Felid Herpesvirus 1 as a Causative Agent of Severe Nonsuppurative Meningoencephalitis in a Domestic Cat

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Meningoencephalitis in a Domestic Cat

Felid herpesvirus 1 is an important respiratory pathogen of domestic cats. This report presents the first case of severe nonsuppurative meningoencephalitis caused by this virus in a cat.

CASE REPORT

A Birman 4-month-old male cat presented with lethargy, anaucrosis, pyrexia, pneumonia, and seizures. At that time, the cat had received only one dose of core vaccine (panleukopenia virus, felid herpesvirus 1 [FHV-1], feline calicivirus), while the vaccination guidelines of the World Small Animal Veterinary Association recommend at least 3 doses at this age (1). Hematology analysis revealed neutrophilia with hypersegmented neutrophils and lymphopenia. The first clinical suspicion of infectious disease was feline retrovirus infection, but the cat was negative for the detection of anti-feline immunodeficiency virus (FIV) antibodies and feline leukemia virus (FeLV) p27 antigen by an enzyme-linked immunonasay (ELISA) kit (SNAP FIV/FeLV Combo Test; IDEXX Laboratories). Aerobic and anaerobic cultures of cerebrospinal fluid were negative, thus excluding bacterial central nervous system infections. Analysis of cerebrospinal fluid (CSF) showed high protein content (75.43 mg/dl; reference range, 6 to 36 mg/dl [2]) with increased cellularity (672 cells/μl; reference range, 0 to 2 cells/μl [2]), both findings possibly due to viral disease. The cat was humanely euthanized due severe clinical manifestations and poor prognosis.

At necropsy, there was approximately 2.0 ml of yellowish serous fluid in the abdominal cavity and 0.5 ml of reddish fluid in the pericardial sac. The mesenteric lymph nodes were slightly enlarged, while the other organs were grossly normal.

Samples of organs (brain, cerebellum, lung, heart muscle, thoracic lymph node, liver, spleen, mesenteric lymph node, and kidney) were collected at necropsy for histopathological analysis. Tissue samples were fixed in 10% buffered formalin and routinely embedded in paraffin. Sections (5 μm thick) were stained with hematoxylin and eosin (HE). Histologically, discrete interstitial pneumonitis was detected. In the brain, tissue lesions were characteristic of severe, diffuse, nonsuppurative meningoencephalitis associated with chromatolysis of neurons, satellitosis, and gliosis with perivascular cuffing of mononuclear cells, mainly lymphocytes, an indication of viral infection (Fig. 1 and 2), but inclusion bodies were not found.

Aqueous humor, effusions, brain, cerebellum, lung, heart muscle, thoracic lymph node, liver, spleen, stomach, mesenteric lymph node, kidney, and large and small intestine samples were surveyed for DNA pathogens (FHV-1 and Toxoplasma gondii) and RNA pathogens (feline coronavirus [FCoV] and rabies virus). These particular pathogens (FHV-1, T. gondii, FCoV, rabies virus, FIV, and FeLV) have been chosen because they are the most likely pathogens involved in the presentation observed in the cat. Despite the absence of FHV-1 meningoencephalitis in cats, we decided to include FHV-1 due to the fact that the cat brain lesions resembled those observed in other animal species with viral meningoencephalitis caused by herpesviruses.

Genomic DNA was extracted from all samples (except aqueous humor and effusions) using a DNeasy Blood & Tissue kit (Qiagen, Valencia, CA) according to the manufacturer’s protocol. A commercial vaccine (Feline-4; Merial Inc., Athens, GA) and UltraPure diethyl pyrocarbonate (DEPC)-treated water (Invitrogen, Carlsbad, CA) were used as positive and negative FHV-1 controls, respectively. PCR for the highly conserved thymidine kinase (TK) gene of FHV-1 was performed using primers and conditions previously described (3), and brain, cerebellum, lung, spleen, and kidney results were positive. Amplicons (267 bp) were purified by using ExoSAP-IT PCR Product Cleanup (USB Products Afymetrix, Cleveland, OH) and submitted to bidirectional DNA sequencing with BigDye 3.1 (Applied Biosystems, Foster City, CA), and the electropherograms were analyzed with Phred at http://asparagin.cenargen.embrapa.br/phph/. Positions with a quality score of >20 were used to generate contiguous sequences with Cap-Contig implemented in the software Bioedit 7.0.9.0 (4). Those sequences were then submitted to BLAST/n at http://www.ncbi.nlm.nih.gov/BLAST to confirm the amplicon identities. The TK gene partial sequences were obtained from all positive samples, except for spleen. Each sequence was aligned with homologous sequences from neuroinvasive herpesviruses retrieved from GenBank with CLUSTAL/W in Bioedit 7.0.9.0 (4), and a phylogenetic tree for the nucleotide sequences was generated with the neighbor-joining distance algorithm and the maximum composite likelihood model with 1,000 bootstrap replicates using MEGA 5.0 (5).

Sequence analysis revealed a ≥99.5% nucleotide identity and 100% amino acid identity among sequences determined in the...
present study. These sequences segregated in a host-specific clustering pattern (Fig. 3) compared to those of neuroinvasive herpesviruses that affect other species and were characterized as felid herpesvirus 1 sequences.

Brain and cerebellum were negative for *T. gondii* by a nested PCR for gene B1 of this protozoan (6). A positive control (RH sample [19]) and a negative control (UltraPure DEPC-treated water; Invitrogen, Carlsbad, CA) were used. *T. gondii* cysts were not observed in these tissues by histological analysis.

All tissue samples for total RNA extraction were prepared as 30% (vol/vol) suspensions in UltraPure diethyl pyrocarbonate (DEPC)-treated water (Invitrogen, Carlsbad, CA) and submitted to 3 freeze-thaw cycles in liquid nitrogen and at 56°C and clarified at 5,000 *g* for 15 min at 4°C. Aqueous humor and effusion cells were concentrated at 12,000 *g* for 15 min at 4°C. The total RNA was extracted from the supernatants of organs and pellets from aqueous humor and effusions with TRIzol reagent (Invitrogen, Carlsbad, CA), according to the manufacturer’s instructions.

All samples were screened for the presence of FCoV M gene mRNA using primers previously described (7), with modifications (2RNAmA, TAAATMCATARACGADCCAGCT, nucleotides [nt] 2,6440 to 2,6461; 2RNAmS, GTGCTAGVTTTGTCTTGGACAMC, nt 60 to 83) (positions referenced to feline infectious peritonitis virus [FIPV] strain 79-1179). A positive RNA control was prepared from the supernatants of organs and pellets from aqueous humor and effusions with TRIzol reagent (Invitrogen, Carlsbad, CA), according to the manufacturer’s instructions.

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Felic herpesvirus 1 (FHV-1) is a member of the *Varicellovirus* genus of the subfamily *Alphaherpesvirinae* (10). The infection causes mainly upper respiratory tract and ocular disease in cats worldwide and generally induces more-severe signs than other feline respiratory pathogens (11). Usually, the infection is followed by fever, depression, and anorexia. Primary pneumonia and a viremic state that can produce severe generalized signs and sometimes death have been identified in particularly susceptible kittens (12). This report describes a case of severe, diffuse, non-suppurative meningoencephalitis in a cat as a consequence of FHV-1 infection.

In the cat, FHV-1 replicates in epithelial cells of both the conjunctiva and the upper respiratory tract and causes infection of trigeminal ganglia. As for other alphaherpesviruses, peripheral nervous system infection leads to lifelong latency after primary infection (11,13). Although infections are often not associated with evident symptoms, severe disease (encephalitis and menigitis) can arise from the active replication of these viruses coupled with their propensity to spread within neural circuits (13). Pathological behavior similar to that observed in this study was reported in cats experimentally infected with equid herpesvirus 9, a highly neurotropic virus. Histologically, the cats showed severe encephalitis, moderate to severe menigitis, and slight interstitial pneumonia (14). Bovine herpesvirus 5 also induces similar histological lesions in cattle, rarely producing inclusion bodies but leading to lethal encephalitis in young animals (15).

Results of a recent study demonstrated that early primary FHV-1 viremia spread the virus to distant connective tissues (16), which could possibly explain the lack of histopathological lesions in the kidney and spleen despite the presence of FHV-1 DNA in these organs. In horses, neurotropic strains of equid herpesvirus 1 have been associated with a higher amplitude and longer duration of viremia, as well as enhanced neuropathogenicity (17, 18).

Other neuroinvasive herpesviruses are associated with life-threatening diseases in humans (herpes simplex virus type 1 and
type 2, varicella-zoster virus), in monkeys (monkey B virus [B-virus]), in pigs (Awjesky disease virus), and in poultry (gallid herpesvirus type 1) (13).

A broad assortment of neuroinvasive herpesviruses infects domestic and wild animals, but this is the first report of nonsuppurative meningoencephalitis caused by FHV-1 and demonstrates the pathogenic potential of this virus to cause central nervous system disorders of cats.

Nucleotide sequence accession numbers. The nucleotide sequences determined in this work have been submitted to GenBank under accession numbers JX559629 to JX559632.

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REFERENCES


