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ORIGINAL PAPER

Canine distemper virus surveillance and sequencing in invasive feral American mink (*Neogale vison*) in Poland

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Abstract Canine distemper virus (CDV) is a significant pathogen with high mortality rate affecting carnivore species worldwide, including both wild and farmed mink populations. Invasive species have been implicated in the spread of CDV, potentially facilitating viral spillover into new populations and exacerbating conservation challenges, especially among wild carnivores. This study investigates the presence of CDV in wild American mink (*Neogale vison*) populations across Poland. Carcasses of 1,205 mink, collected between 2007 and 2021 were examined, through *post-mortem* monitoring. Two mink spleen samples from a different year and area were retrospectively confirmed positive for CDV RNA, and subsequent phylogenetic analysis of the partial Hemagglutinin gene identified both sequences as belonging to the Europe lineage. This finding suggests that American mink can be infected with endemic CDV strains, potentially expanding the spectrum of susceptible host species in the region. The results highlight the need for further research into the ecological implications of invasive species in the spread of infectious diseases within vulnerable ecosystems. Integrated wildlife health monitoring and targeted management interventions are essential for understanding and mitigating the risks posed by biological invasions to wildlife health and biodiversity conservation. These findings offer important insights into the potential role of the invasive American mink in CDV transmission in Europe.

Keywords *Paramyxoviridae* · *Morbillivirus* · *Morbillivirus canis* · Biodiversity conservation · Disease ecology · Wildlife epidemiology

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Introduction

The introduction of invasive species is one of the threats affecting biodiversity (Maxwell et al. 2016). In addition to the various negative impacts on native species, invasion species are often associated with the spread of pathogens potentially altering disease dynamics in ecosystems (Daszak et al. 2000). The American mink (*Neogale vison*) is native to North America and is an invasive alien species in Europe,

Asia and South America (Zalewski and Brzeziński 2026). In Europe, this species is still extensively farmed and has established feral populations. It has become a notable host and amplification host for various viruses, affecting both farmed and feral populations (Liang et al. 2024). Mink pelt producing farms pose therefore significant challenges, including the risk of disease outbreaks and escape of farmed mink, which can transmit diseases to both native species and invasive feral populations, thereby affecting local ecosystems (Zhao et al. 2024).

One disease that is widespread around the world is canine distemper virus (CDV) belongs to the family *Paramyxoviridae*. Multiple genetic lineages of CDV have been identified, primarily based on geographic distribution, with most classifications relying on sequence variation in the viral hemagglutinin (H) gene, due to its high genetic variability and critical role in viral entry and host specificity (Martella et al. 2008). It has been linked to outbreaks and various population declines (Williams et al. 1988; Fournier-Chambrillon et al. 2022), particularly in regions where domestic and wild carnivores interact (Forsyth et al. 1998; Viana et al. 2015). CDV is a significant pathogen impacting several carnivorous species with a high mortality rate, especially within the Mustelidae family. In Europe, for instance, outbreaks of CDV among mink farms in countries such as Denmark, Belgium, and the Netherlands have caused considerable concern. These outbreaks are particularly worrying due to the risk of virus transmission from farmed mink to populations wild native species, further complicating ecosystem stability and conservation efforts (Trebbien et al. 2014; Gregers-Jensen et al. 2015; Molenaar and Buter 2018). Therefore, monitoring of infestation levels in feral and farmed mink is the basis for determining the risk of native species.

In the late 1970s, feral American mink were introduced in Poland, in the north-east of the country. Since then, the mink has spread throughout practically all of Poland, with the exception of the southernmost regions (Brzeziński et al. 2019). In their new range, American mink have used a variety of riparian habitats, such as lakes, rivers, and streams, fish ponds, and seashore (Brzeziński et al. 2018; Zalewski and Brzeziński 2026). In these habitats, American mink coexists with Eurasian otter (*Lutra lutra*), European polecat (*Mustela putorius*), partly with pine marten (*Martes martes*), raccoon dog (*Nyctereutes procyonoides*), red fox, and European badger (*Meles meles*) (Zalewski et al. 2025). The habitat and diet niches highly overlapped with some species, e.g., with the polecat (Zalewski et al. 2021a; Brzeziński et al. 2021). At the same time, the breeding of American mink on farms was growing in Poland, and in 2013 there were already 361 farms in Poland with over 5.7 million mink in the second part of the breeding season (Zalewski et al. 2021a). The number of escapees from mink farms in the feral local population depends on the number of mink kept on the farm in a given region (Zalewski et al. 2010). Furthermore, farms are mainly located close to villages, and most often at the border of natural habitats used by wild carnivores, potentially allowing the transmission of diseases between farmed and wild animals.

In Poland, the presence of canine distemper virus has been detected since the 1940s; however, some researchers suggest that the establishment of large-scale American mink and Arctic fox (*Vulpes lagopus*) farms in the 1950s coincided with a notable increase in reported cases of CDV in different species such as domestic dogs and ferrets (*Mustela furo*) (Rzeżutka and Mizak 2002). Sequence analysis of the CDV phosphoprotein (P) gene from samples collected between 1998 and 1999 provided early insight into the genetic diversity of circulating lineages (Rzeżutka and Mizak 2003). Subsequent investigations of the nucleoprotein (N) gene from foxes, minks, and dogs further demonstrated the presence of distinct CDV variants among different host species (Adaszek et al. 2009). Surveillance of CDV antibody titers among dogs in Warsaw, conducted since the 1990s, highlights the critical role of vaccination in conferring immunity, suggesting that widespread vaccination efforts have likely contributed to lower infection rates in urban dog populations (Jóźwik and Frymus 2002; Jóźwik et al. 2004).

The aim of this study was to assess the presence of canine distemper virus in feral American mink collected across Poland over a 15-year period. The study also focused on identifying the virus lineage through sequencing to better understand the genetic background of the virus. By examining a large sample of carcasses, this research aimed to provide valuable surveillance data on the prevalence of CDV in feral mink and to offer insights into the specific viral lineage circulating in Eastern Europe.

Materials and methods

Study area and animal tissues

American mink carcasses were collected across Poland between 2007 and 2021 (Fig. 1). Most of the specimens were collected from sites where feral mink were eradicated as part of nature protection plans within bird conservation projects, under permission granted by local and government authorities. In addition, some mink were collected as roadkill. No animals were killed specifically for this study. Mink carcasses were frozen and stored at −20 °C before dissection. Next, the mink were dissected, and the spleen samples were extracted and frozen at −20 °C until further processing. A total of 1,205 spleen samples were collected from the carcasses (473 from females and 732 from males) and subjected to further molecular virological analyses. An average of 80 samples per year (range 1–297) were analyzed. The age of 1,194 mink were determined by the annual growth of dental cement layers carried out at Matson Laboratory (PO Box 308, Milltown, MT 59851, USA).

Nucleic acid extraction and RT-PCR reactions

Approximately 5 g of spleen tissue was homogenized in 500 µL of phosphate-buffered saline (PBS) using the TissueLyser II (Qiagen, Hilden, Germany) at maximum speed for 3 min. Two glass beads were added to each sample to enhance tissue disruption. After a brief centrifugation, 100 µL of the supernatant was used for RNA extraction with the Monarch Total RNA Miniprep Kit (NEB, Ipswich, MA, USA). The extracted nucleic acids were analyzed using a CDV-specific real-time PCR assay [10]. All PCR reactions were conducted with the OneTaq® One-Step RT-PCR Kit (NEB, Ipswich, MA, USA), following the manufacturer's protocol.

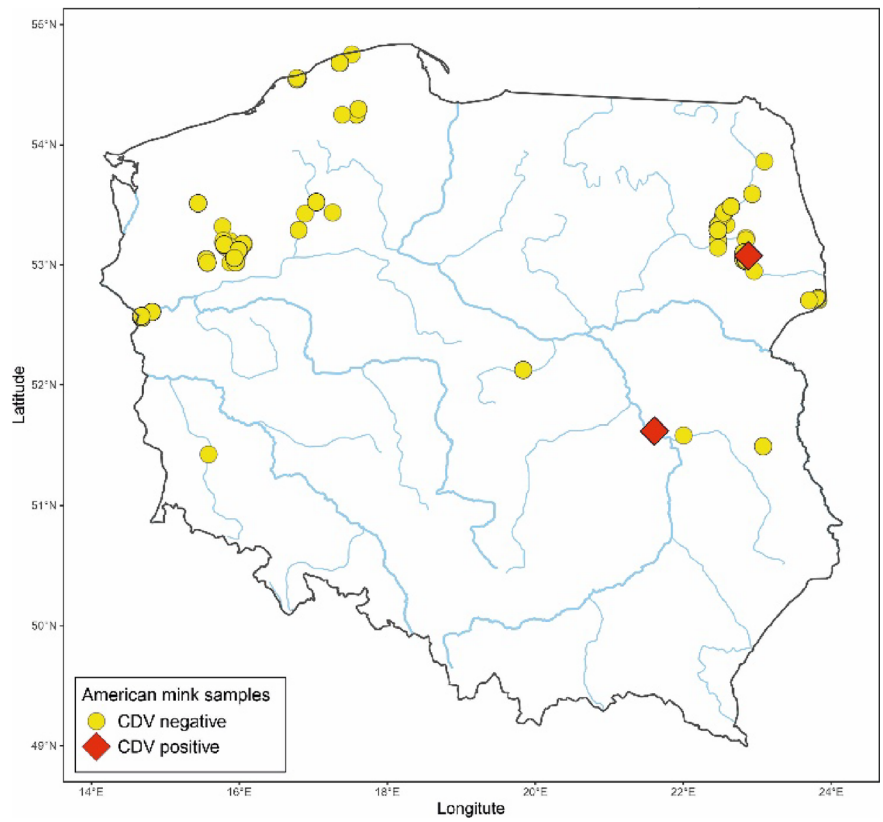

Fig. 1 Geographic distribution of American mink samples collected in Poland between 2007 and 2021. Each yellow circle represents a location where CDV negative individuals were collected, while the red diamond indicates the CDV positive case detected. Blue lines indicate main rivers

MinION sequencing and genome assembly

Complete genome sequencing was conducted using MinION nanopore sequencing technology (Oxford Nanopore Technologies, Oxford, UK). An amplicon-based sequencing approach, which we previously developed and published specifically for CDV genome sequencing (Lanszki et al. 2022c), was employed. Library preparation, sequencing, and in-run basecalling on the MinION platform were carried out following the same methodology. The sequencing was performed for 72 h on a r10.4.1 flow cell (Oxford Nanopore Technologies, Oxford, UK) with the Native Barcoding Kit 96 V14 (Oxford Nanopore Technologies, Oxford, UK). The complete protocol is available on our laboratory's protocols.io page (2021).

Post-run basecalling was carried out with guppy_basecaller (v6.5.7) applying the appropriate super accurate model (dna_r10.4.1_e8.2_400bps_sup.cfg), and demultiplexing of reads by guppy_barcoder (v6.5.7) with the appropriate barcoding kit selected (SQK-NBD114.96). For the quality check of the reads we utilized the NanoPlot software (v1.32.1), and for the filtering of the reads NanoFilt software (v2.8.0) was used. The processed reads were mapped to OP209189.1 with minimap2 (v2.24) and polished consensus was called with medaka (v2.0.1).

Sanger sequencing

The Hemagglutinin (H) gene of CDV was sequenced using a four-overlapping amplicon PCR strategy, targeting the entire H gene. Previously published primer sets (Sekulin et al. 2011) were employed, complemented with additional primers (Lanszki et al. 2021). Amplification was performed under optimized conditions to ensure robust and specific target amplification. The resulting PCR products were purified and subsequently sent to the Microsynth company for Sanger sequencing. The obtained sequences were analyzed using nucleotide BLAST against the NCBI GenBank database to determine their similarity to reference sequences.

Phylogenetic analysis

Before constructing the phylogenetic tree, homologous sequences were obtained from the GenBank database and aligned with our sequences using the MUSCLE alignment algorithm (Edgar 2004). The finalized dataset consisted of 64 partial H gene sequences with a total length of 421 nucleotides. A maximum likelihood phylogenetic tree was generated using IQ-TREE 2 with the K3Pu + F + G4 substitution model, determined through model selection based on information criteria (BIC and corrected AIC) (Minh et al. 2020). Node support was assessed using 1000 bootstrap replicates. The resulting tree was subsequently refined and visualized using the iTOL online platform (Letunic and Bork 2021).

Results

RT-PCR screening

A total of 1,205 mink were collected, of which 60.5% were males and 66.5% were mink less than one year old, 19.6% one year old, 7.0% two years, 3.3% three years, 2.6% four years, and 1.0% were five or six years old. The presence of CDV RNA was detected in two samples (~0.17%) (Table 1). The first sample (ID:748) was collected from a male caught in 2012 in central-eastern Poland along the Vistula river (Fig. 1). The second sample (ID:1038) was obtained from a male caught in 2014 in Narew National Park, located in northeastern Poland (Fig. 1).

Sequencing results

Despite the high Ct values observed during Real-Time RT-PCR, complete genome sequencing was attempted; however, complete genome assembly could not be achieved, and only partial sequences were obtained. The inability to generate complete sequences is likely due to the low viral load and potential sample degradation, as the tissues originated

Table 1 Sample information of CDV positive animals

Accession number	ID	Month	Year	Age (years)	Body weight (g)
PV809618	748	April–May	2012	1	1,040
PV809619	1038	October	2014	1	1,230

from post-mortem examinations and had undergone multiple freeze–thaw cycles. Additionally, the complete H gene could not be recovered; however, a 539 bp fragment of the H gene, followed by a short segment of the adjacent non-coding region, was successfully sequenced from both sample ID:748 and ID:1038, and subsequently submitted to the NCBI database. A comparison of the two sequences revealed two nucleotide differences, one located within the H gene region and the other in a non-coding region; however, no amino acid variation was observed.

Based on the GenBank BLASTn search (421 bp, part of the H gene), ID:748 sequence depicted the highest 99.52% nucleotide similarity with a representative sequence of the CDV Europe lineage (DQ889177) identified previously from one domestic dog in Hungary from 2004 (Demeter et al. 2007). It displayed the same 99.29% nucleotide identity with the sequences from American mink, red foxes and raccoon dogs in Denmark from 2012 and 2013 (Trebbien et al. 2014), European badgers, red foxes in Italy between 2012 and 2017 (Bianco et al. 2020) and two red foxes from Germany in 2008 (Nikolin et al. 2012). The BLAST analysis for the ID:1038 sequence identified the same most similar sequences as for ID:748, with 99.76% identity to DQ889177 and 99.52% to the previously described sequences. During the BLAST analysis, two nucleotide difference was observed between the two sequences one of the H gene region and one of the noncoding region, with no amino acid variation detected.

Phylogenetic analysis

Phylogenetic analysis of the partial Hemagglutinin gene sequences confirmed that both CDV detected mink in this study cluster within the Europe lineage, which is recognized as an endemic lineage in the region. (Fig. 2).

Discussion

In this study, we analyzed spleen samples collected from 1,205 invasive American mink carcasses collected over a 15-year period in Poland and detected canine distemper virus RNA in two individuals. The viral genetic background in both cases confirmed the infection with the Europe lineage of CDV. This virus lineage has also been found in other native species in Europe, including various mustelid species (Trebbien et al. 2014; Molenaar and Buter 2018; Trogu et al. 2021; Lanszki et al. 2022b; Huang et al. 2024). Our finding provides direct evidence of CDV infection in feral American mink populations in Poland, confirming their susceptibility to local CDV lineage in Europe. Given the invasive nature of the species and its extensive distribution in Poland, these results raise concerns about the role of feral American mink in the epidemiology of CDV and the potential for cross-species transmission. Already, these results suggest that this invasive species is susceptible to infection with the CDV Europe lineage and therefore may be a new amplification host in local CDV ecology and epidemiology. However, to better understand their role as amplification hosts and potentially novel members in the CDV transmission chain, more data about the pathogenicity of this CDV strain in these animals is necessary.

In our study, the PCR detection of CDV in wild mustelids was notably low, with only 2 positive cases out of 1,205 samples (~0.17%). This prevalence is substantially lower than those reported in other European investigations based on tissue examination with PCR testing of wild mustelids. In Spain, CDV was detected in 4.5% of feral American mink sampled between 2019 and 2023 (Huang et al. 2024). In northern Italy, CDV prevalence reached 52.6% in European badgers, and 14.3% in stone martens between 2018 and 2020, with viral sequences clustering within the Europe lineage (Trogu et al. 2021) and in southern Italy, between 2022 and 2024, CDV prevalence reached 17.6% in Eurasian badgers, and 33.3% in pine martens (Alfano et al. 2025). In the Czech Republic, prevalence rates reached 10% in stone martens, 20% in pine martens, and 2.5% in European badgers between 2012 and 2020, (Kličková et al. 2022). Furthermore, PCR prevalence of 3.1% in steppe polecats (*Mustela eversmanii*), 2.8% in European polecats, 5.6% in stone martens and 0.6% Eurasian otter were documented in Hungary (Lanszki et al. 2022a, b). In most these studies, CDV sequence belonging to the Europe lineage were detected, confirming the circulation of this lineage across mustelid species in various parts of Europe. In addition, the Arctic-like lineage and European Wildlife lineages have been detected

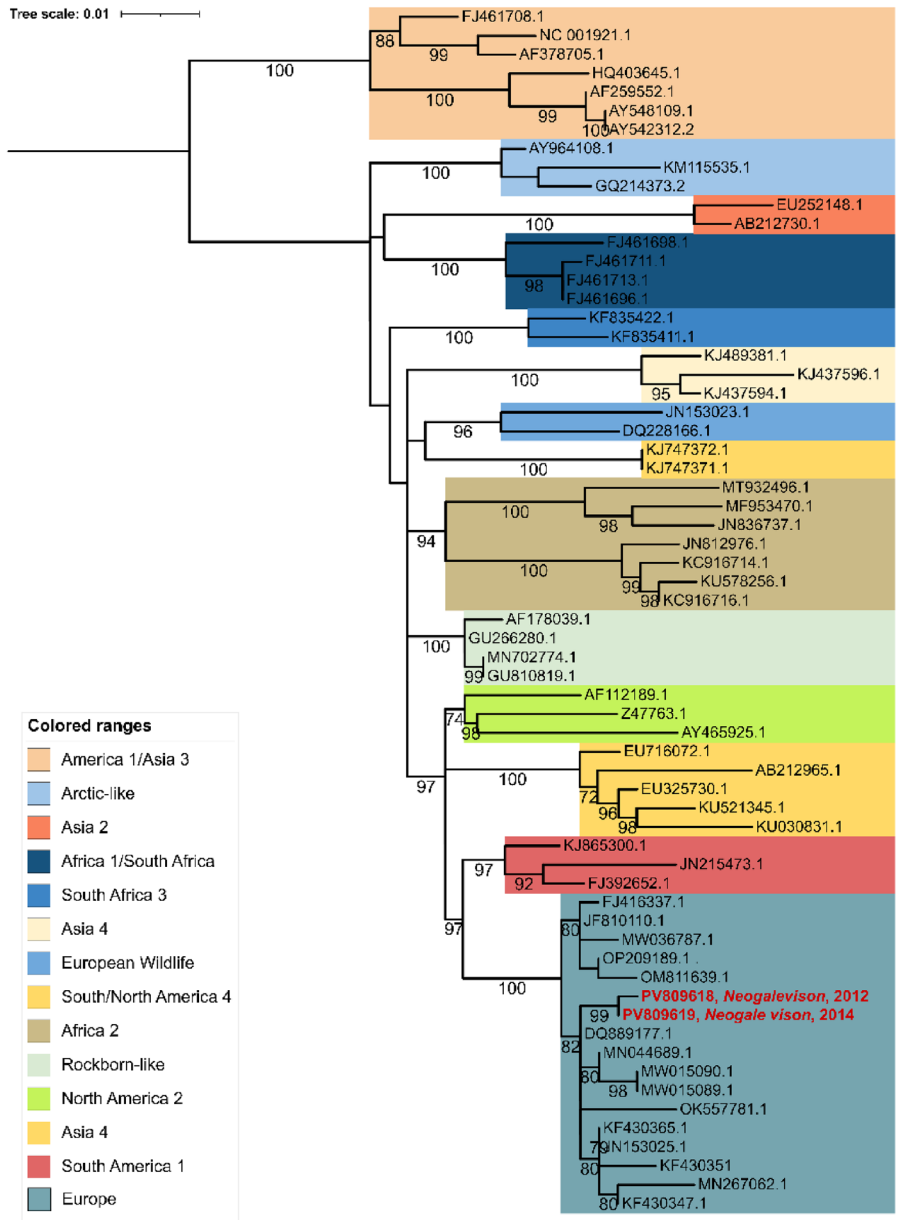

Fig. 2 Maximum-likelihood phylogenetic tree based on 64 partial hemagglutinin (H) gene nucleotide sequences (421 bp) of canine distemper virus. Colored clusters represent the major established CDV lineages. Novel sequences generated in this study are highlighted in red

in some countries (Kličková et al. 2022; Alfano et al. 2025).

In Europe, CDV outbreaks on Denmark mink farms have caused significant mortality, highlighting spillover risks to wildlife (Trebbien et al. 2014). Phylogenetic analyses of the virus between 2011 and 2013 confirmed clustering within the Europe lineage. This lineage was also found in red foxes, raccoon dogs, and feral ferrets, which clustered closely with CDV strains isolated from mink. This genetic proximity indicates cross-species transmission potential among these carnivores, suggesting a shared evolutionary origin. Between 2004 and 2007, CDV strains isolated from Danish mink during outbreaks formed a distinct cluster, grouping separately from the 2011–2013 viruses. During this period, the CDV detected in mink belonged to the Rockborn lineage, which served as the basis for the commercially available vaccine (Trebbien et al. 2014). In 2017, CDV was detected in American mink on four mink farms

in the Netherlands and Belgium, confirming the continued presence of the virus in farmed populations in these countries (Molenaar and Buter 2018). Globally, studies in South America have shown that CDV transmission between domestic dogs, Southern river otter (*Lontra provocax*), and invasive American mink can facilitate the virus's spread across multiple species, demonstrating broader ecological risks associated with CDV in feral mink populations (Sepúlveda et al. 2014).

Mink are susceptible to a wide range of viruses, including those from the *Parvoviridae* family, such as feline panleukopenia virus (FPV), canine parvovirus 2 (CPV-2), and mink enteritis virus (MEV) (Steinel et al. 2001; Barros et al. 2022; Zhao et al. 2022). Both feral and farmed mink are susceptible to influenza A viruses, including human and avian subtypes (Gholipour et al. 2017; Restori et al. 2024; Kuchinski et al. 2024). Mink are also highly susceptible to SARS-CoV-2 infection, with documented cases of both human-to-mink and mink-to-human transmission (Domańska-Blicharz et al. 2023). Aleutian mink disease virus (AMDV) is of particular concern as it complicates disease management strategies, both in farmed and feral mink (Virtanen et al. 2021; Zalewski et al. 2021b, 2025;). Therefore, infection with an additional virus (like CDV) results in the risk of transmission of a large number of pathogens and the circulation of a large number of them in the environment, which increases the threat to native species.

The spread of CDV from farmed mink to feral populations has been particularly concerning due to the role of escaped mink acting as amplification host for the virus, further contributing to the spread of CDV and other pathogens, as these feral populations interact closely with both domestic and wild carnivores (Zhao et al. 2024). Our results highlight the urgent need for more robust, integrative approaches to wildlife management and disease monitoring and pinpoint gaps in the current understanding of the complex eco-epidemiology of CDV. This includes improved surveillance and targeted studies focusing on invasive species, which may act as amplification host for diseases that threaten both wildlife health and conservation efforts. Simultaneous investigations of CDV prevalence in American mink, domestic dogs, and other sympatric wild carnivores are essential to comprehensively assess cross-species transmission dynamics and associated epidemiological risks. Furthermore, detailed genetic characterization of circulating CDV strains is necessary to better understand viral evolution and transmission pathways. Early detection, along with proactive management strategies, is key to preventing further ecological disruption caused by the introduction of such species. Understanding the broader implications of disease transmission by invasive species is crucial for safeguarding biodiversity and strengthening conservation policies.

This study's limitations include the absence of multiple tissue types other than spleen, which restricts the ability to accurately estimate CDV prevalence among the investigated specimens. In most cases, mink carcasses were frozen at −20 °C immediately after dispatch to prevent tissue and virus decomposition. The time between the acquisition of the mink and the virus analysis (max. 13 years) could have had a potential impact on the low prevalence. This may confirm the fact that neither the complete genome nor the complete hemagglutinin gene could be sequenced, likely due to partial sample degradation. Additionally, the disease course in the sampled mink remains unknown since histopathological examinations were not performed due to the low tissue quality. Despite these limitations, the study provides valuable preliminary data that contribute to a better understanding of the potential role of the invasive American mink in the spread of canine distemper virus among wildlife populations. Further research with a more structured sampling approach, including the use of viral transport media and prompt processing of fresh tissue samples, could help to gain deeper insights into the epidemiology and ecological impact of CDV in this species.

Conclusions

The introduction of invasive species poses significant threats to natural ecosystems. However, its association with endemic or emerging infectious diseases adds further complexity, which has been poorly studied. Given the ability of CDV to infect a broad range of species, including rare and protected animals, our results emphasize the need for integrative wildlife management and disease monitoring to mitigate the risks posed by invasive species to vulnerable ecosystems and conservation efforts.

Our retrospective analysis of post-mortem samples reveals that invasive American mink in Poland are susceptible to infection with an endemic CDV strain, highlighting the need for expanded surveillance and in-depth studies to better understand the role of CDV in wildlife populations and its broader ecological impact.

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Author contributions All authors contributed to the study conception and design. Conceptualization was carried out by A.Z., Z.L., and J.L. Sample collection was performed by A.Z. Nucleic acid extraction and PCR reactions were performed by D.P., K.B., Á.Á., Z.V. and Z.L. NGS sequencing was conducted by Á.Á. and Z.L. Sanger sequencing was performed by Z.L. Bioinformatics analysis was carried out by Á.Á. Phylogenetic analysis and visualization were performed by Z.L. and Á.Á. The first draft of the manuscript was written by Z.L., and all authors commented on previous versions of the manuscript. Writing – review and editing were carried out by A.Z., G.K. and J.L., and supervision was provided by A.Z. and Z.L. All authors read and approved the final manuscript.

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Data availability The sequence data obtained in this study have been submitted to GenBank database under accession numbers PV809618-PV809619.

Declarations

Conflict of interest The authors have no relevant financial or non-financial interests to disclose.

Ethical approval No animals were killed specifically for use in this study. American mink carcasses were obtained from various projects that were unrelated with this study and aimed to eradicate invasive species to implement nature protection plans within bird conservation projects. According to the National Ethics Committee for Animal Experiments resolution no. 22/2006, permission from the Local Ethics Committee for Animal Experimentation in Poland is not required for the use of tissue samples from animals acquired in the course of other projects.

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