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Molecular analysis of canine distemper virus H gene in the golden jackal (Canis aureus) population from Serbia

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Abstract

Canine distemper virus (CDV) is a highly contagious and often fatal disease affecting wild and domesticated carnivores. The virus is a single-stranded RNA virus from the genus *Morbillivirus* and the family *Paramyxoviridae*. While domestic dogs are the most common hosts, the virus poses a significant threat to endangered wildlife due to its broad host range. This study aimed to characterize the CDV Haemagglutinin (H) gene in golden jackals and explore the molecular evolution of the virus in an underrepresented host. A total of 88 brain samples from hunted golden jackals were tested for the presence of CDV viral nucleic acid, and the H gene of positive samples was amplified and sequenced using the Sanger method. Phylogenetic analysis, conducted using maximum likelihood methods, revealed that all Serbian sequences clustered within the Arctic lineage. Notably, the analysis identified a tyrosine (Y) at position 549 of the H protein, a mutation commonly associated with wildlife hosts, instead of the histidine (H) typically found in domestic strains. Additionally, a mutation at position 310 was observed, which could potentially affect the protein's function and virus-host interactions. These findings provide valuable insights into the genetic diversity and evolutionary dynamics of CDV in golden jackals, with broader implications for understanding the virus's adaptability to different hosts. Further research is needed to investigate the functional impact of these mutations, particularly their role in vaccine efficacy and disease transmission across wildlife and domestic species.

Keywords Canine distemper virus, Golden jackal, Phylogenetic analysis, Mutation analysis, Serbia

Introduction

Canine Distemper Virus (CDV) is the causative agent of a multisystemic disease in many animal species. CDV has been reported in a wide range of hosts primarily including carnivore species such as *Canidae*, *Felidae*, *Ursidae*, and *Mustelidae* and recently even in rhesus monkeys and other non-human primates [11, 23, 34, 35]. The virus belongs to the genus *Morbillivirus* and the family *Paramyxoviridae* together with the Peste des Petits Ruminants virus, Dolphin morbillivirus, Feline morbillivirus, Measles virus, Rinderpest virus (eradicated), and Phocine distemper virus [25]. CDV has a negative sense, single-stranded RNA, surrounded by a nucleocapsid layer, and a lipid envelope. Viral RNA codes six structural and two nonstructural proteins including nucleocapsid protein (N), phosphoprotein (P), matrix protein (M), fusion protein (F), hemagglutinin (H), and RNA polymerase (L) which make up the structural proteins observed from the 3' end, as well as the virulence factor protein (V), and polymerase cofactor protein (C) making up the nonstructural proteins [34]. Viral entry into the susceptible host

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cell is mediated by the H protein which binds primarily to the signalling lymphocyte activation molecule, CD150 (SLAM) receptor protein on lymphocytes or the nectin-4 protein. Further entry into the cell is controlled by the F protein which allows for the lipid outer layer to fuse with the cell membrane [9, 20]. Clinical manifestations of CDV are initially observed as respiratory and gastrointestinal symptoms, subsequently progressing to neurological complications, which frequently culminate in a fatal outcome [34]. Mortality varies significantly depending on the virus strain and the host species. For example in domestic dogs mortality has been estimated at 50%, while in ferrets it can reach almost 100% [34].

The highest genetic variability has been reported in the H gene, and changes in the genomic structure have been connected to species crossover as well as virulence. Specifically, mutations at the 530th and 549th (Tyrosin to Histidine) position in the amino acid sequence of the H protein have been connected to increased virulence [33]. Twenty-two different genetic lineages have been described based on the analysis of the H gene. Many of these are specific to certain geographical regions, although some overlap does exist [11, 34]. New lineages are often named according to the region (continent, subcontinent) in which they were identified followed by a number or a specific close denomination i.e. *Rockborn-like* CDV [34]. Currently identified lineages are: Arctic-like, Rockborn-like, Caspian, Europe-wildlife, Europe/South America, South America 1–3, America 1–3 (primarily vaccine strains), America/South America 4, Canada 1–2, Asia 1–6, Africa 1–2, and Australia [2, 7, 8, 10, 32]. The differentiation of a new lineage is done based on the nucleotide and amino acid sequence of the H gene/protein.

The golden jackal, an opportunistic carnivore, frequently establishes its habitat in close proximity to human settlements [4]. Their population in the country was severely reduced after WWII in a poisoning campaign aimed at European grey wolf populations. However, from the 1980s onwards, there has been a notable spreading of the golden jackal population, leading to their widespread presence across the majority of the country [3]. Commonly considered as pests, golden jackals are subject to year-round hunting regulations. Their close association with human environments facilitates interactions with local owners and stray dog populations, positioning them as a significant vector for the transmission of diseases [28]. Molecular studies on CDV in Serbia have not been previously done. However, there are reports of high CDV seroprevalence (36.8%) among red foxes in Western Serbia [1]. Attenuated vaccines for CDV are available in the country, however, their administration is not compulsory and is employed at the discretion of domestic dog owners. Currently, no vaccination programs are in place for wildlife populations. With the number of reports of novel strains discovered in wildlife hosts rising [24], this study aimed to conduct a molecular analysis of circulating CDV in the increasing and expanding golden jackal population. In our country, golden jackals are considered an pest species and are legally hunted throughout the year. Given the absence of previous research of this kind locally, our goal was to investigate the presence of CDV in the population of golden jackals. This research will not only provide insights into the molecular evolution of CDV in golden jackals but also has significant implications for wildlife management and disease control policies, particularly in understanding how emerging CDV strains in wildlife could impact domestic animals and public health. Furthermore, we intended to perform molecular analyses to determine the specific CDV strains prevalent among these animals.

Materials and methods

Collection of samples

During regular population monitoring activities, a total of eighty-eight legally hunted golden jackals were randomly collected. Eighty-six bodies of dead golden jackals were brought to the Faculty of Biology at the University of Belgrade for necropsy. The organs of the specimens were carefully preserved in sterile bags at -20° C. Detailed information, including sex, date of capture, age, and coordinates, was meticulously documented for each animal. Samples were collected from 2019 until 2023, from 12 different administrative districts. Samples were collected from juveniles (3–9 months) and adult (1 + years) animals. Two samples were collected by veterinary officials (Private Enterprise of Vojvodina šume) from golden jackals displaying neurological clinical signs and were brought to the Institute of Veterinary Medicine of Serbia for regular Rabies testing within an ongoing government monitoring program in 2023. These two samples originated from the habitat of Deliblatska pescara, and Vrsacke planine. For all collected animals, the date of death (hunting), location, sex, age, body weight, and body dimensions were recorded and measured.

Brain tissue was obtained from each animal, and a 1 cm^3 sample of this tissue was homogenized using a pestle and mortar. Subsequently, the homogenized tissue was suspended in a cell culture medium (DMEM, Dulbecco's Modified Eagle Medium, Thermo Fisher, Massatschusses, USA) at a 1:10 ratio. Further, samples were centrifuged at 1500 g for 10 min, and afterwards, the supernatant was decanted, and stored at -20 °C until further analyses.

Extraction of nucleic acid

Viral nucleic acid was extracted using the IndiSpin Pathogen kit (IndiSpin Pathogen Kit, Indical Bioscience GmbH, Leipzig, Germany) following the manufacturer's instructions. An external VetMax Xeno Internal positive RNA control (Applied Biosystems, Thermo Fisher Scientific, Massachusetts, USA) was included in each sample to confirm a successful extraction of nucleic acid.

Canine distemper virus rtRT-PCR screening

To detect the nucleic acid of CDV the following master mix for *rtRT-PCR* was used: 10 µl of Luna Universal Probe One-Step Reaction Mix (from New England BioLabs, Ipswich, MA, USA) 0.8 µl of each 10 mM primer, 0.4 µl of 10 mM probe, 1 µl of RT Enzyme Mix, 5 µl of template, and 1 µl Xeno Internal positive RNA control primer was used, the rest up to 20 µl was supplemented with nuclease-free water (RT-PCR Grade Water, Thermo Fisher Scientific, Massachusetts, USA). The list of primers and probes used for the detection of CDV is given in the table below (Table 1.). An external positive control, consisting of previously confirmed CDV-positive samples, was included in each run to serve as both an extraction and amplification control. This control was diluted to achieve a Ct value of 32 and was used as a cut-off value. PCR-grade water was used as the negative extraction and amplification control (RT-PCR Grade Water, Thermo Fisher Scientific, Massachusetts, USA). The temperature profile used included an initial reverse transcription at 45 °C for 10 min, and at 95 °C for 10 min, the cycle conditions were as follows 95 °C for 60 s, and 60 °C for 45 s as stated in the kit manufacturers instructions.

RT-PCR amplification for sequencing

Samples which were deemed positive by rtRT-PCR testing were further amplified by gel-based RT-PCR in order to obtain adequate PCR products for Sanger sequencing. For the amplification of the entire Hemaglutinin gene, the previously published primers were used [30] (Table 1. and Fig. 1.). The master mix consisted of 4 µl of 5xQiagen One-Step RT-PCR Buffer, 4 µl of 5QSolution, 0.8 µl of dNTP Mix and the Qiagen OneStep RT-PCR enzyme mix, 1.2 µl of each 10 mM primer, and 2 µl of the template. The rest up to 20 µl was supplemented with nuclease-free water (RT-PCR Grade Water, Thermo Fisher Scientific, Massachusetts, USA). The temperature profile included an initial reverse transcription at 50 °C for 30 min, followed by denaturation at 95 °C, then 40 cycles of denaturation at 94 °C for 15 s, annealing at 51 °C for 30 s, and extension at 68 °C for 1 min, a final extension step at 68 °C for 10 min was also included [29]. An expected base pair length for all segments can be found in Table 1. The results were visualised by electrophoresis on 1.5% agarose gel, later viewed by UV transillumination. Samples which were deemed positive were further purified using the GeneJET PCR purification kit (ThermoFisher Scientific, Waltham, MA, USA) and then sequenced in both directions using a commercial sequencing service (Macrogen, Netherlands).

Table 1 Primers and probes used for the amplification of CDV

Real-time qPCR			
Primers	**Probe**	**Reference**	**Base pair**
3'-AGCTAGTTTCATCTTAACTATCAAATT-5' 3-TTAACTCTCCAGAAAACTCATGC-5'	TAMRA-ACCCAAGAGCCGGATACATAG TTTCAATGC-FAM	Wang et al. 2017 [31]	
Ist 72f 3'-TACTCTGGTCACACGTCTTA-5' 798r 3'-TAGCTCCACTGCATCTGTAT-5'	/	Sekulin et al. 2011 [27]	700 bp
IInd 472f 3'-CCGTACATCACCAAGTCATA-5' 1172r 3'-TAGAACACCACCTTGTGAAC-5'	/		700 bp
IIIrd PCR 1113f 3'-GTAGATGAGAGCACCGTATT-5' 1771r 5'-TGTGTAGGCAACACCACTAA-3'	/		660 bp
IVth PCR 1629f 3'-GGAGACCAGTTCACTGTAAT-5' 2221r 3'-GATGGACCTCAGGATATAGA-5'	/		600 bp

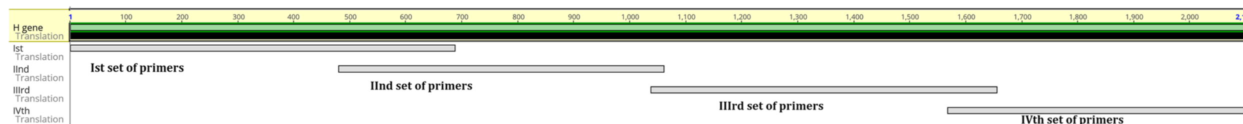

Fig. 1 Primer overlap

Sequence analysis

The acquired sequences underwent quality assessment, utilizing quality scores exceeding 30 as the benchmark. Subsequently, primer sequences were excised employing the 'Trim Ends' feature within the Geneious Prime software suite (Dotmatics, Boston, MA, USA). Alignment of the trimmed sequences was then performed using the MAFFT alignment algorithm, also integrated within the Geneious Prime platform. Afterwards, the consensus sequences were obtained using the Generate consensus sequence option in the Geneious Prime platform and the threshold was set at the highest quality at 75%. These sequences were uploaded to the National Center for Biotechnology Information GenBank (NCBI). The sequences from this study were aligned with 62 publicly available sequences of the CDV Haemagglutinin gene from the NCBI, representing known lineages. All sequences in the alignment were trimmed to the length of 918 bp (IIIrd and IVth segment of the H gene) and then used for phylogenetic analysis in the Molecular Evolutionary Genetic Analysis (MEGA X) software. The Maximum Likelihood method and Tamura Nei model used in the analysis were determined based on the results of the "Find Best DNA/Protein model" feature of MEGA X.

The analysis of glycosylation sites of the sequenced strains was based on the N-X-S/T motif and was conducted using the free NetNglyc server (https://services.healthtech.dtu.dk/service.php?NetNGlyc-1.0). To detect and study possible recombination breakpoints within the Arctic lineage and the Vaccine strain, the Genetic Algorithm for Recombination Detection (GARD) tool was used which is available on the DATAMONKEY web server (https://www.datamonkey.org/). The analysis involved two separate sequence alignments: the first combined Serbian strains (PP151312-PP151318) with strains from the Arctic lineage, and the second paired the same Serbian strains with Vaccine strains. For consistency in comparison, sequences in both alignments were trimmed to a length of 918 bp. To investigate site-specific selective pressures and identify amino acid sites under differential selection between the two groups, we employed the Contrast-FEL method implemented in the DATAMONKEY web server (www.datamonkey.org). For this analysis, the same alignments as in the previous analysis were used [13]. Map were created using the QGIS software.

Results

Out of 88 tested samples, 7 samples (7.9%) were positive for CDV (Fig. 2 Map). Among the CDV-positive samples, a complete sequence of the haemagglutinin gene was successfully extracted from only one sample (16.7%), while partial gene sequences were obtained from six samples (83.3%). Notably, the sequences obtained from the region targeted by the second set of primers were of poor quality and thus, uninterpretable. From all other samples, three regions were obtained (I, III, and IV). Quality sequences were processed and submitted to GenBank (NCBI) under accession numbers PP151306-PP151318.

The phylogenetic analysis of the partial H gene revealed that all Serbian CDV strains can be classified as members of the Arctic lineage, alongside strains from Italy (HM443706, KX943320-KX943326, DQ226087, DQ226088), Switzerland (KR002657 and KR002658), and Greenland (Z47760.1). Within the Arctic lineage, the Serbian strains are divided into two distinct clades: the first includes SRB/CDV/Hgene/Deliblato, SRB/CDV/Hgene 3–4/VrPlanine, SRB/CDV/Hgene 3–4/27, and SRB/CDV/Hgene 3–4/24; the second comprises SRB/CDV/Hgene 3–4/12, SRB/CDV/Hgene 3–4/31, and SRB/CDV/Hgene 3–4/38 (Fig. 3). Notably, the Serbian strains showed the highest genetic similarity with the Italian strains (KX943320.1-KX943324.1) at 97.86%.

In total 19 nonsynonymous mutations were identified when comparing Serbian sequences with others from the Arctic lineage (Online Resource 1). Focusing on mutations at the SLAM protein binding sites, particularly at positions 519, 530 and 549 a consistent presence of arginine (R) at the 519th and asparagine (N) at the 530th across all strains was found. Interestingly, at position 549, the strain SRB/CDV/Hgene 3–4/31 uniquely exhibited histidine (H), whereas tyrosine (Y) was observed in this position in all other strains (Table 2). Nectic – 4 binding sites were the same across the Arctic lineage (478 V, 479 L, 537Y, 539Y).

Glycolisation analysis revealed 8 possible glycosylation sites in all sequenced strains in the positions 19–21, 149–151, 309–311, 391–393, 422–424, 456–458, 587–589, and 603–605. Three distinct amino acid residues – glycine (G), aspartic acid (D), and asparagine (N) – have been identified at the 310th position in the glycosylation site (Table 2), while in other glycosylation sites, no differences were observed.

Recombination analysis using GARD for both alignments revealed the evaluation of 1,223 models at 94.08 models per second. This process investigated 68 potential recombination breakpoints within the alignments, covering 52.13% of the total search space of 2,346 models, each allowing for up to two breakpoints. The algorithm inferred one potential breakpoint in the alignment. Contrast-FEL analysis did not identify any sites (positions in the genetic sequence) that show a statistically significant difference in the rate of non-synonymous to synonymous substitutions between the two groups being compared. This was assessed with a q-value of 0.2 [13].

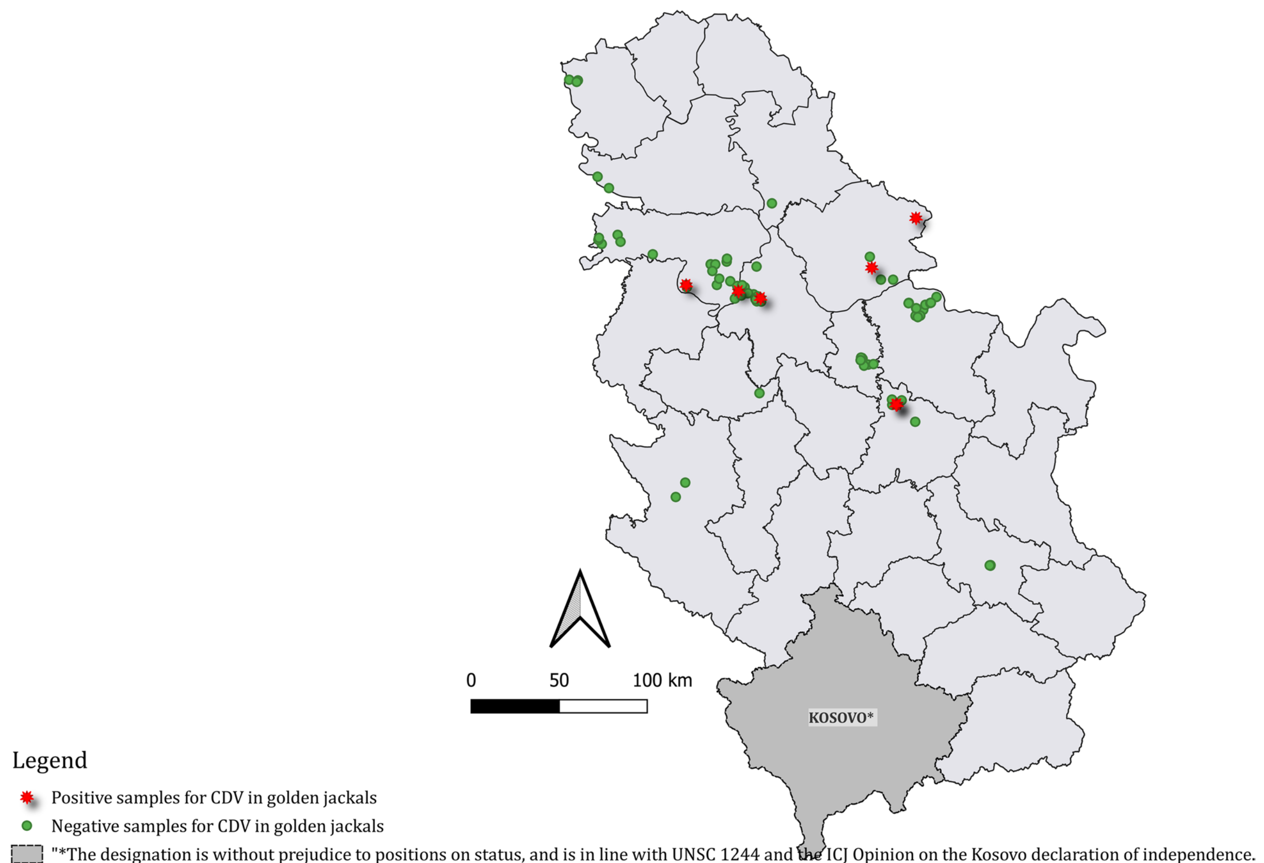

Fig. 2 Map - Distribution of positive and negative samples of CDV in golden jackals from Serbia

Discussion

This study offers the inaugural molecular characterization of the H gene of CDV in golden jackals within the country, marking a significant first. Due to the absence of prior publications on the genetic traits of the H gene, there was no existing reference point to compare the currently circulating strains. The phylogenetic analysis involved 10 different lineages of CDV including the Vaccine strains, and grouped Serbian strains in the Arctic-like lineage. This lineage was first described after a mass mortality event in Siberian seals (*Phoca sibirica*) in 1987/88 with highly similar clinical signs as CDV in dogs [15]. The same lineage was later reported in Harbour seals (*Phoca vitulina*) in the Baltic Sea [21]. The presence of this lineage has been described in many European and non-European countries including Italy, Hungary, Switzerland, Austria, and Turkey, also there were reports of the Arctic lineage in China, Russia, Iran and the US [6, 12, 29, 30]. Besides a broad geographical distribution, the lineage has been detected in various hosts such as badgers, wolves, dogs, and golden jackals [6, 12, 29, 30]. It is presumed that the lineage spread across the world through the canine population [16]. Authors Lanszki et al. [14, 18] suggest that the possible introduction of the lineage into Italy might have been from Eastern European and Asian imported with dogs. The origins of this lineage in Serbia remain unclear. However, its prior identification in Turkey and Hungary suggests it has been present in

(See figure on next page.)

Fig. 3 A phylogenetic tree showing the genetic relationships between Serbian strains (PP151312-PP151318) and strains from the NCBI representing other lineages of CDV. The study first identified the optimal model using the "Find Best DNA/Protein Models" function in MEGA X. Based on this, the Maximum Likelihood Method with the Tamura-Nei model was applied to construct the tree. To enhance accuracy, 1000 bootstrap replicates were performed, and a Gamma distribution (+G) across five rate categories was used to account for variation in rates across sites, along with the assumption that some sites remain unchanged (+I). Branches supported by less than 70% of bootstrap replicates were condensed. Serbian sequences were marked with black dots. The proposed phylogeny was addapted from Nikolin et al. [19]

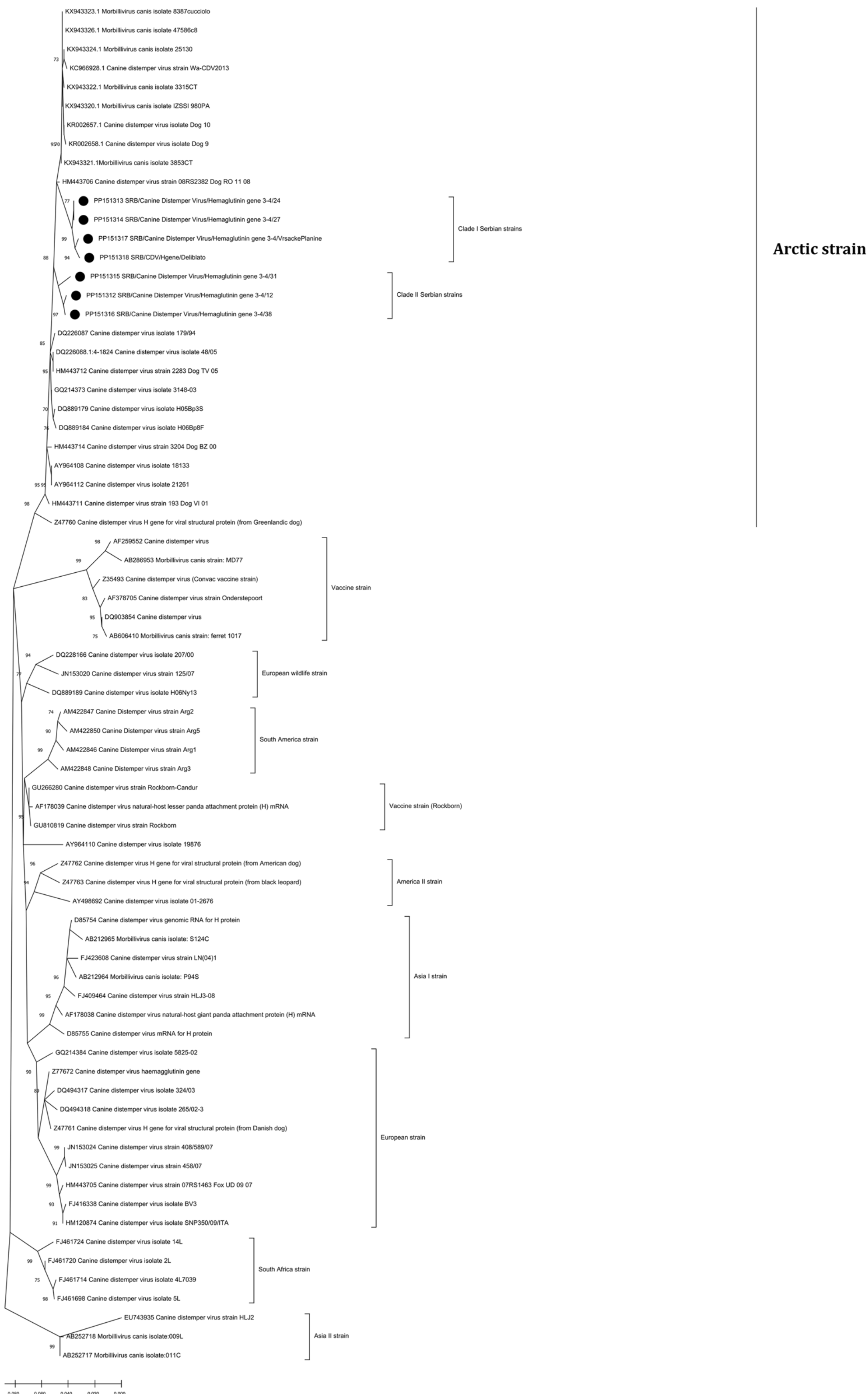

Fig. 3 (See legend on previous page.)

Table 2 List of sequences and amino-acid residues at the 310 glycolisation site, 519 and 530 SLAM binding site, and 549 positions of the H gene in the Serbian strains, the Arctic lineage, and the vaccine strains

Sequence name	Glycolisation site-changes	SLAM binding site			
	Amino acid position				
	310	519	530	549	
PP151306	-	-	-	-	**Serbian strains Arctic lineage**
PP151307	-	-	-	-	
PP151308	-	-	-	-	
PP151309	-	-	-	-	
PP151310	-	-	-	-	
PP151312	**N**	R	N	Y	
PP151313	D	R	N	Y	
PP151314	D	R	N	Y	
PP151315	D	R	N	**H**	
PP151316	**N**	R	N	Y	
PP151317	D	R	N	Y	
PP151318	**G**	R	N	Y	
Z47760	G	R	N	Y	**Arctic lineage**
HM443711	G	R	N	Y	
KR002657.1	D	R	N	Y	
KR002658.1	G	R	N	Y	
KC966928.1	D	R	N	Y	
KX943320.1	D	R	N	Y	
KX943322.1	D	R	N	Y	
KX943324.1	D	R	N	Y	
KX943321.1	D	R	N	Y	
KX943323.1	D	R	N	Y	
KX943326.1	D	R	N	Y	
HM443714	D	R	N	Y	
DQ226087	G	R	N	Y	
DQ226088	G	R	N	Y	
HM443712	G	R	N	Y	
GQ214373	G	R	N	Y	
DQ889179	G	R	N	Y	
DQ889184	G	R	N	Y	
Z35493	G	R	**S**	Y	**Vaccine strain**
AF378705	G	R	**S**	Y	
AB606410	G	R	**S**	Y	
DQ903854	G	R	**S**	**H**	
AF178039	G	R	**D**	**H**	
GU266280	G	R	**D**	**H**	
GU810819	G	R	**D**	**H**	

the region for a while [5, 12]. This is further supported by a high similarity observed between these strains, despite them being identified and described decades apart. Interestingly, the Serbian strains have diverged into two distinct clades within the Arctic lineage. This divergence hints at the potential presence of a unique sublineage that has been circulating within the local golden jackal population. This complex evolutionary relationship within the Arctic lineage is supported by recombination analysis conducted using the GARD method in which a potential recombination breakpoint has been identified.

The SLAM protein, a membrane glycoprotein found on human T and B lymphocytes, is identified as a common cellular receptor for the measles virus. While its exact function in the pathogenesis of CDV remains unclear, it is suggested that SLAM might play a significant role in the cell-to-cell transmission of the virus especially in the lymphoid system [9]. Analysis of mutations within the Serbian strains, when compared to the rest of the Arctic lineage revealed 19 nonsynonymous mutations. The Contrast-FEL analysis, which compares non-synonymous to synonymous substitution rates between the Arctic lineage and Serbian strains, failed to identify any sites demonstrating a preference for specific mutations. This suggests an absence of mutation bias at any particular point within the lineage. However, among these mutations, a single one occurred at the SLAM binding site (position 549), specifically in the strain PP151315, which exhibited histidine (H) at this position. In contrast, all other strains displayed tyrosine (Y) at the same site. In previous studies, it has been suggested that the 530th and 549th binding sites in the H gene could be connected to increased virulence in certain species [17]. However, Nikolin et al., [19] found no evidence to support this claim and suggests that the 530th binding site is conserved among different lineages. In our study, consistent with these observations, all strains from the Arctic lineage, encompassing the Serbian strains, display asparagine (N) at position 530. Furthermore, analysis of Vaccine strains reveals the presence of two distinct amino acids at this position: serine (S) and aspartic acid (D). Alterations in amino acids at position 549th of the binding site have been associated with host species specificity. Specifically, the presence of tyrosine (Y) at this position is predominantly linked to domestic dog hosts, whereas histidine (H) is associated with wildlife hosts [19]. In this study, 6 out of 7 strains exhibited tyrosine (Y) at the 549th position, while only one strain exhibited histidine (H). Since golden jackals have frequently high density in proximity to human settlements and possibly near infected dogs, this could account for the high prevalence of the domestic dog-specific amino acid tyrosine (Y) at 549th position in Serbian strains. The PP151315 strain, characterized by the presence of histidine (H) at the 549th amino acid position, may be more closely associated with wildlife origins. In contrast, other strains appear to represent introductions from domestic dog populations. Previous research on the dietary habits of the golden jackal in Serbia has demonstrated that

a significant portion of their winter diet is derived from anthropogenic sources, including frequently discarded carcasses and domestic animals leftovers in proximity to human settlements [4]. Additionally, other studies have indicated that domestic dogs are commonly preyed upon by golden jackals, constituting a substantial component of their diet [4, 22]. The frequent proximity between species may elucidate the predominance of a domestic dog-specific amino acid at the 549th binding site observed in the majority of Serbian strains. Nonetheless, as the samples analyzed in this study were not obtained during a single outbreak, it becomes challenging to conjecture about the origin of the virus strains.

N-glycosylation is a crucial post-translational modification for Paramyxovirus glycoproteins, notably the H protein. This process significantly influences the protein's function, folding, and transport. It entails the addition of N-glycans to asparagine (N) residues. These residues are located within the consensus sequences of N-X-S/T, where 'X' represents any amino acid except proline [26]. In this study, a total of eight glycosylation sites have been found, which is in accordance with previously published research [5, 12, 18]. In Serban strains, three different amino acids, aspartate (D), asparagine (N), and glycine (G) have been found at the 310th position. In the analysed Arctic lineage only aspartate (D) and glycine (G) have been found while in the Vaccine strain, glycine (G) is the dominant amino acid. Glycine (G) at the 310th glycosylation site has been found in other analysed strains from the Arctic lineage, HM443711, HM443712, DQ889179, and DQ889184, which were previously described in Italy and Austria [18]. Asparagine (N) has not been found in other sequences from other lineages and is a unique feature of the sequenced Serbian strains. In analyzing mutations at the 310th position within the Arctic lineage, including sequences from Serbia, three distinct amino acid residues were identified. This finding indicates a potential bias for mutations in this region. Notably, these mutations affect a predicted glycosylation site, suggesting their significant impact on this molecular feature. However, additional research is necessary for a deeper analysis of the evolutionary characteristics of the H gene.

A key limitation of the study is the absence of the second segment in the alignment for most Serbian sequences. This could stem from several factors, including the partial degradation of nucleic acids, potentially due to incorrect storage of deceased golden jackals post-mortem. Additionally, primer mismatch in this specific region for these strains is another possibility. However, the concern of improper synthesis of primers is less likely, given that the second region of the H gene was successfully amplified in the strain PP151318.

Conclusion

In conclusion, this study represents the first comprehensive molecular analysis of circulating CDV strains in Serbia, focusing on an underrepresented host, the golden jackal. The sequenced CDV strains were classified within the Arctic lineage, which has previously been identified in wild canid populations in the Alps, Central Europe, and Turkey. Comparative analysis revealed 19 nonsynonymous mutations in the Serbian strain, including three in the 310th glycosylation site and one in the 549th SLAM binding site. Notably, the presence of asparagine (N) at the 310th glycosylation site had not been previously reported within the broader Arctic lineage. The observed variation at position 549 suggests potential differences in the virus's host adaptation within the golden jackal population. These findings pave the way for further investigations into CDV's pathogenesis and host interactions. This study highlights the genetic complexity of CDV and emphasizes the need for continued research to better understand its transmission dynamics and evolutionary adaptations, which could have implications for disease control strategies in both wildlife and domestic species.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12917-024-04284-5.

Supplementary Material 1.

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The sequences were generated through the Sequencing Service of the Animal Production and Health Sub-Programme of the Joint FAO-IAEA Centre in Vienna, Austria.

Authors' contributions

All authors contributed to the study's conception, design, and manuscript writting. D.G. was the principal investigator, molecular tests were carried out by M.K. and S.Š., sequences were analysed by D.G. and S.Š., and the sample collection and pathological examination were carried out by M.K., J. M., and D.Ć., while V.M. supervised the study.

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Availability of data and materials

The datasets generated during and/or analysed during the current study are available from the corresponding author upon reasonable request.

Data availability

The datasets generated during and/or analysed during the current study are available from the corresponding author upon reasonable request. Quality sequences were processed and submitted to GenBank (NCBI) under accession numbers PP151306-PP151318.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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