Naturally Occurring Herpes Simplex Encephalitis in a Domestic Rabbit (*Oryctolagus cuniculus*)

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BRIEF COMMUNICATIONS and CASE REPORTS

Naturally Occurring Herpes Simplex Encephalitis in a Domestic Rabbit (*Oryctolagus cuniculus*)

H. WEISSENBÖCK, J. A. HAINFELLNER, J. BERGER, I. KASPER, and H. BUDKA

Abstract. An approximately 1-year-old domestic rabbit showed severe neurologic signs with circling and turning somersaults. Histologically, a nonsuppurative meningoencephalitis with neuronal cell necrosis and numerous intranuclear inclusion bodies in neurons and glial cells was found. Electron microscopic examination revealed herpesvirus particles in affected cells. A human herpes simplex virus was identified by means of immunocytochemistry and in situ hybridization as the causal agent and was further classified as herpes simplex virus 1 by polymerase chain reaction analysis. Because encephalitis is easily induced in rabbits by experimental infection with herpes simplex virus, the source of infection is suspected to be a human with herpes labialis who had close contact with the rabbit.

Key words: Encephalitis; herpes simplex virus 1; immunocytochemistry; in situ hybridization; polymerase chain reaction; rabbits.

Herpesviruses are uncommon pathogens in rabbits. In domestic rabbits (*Oryctolagus*), *Herpesvirus cuniculi* (in older papers referred to as virus III or herpes-like virus) has been shown to be apathogenic.[13] A second leporid herpesvirus, *Herpesvirus sylvilagus*, is only pathogenic for cottontail rabbits (*Sylvilagus* spp.) and causes lymphoproliferative disease.[3] Recently, lethal infections of domestic rabbits were characterized by hemorrhagic dermatitis, pneumonia, and splenic necrosis and were attributed to a hitherto unclassified herpesvirus.[8]

Naturally occurring herpesvirus-induced encephalitis has not been reported. However, rabbits are used as experimental animals to study herpesvirus diseases of other species, e.g., Aujeszky's disease,[1] malignant catarrhal fever,[2] or *Alphaherpesvirus saimiri* infection.[6] The rabbit is of unique importance as an animal model of human herpes simplex encephalitis,[11] which is the most frequent sporadic acute encephalitis in temperate parts of the world. The experimental disease is easily induced by corneal scarification[4,9] and by intraocular[7] or intracerebral[11] inoculation. In this paper, we report on a spontaneous infection with human herpes simplex virus (HSV) that caused encephalitis in a domestic rabbit.

An approximately 1-year-old castrated male domestic rabbit was presented to a private animal hospital with a 2-day history of anorexia and neurologic signs of circling, raising on hind legs, and turning somersaults. It had been purchased 3 weeks earlier from a pet shop and was apparently healthy then. The condition deteriorated within hours of presentation at the clinic; the animal became laterally recumbent and was euthanatized.

Necropsy revealed no conspicuous gross lesions. Histologic examination of hematoxylin and eosin-stained, formalin-fixed, paraffin-embedded material and toluidine blue-stained Epon-embedded semithin sections revealed lesions that were confined to the brain and meninges and were most prominent in the gray matter of cerebral hemispheres. Rhinencephalon, hippocampus, basal ganglia, and mesencephalon were less severely affected. Lesions in metencephalon and medulla oblongata, including trigeminal nuclei, were minimal. Spinal cord was not examined. There was moderate diffuse leptomeningitis and moderate perivascular cuffing. The infiltrates consisted of histiocytes, lymphocytes, plasma cells, and single neutrophils. Widespread necroses of neuronal cell bodies, frequently accompanied by single or groups of neutrophils, were found. The most outstanding histologic feature, however, was the presence of numerous intranuclear inclusion bodies (IIB) in neurons and glial cells. In neurons, different stages of IIB could be distinguished. There were either several or single eosinophilic, round, ovoid, or bizarrely shaped bodies separated from the hyperchromatic and often fragmented nuclear wall by a halo. Frequently, the whole nucleus was occupied by an amphophilic body, which was usually adjacent to the nuclear wall (Fig. 1). Necrotic neurons regularly contained dark eosinophilic IIB in their shrunken nuclei that reached the hyperchromatic and fragmented wall (Fig. 2). Glial cells contained predominantly homogeneous, weakly basophilic bodies that occupied the nucleus completely. Few glial cells contained eosinophilic IIB that occupied only the center of the nucleus and were separated from the condensed and often fragmented nuclear wall by a halo.

For electron microscopy, formalin-fixed cubes of cerebrum were postfixed in glutaraldehyde and osmium tetroxide and embedded in Epon. Ultrathin sections were stained with uranyl acetate and lead citrate. IIB were found to consist of osmiophilic granular material and more or less large

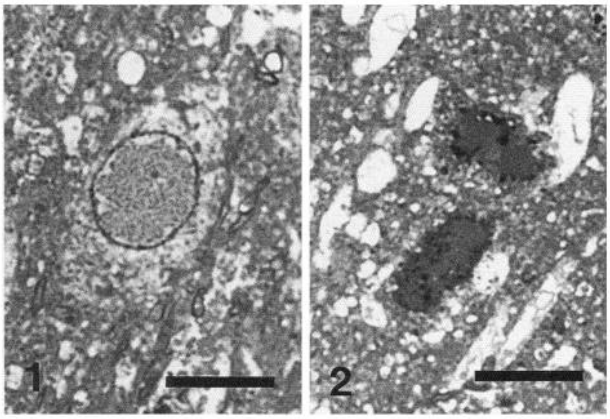

Fig. 1. Cerebrum; rabbit. Neuron containing a single pale intranuclear inclusion body reaching to the nuclear wall. Semithin section; toluidine blue. Bar = 10 μm.

Fig. 2. Cerebrum; rabbit. Two necrotic, shrunken neurons containing dense bodies adjacent to the hyperchromatic and fragmented nuclear wall. Semithin section; toluidine blue. Bar = 10 μm.

amounts of incomplete herpesvirus particles, singly or in groups (Fig. 3). The virus particles were 100–120 nm.

Anti-HSV immunocytochemical analysis (ICC) was performed using rabbit antisera against HSV-1 and HSV-2 (Dako, Glostrup, Denmark). Incubation with the primary antibodies was followed by the peroxidase–antiperoxidase technique with diaminobenzidine as chromogen. Both antisera strongly labeled all cells containing IIB. In neurons, frequently nucleus and cytoplasm were homogeneously stained. Occasionally, the cytoplasmic staining was comparatively weaker (Fig. 4). In glial cells, predominantly nuclei were positive. Many neurons without morphologic changes adjacent to necrotic and IIB-bearing neurons showed specific staining either of the nucleus or of both the nucleus and cytoplasm. There was no positive reaction using rabbit-raised antibodies to pseudorabies virus (M. Pensaert, University of Ghent, Ghent, Belgium) or equine herpesvirus 1 (N. Nowotny, University of Veterinary Medicine, Vienna, Austria). In situ hybridization (ISH) using a biotinylated probe for HSV-1 and HSV-2 DNA (Enzo Biochem, New York, NY) was performed according to previously described protocol.[12] Viral nucleic acid sequences were detected within the nuclei of numerous neurons and some glial cells. The staining either comprised the whole IIB or was confined to multiple intranuclear granules (Fig. 5). The number of cells stained with ICC and ISH techniques was approximately equal.

For polymerase chain reaction (PCR) amplification, genomic DNA was extracted from 20 sections (5 μm thick) of paraffin-embedded, formalin-fixed brain material according to a previously described method.[5] Thereafter, a HSV-1 UL42-specific PCR was performed as previously described[10] with the exception that in addition the amplified samples were diluted 1:10 in PCR buffer containing the same set of primers and subjected to an additional 32 cycles. The specificity of the 277-bp HSV-1 UL42 PCR product was confirmed by *Sac* 1 restriction enzyme

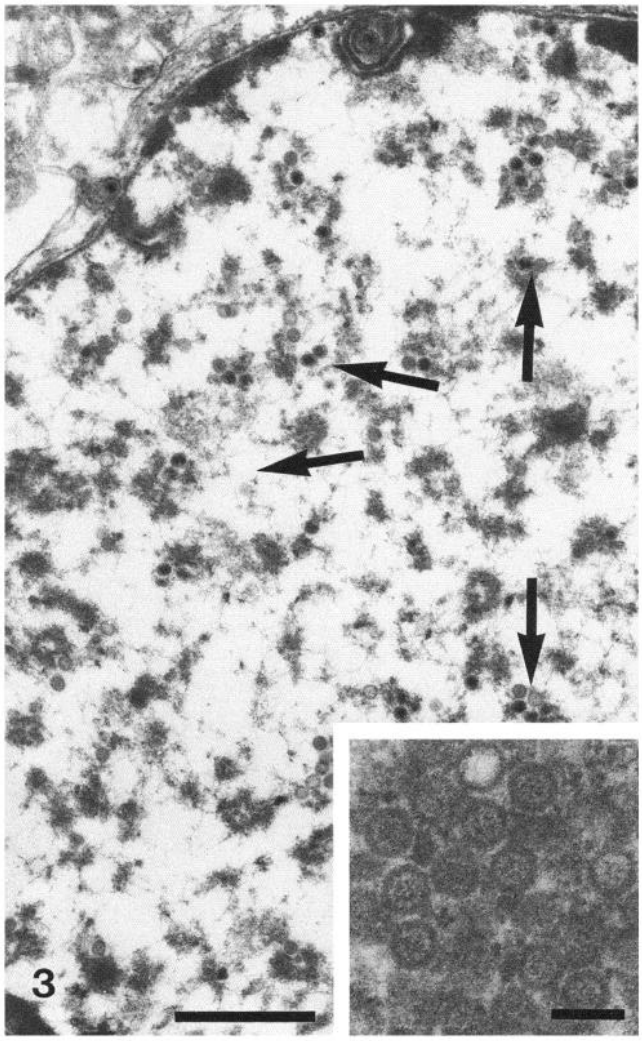

Fig. 3. Electron micrograph. Cerebrum; rabbit. Nucleus of a neuron containing scattered herpesvirus particles (arrows). Bar = 1 μm. *Inset:* Higher magnification of an intranuclear cluster of herpesvirus particles. Bar = 0.2 μm.

digestion, resulting in the predicted fragments of 187 and 91 bp, respectively (Gene/Embl data bank accession number M19121). Negative controls were DNA from human uninfected brain and HSV-2-infected skin extracted simultaneously with the rabbit samples. DNA extracted from human HSV-1-infected brain served as a positive control (all control tissues from the archives of the Institute of Neurology, Vienna). Somatic tissues, which were all histologically unchanged, were not examined for the presence of HSV.

This is the first reported case of naturally occurring herpesvirus encephalitis caused by human HSV-1 in a rabbit. The histologic lesions of the brain were largely consistent with experimental herpes simplex encephalitis in the rabbit.[9,11] The distribution pattern, however, was different. In experimental infections, focal lesions are found in defined brain regions, depending on the route of inoculation. Following corneal inoculation, the virus enters the brain by the

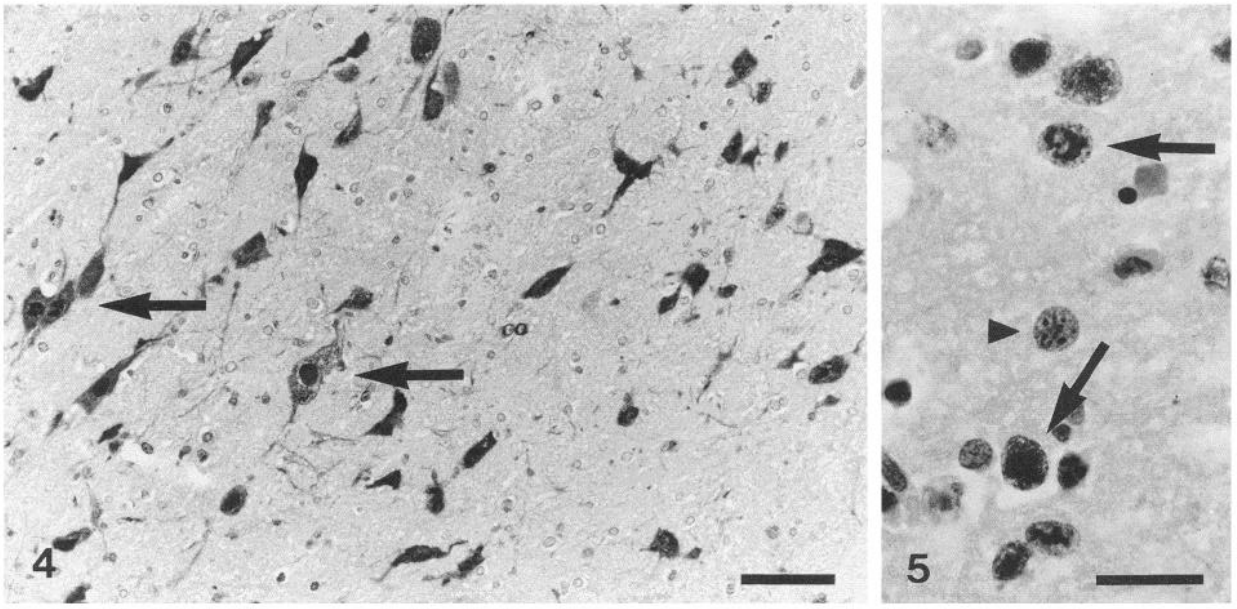

Fig. 4. Cerebrum; rabbit. Numerous neurons labeled for HSV-1. In most cells, both nucleus and cytoplasm are homogeneously stained. Occasionally, the staining reaction is more prominent in the nucleus (arrows). Peroxidase–anti-peroxidase technique, Mayer's hematoxylin counterstain. Bar = 50 μm.

Fig. 5. Cerebrum; rabbit. Intranuclear HSV-specific nucleic acid sequences appearing as compact or irregularly shaped bodies (arrows) or as round granules (arrowhead). In situ hybridization, Mayer's hematoxylin counterstain. Bar = 20 μm.

trigeminal nerve and causes lesions in brain stem and cerebral hemispheres.[9] Experimental intraocular infection remains restricted to optic nerve, chiasma, and geniculate body.[7] Inoculation into the olfactory bulb is followed by focal lesions in the temporal lobes.[11] Furthermore, variation in neurovirulence among HSV strains has been observed.[6] We did not find convincing evidence for either olfactory or trigeminal entry of the virus because lesions in both the olfactory system and the brain stem, including trigeminal nuclei, were insignificant. Optic nerves and chiasma were not examined.

A human with a herpetic lesion who had close contact with the rabbit is most likely the source of this infection. A child of the owner that was the family member with closest contact with the rabbit was frequently affected with herpes labialis. Because rabbits are easily infected experimentally and horizontal transmission among rabbits occurs,[4] contact infections between herpes-infected humans and rabbits appear possible. It is surprising that natural infection with HSV in the rabbit has never been described before.

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T-cell-rich B-cell Lymphoma in a Cat

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Abstract. T-cell-rich B-cell lymphoma is a neoplasm recognized in humans in which a neoplastic proliferation of large B lymphocytes is present amid a background of reactive T lymphocytes. A 13-year-old Domestic Shorthair cat developed a mass in the region of the left parotid gland. Histologically, the mass was composed of scattered large atypical cells within a dense background of uniform small lymphoid cells. Immunohistochemically, the large cells were uniformly labeled using antiserum directed against the B-lymphocyte marker BLA.36, whereas labeling of nearly all of the small cells was limited to the T-lymphocyte marker CD3. The histomorphologic and immunohistochemical features of this unique feline neoplasm are characteristic of T-cell-rich B-cell lymphoma of humans.

Key words: B cells; cats; immunohistochemistry; lymphoma; T cells.

Schemes devised for the classification of lymphoid neoplasms in animals often rely on morphologic criteria similar to those used for their human counterparts.[4,11] T-cell-rich B-cell lymphoma (TCRBL) is a neoplasm recognized in humans in which a neoplastic proliferation of large B lymphocytes is present within a background of reactive T lymphocytes, which comprise the majority of the cell population.[1,3,5,7,9] TCRBL has not been reported in nonhuman species. In this report, we describe a unique feline neoplasm with morphologic and immunohistochemical features characteristic of TCRBL of humans.

A 13-year-old Domestic Shorthair cat presented for a mass in the region of the left parotid gland. The cat had been vaccinated for feline leukemia virus at recommended intervals throughout its life but had not been tested for feline leukemia virus or feline immunodeficiency virus infection. The mass, which was 2 cm in greatest dimension, was surgically removed, fixed in 10% neutral buffered formalin, and submitted for pathologic evaluation. Tissue samples were processed routinely and embedded in paraffin blocks. Sections were cut at 5 μm, mounted on positively charged glass slides (Fisher Scientific, Pittsburgh, PA) and stained with hematoxylin and eosin (HE). Immunohistochemical staining for reactivity to commercially available antibodies raised against human B-lymphocyte antigen 36 (BLA.36), the pan T-lymphocyte marker CD3, kappa and lambda light chains, cytokeratins, and lysozyme was performed using the avidin–biotin–peroxidase complex (ABC) method. The dilutions of primary antisera and enzyme pretreatment of tissue sections are summarized in Table 1. Appropriate tissues containing the antigen of interest were stained in parallel (positive controls), and sections of tumor were also stained by replacing the primary antibodies with normal mouse or rabbit sera (negative controls). Additionally, sections of formalin-fixed, paraffin-embedded normal feline spleen and lymph node were stained using the BLA.36 antibody.

Histologically, the mass was well demarcated and composed predominantly of solid sheets of small round cells with scant cytoplasm and dense round to indented nuclei of uniform size. The morphology of these cells was typical of mature lymphocytes. Throughout this background of lymphocytes, there were scattered round to oval cells, 15–40 μm in diameter, that contained abundant pale eosinophilic cytoplasm and round to oval nuclei with finely stippled chromatin and occasionally a single small nucleolus (Fig. 1). These large cells were commonly binucleate, and some had large irregular to lobated nuclei with vesicular or clumped chromatin. Mitoses averaged 1/400× field and were only identified in large pale cells. The large cells comprised less than 10% of the cell population. The mass was enveloped by a thin capsule of fibroadipose tissue that was infiltrated by small lymphocytes but not by the larger pale cells.

Immunohistochemically, the large cells uniformly reacted with the BLA.36 antibody (Fig. 2) but were negative with CD3 antiserum. The entire cytoplasm of cells positive with BLA.36 exhibited staining. Nearly all of the small lymphoid cells throughout the mass reacted strongly with CD3. A few small cells were immunoreactive with BLA.36. A few large cells also demonstrated weak reactivity for lambda light chains, but neither the small lymphocytes nor the large cells