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Communication

Natural Infection of Domestic Dogs with Raccoon Dog and Fox Amdoparvovirus Resulting in a Severe Disease Outbreak

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Simple Summary

A severe disease affected several Dobermann dogs in a kennel in Serbia, near the city of Novi Sad. Symptoms in the affected dogs included eye and nasal discharge, weight loss, poor coat condition, liver abnormalities and, in some advanced cases, nervous system signs. Routine testing did not identify any of the common pathogens that could explain the disease. The aim of this study was to determine if the cause of this disease is infectious in nature. Using a method that examines all genetic material in a sample, we detected a virus previously reported in raccoon dogs and foxes. Further laboratory testing confirmed the presence of this virus in several affected dogs and in blood, urine and multiple organs, including the kidney, lung, spleen and brain. Healthy dogs from unrelated locations tested negative. These findings provide the first evidence that domestic dogs can become naturally infected with this virus. The results are important for veterinarians because they highlight a possible new infection in dogs and the need to investigate virus transmission between wildlife and domestic animals.

Abstract

Raccoon dog and fox amdoparvovirus (RFAV) has been reported in raccoon dogs and foxes, but natural infection in domestic dogs has not previously been documented. During March-April 2026, samples from affected Dobermann dogs from a kennel near Novi Sad, Serbia, were submitted for laboratory investigation. After negative testing for canine adenovirus, canine coronavirus, herpesvirus, parvovirus, distemper virus, influenza A virus, and leptospirosis, metagenomic sequencing was performed on selected tissues, followed by bioinformatic analysis and targeted RFAV PCR screening of additional outbreak-associated samples. Affected dogs had prolonged illness characterized by conjunctivitis with ocular and nasal discharge, occasional blue eye appearance, progressive weight loss, poor coat quality, biochemical evidence of hepatic injury, and neurologic signs including paraplegia in advanced cases. Sequencing generated 434,220 reads and identified multiple RFAV hits; pooled assembly produced a 4,799-bp consensus genome with approximately 97% similarity to known RFAV strains and genome organization consistent with the genus *Amdoparvovirus*. RFAV DNA was subsequently detected by virus-specific PCR in a epidemiologically linked dog and across diverse specimen types including blood, urine, kidney, spleen, brain, lung, testicle, ileocecal lymph node, and throat swabs, whereas clinically healthy unrelated dogs were PCR-negative.

Keywords: amdoparvovirus; infection; canis lupus familiaris; metagenomic analysis; sequencing; cross-species transmission; novel host; wildlife; spillover; Serbia

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1. Introduction

Amdoparvoviruses are members of the family *Parvoviridae*, subfamily *Parvovirinae*, genus *Amdoparvovirus* [1]. They comprise a growing group of carnivore-associated viruses with small genomes of approximately 4.8 kb containing major open reading frames encoding nonstructural and capsid proteins [2]. The prototype amdoparvovirus, Aleutian mink disease virus (AMDV), is the best-characterized member and causes persistent infection with outcomes ranging from subclinical infection to fatal inflammatory disease in mink and related hosts [2]. Amdoparvoviruses are epidemiologically important because they are multi-host pathogens and several members of the genus are capable of crossing host species barriers [2-4. Cross-species transmission has been documented for AMDV, skunk amdoparvovirus (SKAV), Labrador amdoparvovirus 1, and other recently described carnivore amdoparvoviruses, indicating that host plasticity is a recurring feature of this genus rather than an exception [3,4]. This broad host range is thought to be linked to macrophage tropism and antibody-dependent enhancement of infection through Fc receptor-mediated uptake of antibody-coated virions [4,5]. Amdoparvoviruses have been identified in mink, skunks, foxes, raccoon dogs, red pandas, martens, badgers, and felids, underscoring expanding host breadth [1–8]. Similar or closely related viruses have infected multiple carnivore hosts in wildlife systems, including spillover into non-maintenance hosts [6,7]. Amdoparvoviruses are linked to wasting syndromes, nephritis, vasculitis, hepatitis, pneumonia, and neurologic disease, though severity varies by host and strain [2,8,9]. Viral persistence, shedding, environmental stability, and shared habitats can create opportunities for spillover to sympatric wildlife and domestic animals [5,6]. RFAV is one of the amdoparvoviruses recognized in canids and has previously been reported in raccoon dogs and foxes [10]. The epidemiology, tissue tropism, and disease expression of newly described amdoparvoviruses outside mink remain incompletely defined [6,9]. Natural spillover of pathogens between wildlife and domestic carnivores has become a recurring concern in veterinary and conservation medicine [11,12] Studies of carnivore viruses at human-modified or farm-linked interfaces show that domestic animals, wildlife, and captive populations can exchange pathogens, although transmission intensity varies by virus, host ecology, and contact structure [2,12,13]. Against this background, unexplained outbreaks in dogs with negative routine diagnostic testing warrant investigation for atypical or previously unrecognized agents. The present study investigated a severe outbreak of prolonged multisystemic disease in a Dobermann kennel in Serbia and aimed to identify the etiologic agent using metagenomic sequencing, characterize the detected virus genomically, and assess its distribution among affected dogs and specimen types.

2. Materials and Methods

This study was an outbreak investigation of a severe naturally occurring disease affecting epidemiologically linked Dobermann dogs kept by a professional dog trainer near Novi Sad, Serbia, during March-April 2026. The investigation combined conventional diagnostic testing, untargeted metagenomic sequencing, genome assembly and characterization, and targeted PCR screening of additional samples collected during outbreak follow-up.

2.1. Sampling and Animals

Initial submissions included kidney and whole blood from one affected dog and samples from an adult female dog and her two puppies; liver and lung tissues were also received from the latter animals. As the outbreak progressed, additional cases were recognized and a subsequent submission from another dog included kidneys, liver, spleen, lungs, brain, intestines, tonsils, ileocecal lymph nodes, whole blood, urine, and throat and rectal swabs Clinically affected dogs exhibited prolonged disease with ocular and nasal discharge, conjunctivitis, occasional blue eye appearance, progressive weight loss, poor coat quality, biochemical evidence of hepatic injury, and neurologic signs in advanced stages.

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2.2. Diagnostic Testing

Because the clinical syndrome was nonspecific, testing for several common canine infectious diseases was performed. Samples tested negative on repeated occasions for canine adenovirus, canine coronavirus, herpesvirus, parvovirus, distemper virus, influenza A virus, and leptospirosis.

2.3. Nucleic Acid Extraction and Metagenomic Sequencing

After routine tests were unrevealing, metagenomic high-throughput sequencing was used to search for a candidate etiologic agent. Nucleic acids were extracted using the IndiSpin Pathogen Kit (Qiagen, Germany). DNA was extracted from lung, kidney, and liver tissues from an affected dam and pup. First-strand cDNA synthesis was performed using the RevertAid First Strand cDNA Synthesis Kit (Thermo Fisher Scientific, USA) with random hexamer primers. DNA was quantified using a Qubit 4.0 instrument, and libraries were prepared using the Rapid Sequencing Kit V14 (Oxford Nanopore, England) according to the manufacturers' instructions. Sequencing was conducted on an Oxford Nanopore MinION Mk1D device.

2.4. Bioinformatics and Phylogenetics

A total of 434,220 sequencing reads were generated. Reads were filtered using NanoFilt (15) with quality threshold ≥9 and minimum length ≥200 bp. Host reads were removed by mapping against the dog reference genome (NCBI GenBank accession no. NC_049222.1) using minimap2[16] and samtools [17]. Cleaned reads were searched against a custom viral-only DIAMOND [18] database, which yielded multiple amdoparvovirus hits in each sample. De novo assembly with flye [19] yielded two contigs, and BLASTn analysis showed approximately 97% similarity to raccoon RFAV. To improve assembly completeness, clean reads from all three samples were pooled and assembled, followed by two polishing rounds with Medaka [20] to produce a final 4,799-bp consensus genome. The consensus sequence was deposited in GenBank under accession number PZ348355. BLASTn reanalysis showed approximately 97% similarity to known RFAV sequences, and genome organization was consistent with amdoparvoviruses, including two major ORFs encoding NS1 and VP proteins and an additional smaller ORF. Genome alignment was performed using mafft [21], the phylogenetic tree was built using iqtree [22] and the resulting analysis was viewed with ITOL [23].

2.5. PCR Screening

Following metagenomic identification of RFAV, a virus-specific PCR described previously [10] was applied to additional samples collected during the outbreak investigation. PCR used the HotStarTaq Master Mix Kit (Qiagen, Germany) with annealing at 53 °C for 30 seconds. Each 25-µL reaction contained 12.5 µL HotStarTaq Master Mix, 0.8 µM of each primer, and 5 µL DNA template.

3. Results

Clinical illness in affected Dobermann dogs was prolonged and multisystemic rather than peracute. Reported manifestations included conjunctivitis with ocular and nasal discharge, occasional corneal opacity described as blue eyes, progressive weight loss, poor coat quality, biochemical evidence of hepatic injury, and neurologic abnormalities including paraplegia in advanced disease. Repeated targeted testing did not identify common canine viral or bacterial causes considered relevant to the presentation. Samples were negative for canine adenovirus, canine coronavirus, herpesvirus, parvovirus, distemper virus, influenza A virus, and leptospirosis on several testing occasions. Metagenomic sequencing was pursued after routine diagnostics were repeatedly negative. De novo assembly from lung and liver samples yielded contigs with approximately 97% BLASTn similarity to raccoon dog and fox amdoparvovirus. Pooling of cleaned reads enabled reconstruction of a polished 4,799-bp consensus genome, and genome annotation showed the expected amdoparvovirus organization with major ORFs encoding NS1 and VP proteins plus a smaller ORF. Phylogenetic analysis supported classification of the strain as a separate RFAV

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variant (Figure 1). Targeted PCR extended the metagenomic finding to additional outbreak-associated animals and specimen types. RFAV DNA was detected in whole blood, urine, kidney, spleen, brain, lung, testicle, ileocecal lymph node, and throat swabs from a epidemiologically linked Dobermann dog. In contrast, clinically healthy dogs from unrelated locations and different breeds were PCR-negative, and no amplification was seen in tested samples from birds, pigs, bovines, or cats used during assay evaluation.

4. Discussion

This study provides evidence that domestic dogs can be naturally infected with RFAV originally described in farmed raccoon dogs and arctic foxes, while subsequent studies identified RFAV or closely related viruses in additional carnivore hosts [6,10]. Detection in domestic dogs therefore further expands the recognized host range of this virus. Infection of dogs is biologically plausible given the multi-host ecology of amdoparvoviruses. Closely related viruses have been detected in different carnivore species, including maintenance and spillover hosts [3,5,6]. However, the mechanism enabling RFAV infection of dogs was not investigated in the present study. The clinical presentation of the affected dogs showed some similarities to disease associated with other amdoparvoviruses. Wasting and renal involvement have been described in Aleutian disease in mink and ferrets, while renal, vascular and neurological lesions have been associated with SKAV infection in striped skunks [8,13,24]. Meningoencephalitis and viral detection in the brain have been reported in AMDV-infected mink and SKAV-infected skunks [8,24]. These observations provide a relevant comparison with the paraplegia and brain PCR positivity described above. Detection of RFAV DNA in blood, urine and multiple internal tissues, including lymphoid tissue, kidney, lung and brain, supports systemic viral distribution. Detection in throat swabs and urine is epidemiologically relevant because SKAV has been localized to gastrointestinal, urinary-tract and skin epithelium, suggesting several potential routes of shedding [7]. Several limitations should therefore be considered. The investigation included a limited number of outbreak-associated animals, and complete clinical and pathological data were not available for all dogs. The study did not include histopathological colocalization of RFAV with lesions, viral isolation, serological testing or longitudinal measurement of viral loads. The findings therefore establish natural RFAV infection in domestic dogs and identify a plausible association with multisystemic disease.

5. Conclusions

We identified a RFAV variant in multiple Dobermann dogs during a severe disease outbreak in Serbia, providing evidence that domestic dogs can become naturally infected with RFAV. The detection of viral DNA in multiple tissues and epidemiologically linked animals suggests that RFAV is associated with causing clinical disease in dogs and highlights the potential for cross-species transmission among carnivores. Additional studies are underway to determine the pathogenicity, epidemiology, and geographic distribution of RFAV in domestic and wild canids in Serbia.

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Conflicts of Interest: The authors declare no conflicts of interest.

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