected. Moderate lesions were confined to subcortical zones and slight lesions were present in the tractus of the brain stem, above all the pyramis. The choroid plexus was free of inflammation.

Lesions in the spinal cord of the one examined dog were predominantly found in the gray matter with the ventral horns most severely affected. The white matter and the leptomeninges showed slight, either perivascular or diffuse, lymphocytic infiltration.

Table 2 Quantity and distribution of TBEV antigen in immunohistologically positive cases of TBE in dogs (*nucl.* nucleus, *tract. spin. n. trigem.* tractus spinalis nervi trigemini)

No.	Quantity of antigen	Localization of antigen	Intra- and extracellular distribution of antigen
1	+	Mesencephalon, nucl. tract. spin. n. trigem.	Perikarya of neurons, cytoplasm of macrophages
2	+	Nucl. vestibulares, nucl. pontis	Perikarya of neurons
3	+++	Nucl. tract. spin n. trigem., nucl. vestibulares, cerebellar roof nuclei, Purkinje cells, neocortex	Perikarya of neurons, neurites, cytoplasm of macrophages, extracellular in glial nodules
4	++	Nucl. tract. spin. n. trigem., nucl. vestibulares, Purkinje cells, thalamus, hippocampus	Perikarya of neurons, neurites, cytoplasm of macrophages, extracellular in glial nodules
5	+	Periventricular gray matter around IVth ventricle, mesencephalon	Cytoplasm of macrophages, neurites

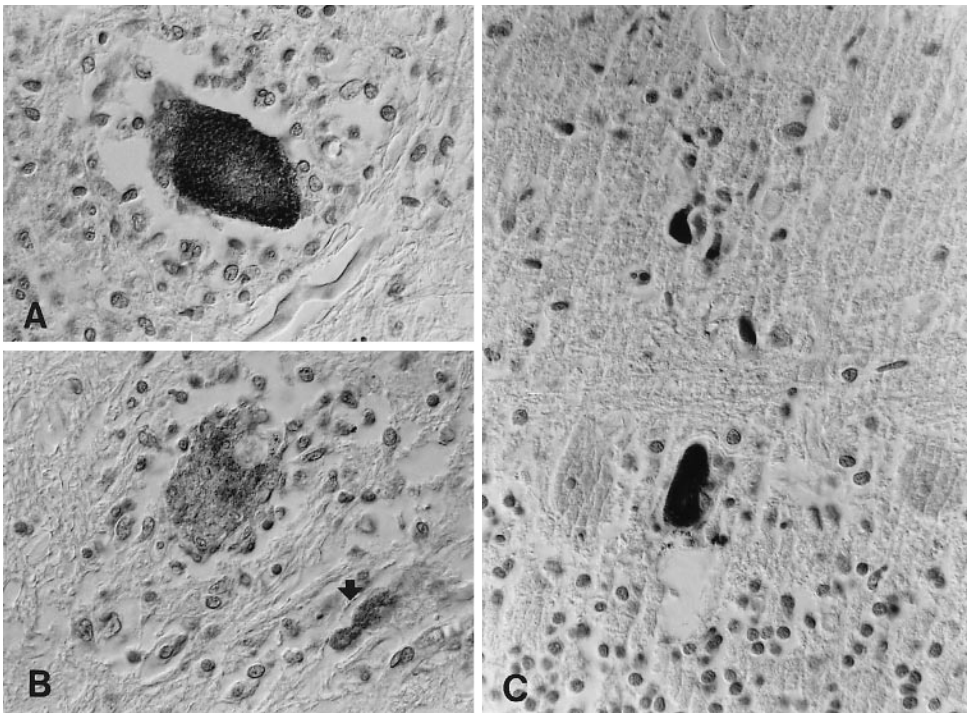

Fig. 3 A TBEV antigen in a degenerate neuron surrounded by microglial cells; nucleus tractus spinalis nervi trigemini, dog 3. **B** TBEV antigen in a necrotic neuron with neuronophagia and within a neurite *(arrow);* nucleus tractus spinalis nervi trigemini, dog 3. **C** TBEV antigen in perikaryon and dendrites of a degenerate Purkinje cell; cerebellum, dog 3. **A**–**C** Avidin-biotin-peroxidase complex technique, × 410

Immunohistological findings

In the positive control (brain of an experimentally infected mouse) viral antigen was present in the cytoplasm of many neurons of all brain regions.

TBEV antigen was found in five dogs (see Table 1). The quantity, distribution and cell tropism of antigen is shown in Table 2. Antigen was found in the cytoplasm of usually degenerate or necrotic neurons where it showed a fine granular staining pattern (Fig. 3 A, B). A frequent finding was the presence of antigen in axons or dendrites, particularly of the Purkinje cells in the molecular layer (Fig. 3 C). Antigen apparently set free from neurons undergoing neuronophagia was found as fine granular extracellular positive material. In some neuronophagic nodules antigen was present as coarsely granular material in the cytoplasm of macrophages. Antigen was never detected in ependymal cells, mononuclear infiltrates, endothelial cells or meninges.

The locations with the most consistent presence of antigen – even in cases weakly positive – were the nuclei in the metencephalon and medulla oblongata, particularly the nucleus tractus spinalis nervi trigemini and the nuclei vestibulares.

Discussion

TBEV is primarily pathogenic for humans, but there is a considerable pathogenic potential for other mammals. Hitherto, only clinical disease of dog and horse has been noticed [19, 20, 22], although seropositivity could be demonstrated in a large range of insectivores, rodents, game and farm animals [18]. The risk for a tick-infested dog from an endemic area of developing clinical manifest TBE seems to be rather small, taking into account that in endemic areas seroprevalence is higher in dogs than in men [13, 18]. The majority of TBE infections in dog must, therefore, be clinically inapparent or produce only slight unspecific clinical signs not considered suspicious of TBE. TBE infections in dogs have been reported from Switzerland, Austria and Germany [15, 19, 22]. Clinical disease is described as acute, highly febrile condition with multifocal neurological implication leading to convulsions, paresis, paralysis and deficits of head nerves [19]. In the present study the disease led to spontaneous death in two cases and euthanasia was indicated or requested in the others. Recently, we got knowledge of serologically proven cases of TBE in dogs that had survived the acute phase, which was followed by a slow recovery period supported by intensive care (G. Kirtz, B. Reiner, personal communication).

The neuropathology of TBE in dogs is largely consistent with the neuropathology of TBE in man and experimentally infected mice, as well as with CNS lesions caused by Flaviviruses closely related to TBEV, e.g., LI virus [1, 2, 6, 7, 9]. The predominance of neuronal necroses accompanied by neuronophagia, gliosis and nonsuppurative inflammation in trigeminal and vestibular nuclei is a striking feature of all these descriptions. Lesions of the cerebellum consisting of Purkinje cell necroses and gliosis or glial shrubbery in the molecular layer are considered typical of human TBE and LI and were also present in dogs. The findings in the one examined spinal cord of this study with most prominent inflammation in the ventral horns are consistent with spinal cord lesions in human TBE and in LI [2, 6]. TBEV has a tropism for neurons which are subsequently damaged and undergo neu-

ronophagia. The antigen is set free, phagocyted and eliminated. In some of the present cases the inflammatory infiltrates were predominantly composed of plasma cells, which also diffusely infiltrated the neuropil and accompanied microglial proliferation. This could be an explanation for the rapid seropositivity of liquor and consecutive virus clearance. These mechanisms considerably hamper postmortem detection of TBEV antigen in brain tissue. In humans, immunohistological demonstration of antigen in fatal cases of TBE is constantly negative due to the usually long-lasting period between infection and death because of intensive care (H. Budka, personal communication).

In dogs, however, immunohistological demonstration of TBEV antigen is a useful method for proving the diagnosis. In two cases readily detectable amounts of antigen were found. In three cases only little antigen was present, requiring careful and time consuming examination. Nevertheless, distinct specific reaction predominantly in the cytoplasm of macrophages enabled unequivocal diagnosis in these cases. The same observation was reported for previously diagnosed cases of TBE [19] and LI [10].

Although disease durations in the affected dogs were short, thus leaving little time for full development of clearance mechanisms, there was no detectable antigen in three cases. These dogs were also negative for other viruses capable of causing nonsuppurative encephalitis, such as SHV-1, CDV and RV. The neuropathological findings in these cases were largely consistent with those in the immunohistologically proven cases of TBE. We consider the diagnosis TBE to be justified in these dogs as well, as much as it is supported by origin from endemic areas, occurrence during the warm season and clinical signs. The etiological diagnosis in such cases could perhaps be achieved in the future by more sensitive techniques for detection of viral pathogens, such as in situ polymerase chain reaction. At present, diagnosis must be based on clinical history, examination of serum and liquor for TBE antibodies, and neuropathology.

Summarizing, TBE must be added to the list of etiologically defined nonsuppurative encephalitides in dogs, particularly in endemic areas. The characteristic neuropathological findings, supported by immunohistology, enable an etiological diagnosis. The disease can be distinguished from encephalitides of other defined etiologies (e.g., rabies, Aujeszky's disease, canine distemper) and from a number of still etiologically undefined cases of nonsuppurative encephalitis of dogs.

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