Case Report

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Huong (Amy) Huynh , Pamela Moraguez , Logan M Watkins , Jonathan H. Wood , Ximena A Olarte-Castillo , Gary R. Whittaker *

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Differential Diagnosis and Rapid Clinical Resolution of a Neurological Case of Feline Infectious Peritonitis (FIP) Using GS441524

Amy Huynh *,1, Pamela Moraguez*,2, Logan M. Watkins [3], Jonathan H. Wood [1], Ximena A. Olarte-Castillo [2] and Gary R. Whittaker [# 2, 3, 4]

Departments of Clinical Sciences [1], Microbiology & Immunology [2] and Public & Ecosystem Health [3] and Feline Health Center [4], College of Veterinary Medicine, Cornell University, Ithaca NY 14,853 USA; hh587@cornell.edu (H.H.); pamoraguez@gmail.com (P.M.); lmw255@cornell.edu (L.M.W.); jhw29@cornell.edu (J.H.W.); xao2@cornell.edu (X.A.O.C.)

* Correspondence: rw7@cornell.edu

* Contributed equally.

Abstract: Case summary: A 2-year-old male neutered domestic shorthair cat was presented with a progressive history of tetraparesis, ataxia, and inappetence over 4 days. A physical exam revealed mucopurulent nasal discharge and stertor. Neurologic exam revealed a multifocal neurolocalization. The cat was non-ambulatory tetraparetic and developed seizures while in-hospital. Hematologic assessment revealed anemia, hypoalbuminemia and hyperglobulinemia. Magnetic resonance imaging (MRI) of the brain revealed multifocal meningeal contrast enhancement in the brainstem and cervical spine, as well as mandibular and retropharyngeal lymphadenopathy. Cerebrospinal fluid revealed marked neutrophilic pleocytosis, no infectious organisms were seen. Toxoplasma IgG/IgM and cryptococcus antigen latex agglutination were negative. Mandibular and abdominal lymph nodes were aspirated, and cytology revealed mixed inflammation. Nanopore sequencing specifically identified FCoV-1 RNA in spinal fluid and anal swab, but not in urine. This method serves as a novel and rapid PCR sequencing technique for the antemortem diagnosis of feline infectious peritonitis. The cat was treated with anticonvulsants (phenobarbital and levetiracetam), an antibiotic (ampicillin/clavulanic acid), and GS-441524. Neurologic signs did not improve on an antibiotic alone but improved significantly after two subcutaneous injections of GS-441524. The cat received an 84-day course of GS-441524 and, at the time of manuscript preparation (over 12 months after diagnosis), remained ambulatory and seizure-free without recurrence of neurologic signs and no detectable viral shedding in feces.

Keywords: FIP; neurology; antiviral drug

Relevance and Novel Information

This report suggests a novel method for detecting and differentiating feline coronavirus RNA. Our Nanopore-based sequencing approach has the potential to be used as a rapid and cost-effective clinical test for an accurate and timely diagnosis of feline infectious peritonitis, including in the central nervous system. This report also demonstrates the rapid resolution of an FIP case using a relatively low dose of 10mg/kg SQ GS-441524.

Case Description

Presentation: A 2-year-old male neutered domestic shorthair cat (ID#352662) was presented following a 4-day history of tetraparesis, ataxia, and hyporexia. He had a history of an ear infection when he was 1-year-old that resolved with oral antibiotics. Post successful treatment, he maintained a residual right head tilt. He was an otherwise healthy indoor cat, had received routine vaccinations, and had no other major medical history.

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A general physical examination revealed mild serous ocular discharge, scant mucopurulent nasal discharge, stertor, and persistent tachypnea with increased bronchovesicular sound in all quadrants. A neurologic exam revealed a right head tilt and non-ambulatory tetraparesis. He had absent menace response in both eyes, absent placing and hopping in both thoracic limbs, and reduced hopping and placing in both pelvic limbs. The rest of the neurologic exam was normal. Based on the seizure and exam findings, the patient's neurolocalization was multifocal: forebrain, central vestibular, and C1-C5 myelopathy. Primary differential diagnoses were infectious (e.g., Toxoplasma gondii, Cryptococcocus neoformans), infectious-inflammatory (e.g. feline infectious peritonitis), and neoplasia (e.g., lymphoma) among other less likely causes of this constellation of clinical signs. The cat had a generalized seizure that was treated with a single dose of midazolam (Hospira) 0.3 mg/kg intravenously (IV) and started on levetiracetam (Auromedics) 30 mg/kg IV every 8 hours

A complete blood count revealed a normocytic normochromic non-regenerative anemia ((HCT 28%, MCV 398 fL, MCHC 31 g/dL, absolute reticuloytes 12,400/uL), inflammatory leukogram with left shift and mild toxic changes (segmented neutrophils 20,800/uL, band neutrophils 500/uL), concurrent stress leukogram (lymphocyte 500/uL, monocytes 2000/uL), and mild thrombocytopenia (161,000/uL). Biochemistry revealed mild hypoalbuminemia (2.5 g/dL), severe hyperglobulinemia (6.8 g/dL) with albumin-to-globulin ratio of 0.4, mildly elevated AST (61 U/L), and moderate hypercholesterolemia (149 mg/dL). Thoracic radiographs revealed a moderate diffuse bronchial pattern and sternal lymphadenopathy. The patient was placed under general anesthesia and magnetic resonance imaging (MRI) of the brain was performed. The MRI (1.5 T, Siemens Skyra, Malvern, Pennsylvania) revealed multifocal meningeal contrast enhancement of the brainstem and cervical spine as well as mandibular and retropharyngeal lymphadenopathy. Cerebrospinal fluid (CSF) was acquired at the atlantooccipital space. CSF analysis revealed marked neutrophilic pleocytosis with a total nucleated cell count of 583 cells/uL, red blood cell 8 cells/uL, and total protein 235 mg/dL. No infectious organisms were seen. CSF culture and sensitivity was not performed.

Fine needle aspirates (FNAs) of the left mandibular lymph node were performed, and cytology revealed mixed inflammation and reactive lymphoid tissue with a moderate amount of contracted purple material that is occasionally surrounded by clear space, concerning for Cryptococcus versus necrotic debris. Cryptococcus antigen latex agglutination and Toxoplasma IgG/IgM antibodies were performed on serum and were negative. Abdominal ultrasound revealed moderate lymphadenopathy of the mesenteric (jejunal, ileocolic), pancreaticoduodenal, and right medial iliac lymph nodes. Innumerable small hypoechoic splenic nodules with normal splenic size and a mass in the right ventral aspect of the liver that distorted the hepatic margin were noted. Both kidneys had poor corticomedullary distinction. FNAs of the jejunal lymph nodes revealed mild non-degenerative neutrophilic inflammation. FNAs of the liver mass revealed marked pyogranulomatous inflammation and hepatocytes with mild atypia and mild vacuolar change.

Molecular Diagnosis: Samples of the cerebrospinal fluid, urine, and anal swab were collected for FCoV-1 screening by PCR followed by next-generation sequencing using the Oxford Nanopore technology, via a research study at Cornell University. The anal swab was placed in DNA/RNA Shield (ZYMO Research) for a week at 4°C, and then frozen at -20°C for further use. The CSF and urine samples were immediately processed and then stored frozen at -80°C. Total RNA was extracted as previously published [1], and FCoV screening was performed by real-time RT-PCR using previously described primers/probe [2] and the iTaq Universal probes one-step kit (BIORAD). This real-time RT-PCR assay was also used to quantify the FCoV-1 RNA load using an RNA standard of FCoV-1 quantified by ddPCR as previously described [3]. This quantification was performed in duplicates. FCoV-1 RNA was detected in the CSF and anal swab samples, but not in the urine sample (Table 1). Four additional fecal samples or anal swabs were collected 7, 15, 17, and 252 days after the initial treatment with GS-441524 (Table 1). FCoV-1 RNA was not detected in any of these samples (Table 1).

For the two samples that tested positive for FCoV-1 (CSF and anal swab), a partial region of the spike (S) gene that includes three regions of relevance for pathogenicity (S1/S2 and S2' cleavage sites,

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and the "1058" residue), was amplified using the primers 1263F (5'- TCCTTTCTCACCACAGCAGT-3') and 1263R (5'- TGCATAGCGAAAGGAACAGC-3'). For this purpose, cDNA was synthesized using the LunaScript® RT SuperMix Kit (NEB), and the PCR was performed using the Phusion Hot Start II High-Fidelity Master Mix (Thermo Fisher Scientific). The resulting amplicons were cleaned using the QIAquick PCR Purification Kit (Qiagen), barcoded using the Native Barcoding Kit 24 V14 (Oxford Nanopore Technologies, ONT), and sequenced in the MinION Mk1B (ONT) using a Flongle Flow Cell R10.4.1 (ONT). After sequencing, super-accurate basecalling was performed using the Dorado v7.1.4 basecaller in the MinKNOW™ software. The resulting sequences were aligned and translated to amino acid sequences Geneious Prime 2023.0 (Dotmatics). The obtained nucleotide sequences were uploaded to GenBank with accession numbers PQ565822 and PQ565823. The sequences of the partial region of the S gene (1,693nt) obtained from the anal swab and CSF were 98.3% similar. The sequence of the S1/S2 and S2' cleavage sites were identical in the two samples and confirmed the presence of FCoV-1 but did not reveal any mutations indicative of a high pathogenicity virus (Table 1). However, one of the nucleotide differences between the sequences is an A->T mutation in position 1,495, resulting in an L residue in site "1058" in the sample from the CSF and an M in the anal swab sample. RNA quantification revealed a lower viral RNA load in the CSF (109.94 ± 0.6 copies/µl, Ct 33.95) than in the anal swab (1,635.9 ± 168.7 copies/µl, Ct 30.05).

Table 1. Summary of the samples collected and the result of the FCoV-1 screening.

Sample collection date	*Sample type*	*FCoV-1 status*	*Amino acid sequence of the S1/S2 furin cleavage site*	*Amino acid sequence of the S2' cleavage site*	*Amino acid residue at position "1058"*
*Aug 29th 2023**	*CSF*	***Positive***	***SRRSRR|STSESV***	***KR|S***	***L***
*Aug 29th 2023**	*Anal swab*	***Positive***	***SRRSRR|STSESV***	***KR|S***	***M***
*Aug 29th 2023**	*Urine*	*Negative*	*N/A*	*N/A*	*N/A*
Sept. 5th 2023	*Feces*	*Negative*	*N/A*	*N/A*	*N/A*
Sept.13th 2023	*Feces*	*Negative*	*N/A*	*N/A*	*N/A*
Sept.15th 2023	*Anal swab*	*Negative*	*N/A*	*N/A*	*N/A*
May 7th 2024	*Anal swab*	*Negative*	*N/A*	*N/A*	*N/A*

*indicates samples that were taken before the treatment with GS-441524.

Treatment: In hospital, the cat was maintained in oxygen supplementation due to persistent tachypnea, which resolved after 24 hours of supplementation. Ampicillin/sulbactam (Unasyn, West-Ward Pharmaceutical) was started at 30 mg/kg IV every 8 hours due to the inflammatory leukogram with left shift and the cat was switched to amoxicillin/clavulanic acid (Clavamox, Zoetis) at 14 mg/kg by mouth every 12 hours prior to discharge. Levetiracetam (Auromedics) at 30 mg/kg every 8 hours was also started in-hospital and he was discharged with levetiracetam (oral suspension, Camber) at 35 mg/kg every 8 hours. He had another generalized seizure in hospital and was also started on phenobarbital (Cameron) with a loading dose of 4 mg/kg IV every 6 hours for 4 doses, transitioned to 2 mg/kg IV every 12 hours, and was discharged with phenobarbital (Cornell Pharmacy compounded oral suspension) at 2 mg/kg every 12 hours. The owner elected to discharge the cat so that he could receive GS-441524 injections. At the time of discharge, the cat remained non-ambulatory tetraparetic.

The cat received the first injection of GS-441524 on the evening of discharge, sourced by the owner. Based on the owner's communications, the cat received GS-441524 at a dose of 10 mg/kg subcutaneously once daily. The owner sent a video update showing the cat's condition had improved significantly and was now ambulatory tetraparetic with proprioceptive ataxia. At this point, the cat had received 2 injections of GS-441524 at the dose stated above.

Follow up: The patient was rechecked 15 days after his initial discharge. Physical exam revealed resolved ocular and nasal discharge but persistent stertor. The neurologic exam revealed that he was ambulatory without paresis but had persistent proprioceptive ataxia, absent menace in both eyes, and had a persistent right head tilt. The patient had no further witnessed seizure activity since

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discharge. A complete blood count revealed a resolved inflammatory leukogram. A serum biochemistry revealed improved hypoalbuminemia (3.0 g/dL) and hyperglobulinemia (5.1 g/dL) with albumin-to-globulin ratio of 0.6. His phenobarbital level was 18 ug/mL (therapeutic range 10-30 ug/mL). Fecal samples swabs were collected seven, thirteen, and fifteen days after antiviral treatment for FCoV-1 screening as described above. None of these three samples was positive for FCoV-1 (Table 1). No additional samples of spinal fluid were taken due to no clinical indication to perform this test, given the patient's improvement and the risk of generalized anesthesia required to obtain further CSF samples. Amoxicillin/clavulanic acid (Clavamox, Zoetis) was discontinued, and the cat was maintained on phenobarbital and levetiracetam at the same doses listed above. The cat was maintained on subcutaneous injection of GS-441524 of 10 mg/kg for 22 days and then transitioned to oral GS-441524 with the same dose thereafter.

At an 11-week recheck exam the cat continued to be mildly stertorous, and the remainder of the physical exam was normal. The neurologic exam revealed that he continued to be ambulatory without paresis but has persistent proprioceptive ataxia, absent menace in both eyes, and right head tilt. A complete blood count showed new eosinophilia (4100/uL), and biochemistry revealed normalized albumin and globulin levels. Cholesterol level continued to be low (109 mg/dL). He was incidentally found to be positive for feline leukemia virus and giardiasis and negative for feline immunodeficiency virus and heartworm. The cat had remained seizure-free since discharge. The cat was no longer on levetiracetam as the client had stopped giving this medication after one month. He was continued on phenobarbital at the same dose, started on fenbendazole (oral powder, Merck) at 50 mg/kg once daily for 5 days, and started on a hydrolyzed diet (Purina Pro Plan HA, St. Louis, Missouri). Oral GS-441524 was continued for a full 84-day course of treatment.

At a 6-month recheck, the cat had persistent mild stertor and the rest of the physical exam was normal. The neurologic exam revealed that he was ambulatory without paresis or ataxia, persistent absent menace in both eyes and persistent right head tilt. A complete blood count and biochemistry were both within normal limits. Conjunctival and rectal swabs were obtained and were negative for FCoV RNA (Table 1). Phenobarbital was tapered off due to no seizures since discharge from the hospital.

The cat remained ambulatory without paresis or ataxia and was seizure-free during the 12-month follow-up period.

Discussion

Feline infectious peritonitis (FIP) is relatively common in young cats with neurologic signs [4]. This disease presents challenges for clinicians, particularly in early diagnosis and establishing effective therapeutic interventions. Clinical management of FIP increasingly involves treating affected cats with antiviral drugs [5-7]. Currently, IV or SQ formulations of GS-441524 are not legally available, although IV Remdesivir® has been used as an alternative nucleoside analog [6] and remains in use in specific cases. Oral GS-441524 formulations are now routinely available, and their efficacy has been demonstrated in multiple studies [8,9]. Cats exhibiting neurological signs, however, are relatively understudied and often require higher doses and extended treatment durations [7]. Here, we report a case of rapid diagnosis and clinical resolution in a cat with confirmed central nervous system FIP, treated with owner-sourced antiviral GS-441524. We are also describing a novel method for a rapid antemortem diagnosis of FIP.

In this study, we employed Nanopore-based next-generation sequencing to detect FCoV-1 in the cerebrospinal fluid (CSF) of a cat with neurological symptoms. Previous studies from our lab used Sanger-based sequencing [10,11] which posed delays due to the need for centralized processing. Here, we demonstrated the use of portable MinION (Oxford Nanopore) sequencing at a clinical facility, offering significant advantages in speed, cost, and convenience, allowing for rapid screening for FCoV-1. The PCR method in this study targeted the S1/S2 cleavage site (the "furin cleavage site") in the spike domain D, the S2' region adjacent to the fusion peptide, and residue "1058" (residue 1046 in reference strain UU4 [12], allowing robust differentiation between type 1 and type 2 viruses. Results suggested a low-pathogenicity virus, indicated by non-mutated cleavage sites compared to

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reference FECV strains, with mutation "M1058L" consistent with systemic spread. Additional sequencing may reveal further mutations in other genomic "hot spots." RNA quantification indicated a low FCoV-1 RNA load in the CSF (109.94 ± 0.6 copies/µl, Ct 33.95), suggesting an early infection stage where the virus had not yet acquired mutations associated with macrophage tropism and widespread tissue distribution. A previous study also detected very low viral loads of FCoV in the CSF of cats with confirmed FIP and displaying various signs [13] . Due to the low viral load, detection of mutations on S was only possible in 10% of the screened samples, all of which had the M1058L mutation. Another study also found low viral loads in the CSF, indicated by high Ct values in the qPCR (>26.5), in cats showing neurological signs. Nevertheless, qPCR in CSF was highly specific for detecting FCoV in the central nervous system, confirming an FIP diagnosis [14].

While lymph node FNAs were collected, they were unavailable for this study. However, they could potentially be predictive for diagnosis via cytology or molecular methods [15,16] and could serve to validate our CSF sequencing results, offering insights into FCoV-1 evolution in this case, and mutational changes associated with FCoV-1 pathogenesis [17]. We did not assess ORF3 or ORF7 due to their limited predictive value in FIP diagnosis. This study underscores the utility of evaluating both "low-path" and "high-path" viral sequences alongside clinical parameters. In a previous study from our lab, we identified mutations in the S1/S2 cleavage site consistent with an "internal mutation" of FCoV-1 [18]; however, this was in a fatal neurological case with virus evolution in macrophage-like cells within the neural tissue but not in other organs.

The cat in this study achieved clinical and diagnostic remission by the time of publication. The patient will continue to be monitored to assess the long-term efficacy of GS-441524 in suppressing FCoV replication and shedding.

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Conflicts of Interest: We declare no conflicts of interest.

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Ethical approval: The work described in this manuscript involved the use of non-experimental (owned or unowned) animals. Established internationally recognized high standards ('best practice') of veterinary clinical care for the individual patient were always followed and/or this work involved the use of cadavers. Ethical approval from a committee was therefore not specifically required for publication. Although not required, where ethical approval was still obtained, it is stated in the manuscript.

Informed consent: Informed consent (verbal or written) was obtained from the owner or legal custodian of all animal(s) described in this work (experimental or non-experimental animals, including cadavers, tissues and samples) for all procedure(s) undertaken (prospective or retrospective studies). No animals or people are identifiable within this publication, and therefore additional informed consent for publication was not required.

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