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First detection and molecular characterization of *Chaphamaparvovirus galliform* in broiler and turkey flocks in Türkiye

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Abstract

Background A newly uncovered parvovirus, *Chaphamaparvovirus*, continues to be reported across various species. This study investigated the detection and genetic characterization of *Chaphamaparvovirus galliform* (GaChpV) in poultry, specifically broilers and turkeys, from various regions in Türkiye. To address this, comprehensive sampling and analysis were conducted to better understand the virus's distribution and impact in these avian populations.

Results In 2023, a total of 1060 fecal samples were collected from 76 broiler flocks (10 healthy and 66 with enteritis) and 30 turkey flocks (10 healthy and 20 with enteritis). Using nested PCR with specific primer sets, the study detected GaChpV in 36 out of 76 broiler flocks (47.3%) and 2 out of 30 turkey flocks (6,6%). Although GaChpV was detected at notable frequencies, the analysis revealed no statistically significant association between GaChpV and enteritis cases ($p = 0.617$). In this study, the nucleotide sequences (nt) of the capsid genes from GaChpV strains isolated from broilers and turkeys were 99 to 100% identical. Furthermore, these strains exhibited a high degree of genetic similarity ranging from 73 to 98% to *Chaphamaparvovirus galliform* 2 (GaChpV-2) strains from Europe, China, and Brazil. Complete genome sequencing of a broiler strain (CkChPV/2023/UN-2-TR) yielded a genome of 4,229 nucleotides, with sequence identity ranging from 78.93 to 98.82% compared to other GaChpV strains. Phylogenetic analysis further revealed that the CkChPV/2023/UN-2-TR strain clustered with GaChpV-2 strains, highlighting its genetic relatedness and diversity within the GaChpV family. The study also investigated genetic recombination signals and identified potential B-cell linear epitopes, contributing to a better understanding of the virus's genetic diversity and antigenic characteristics.

Conclusions This report represents the first detection of GaChpV in turkey and broiler flocks in Türkiye. Notably, research on this topic in turkeys is quite limited. The data derived from this study will contribute to elucidating the molecular epidemiology and evolutionary dynamics of GaChpV.

Keywords Chaphamaparvovirus, Broiler, PCR, Turkey, Türkiye

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Background

Parvoviruses within the family *Parvoviridae* possess a linear, single-stranded DNA genome approximately 4–6 kb in length. These non-enveloped viruses exhibit cubic symmetry [1] and contain two major genes: a non-structural (NS) replicase gene and a capsid (VP) gene [2]. The family *Parvoviridae* is further divided into three subfamilies: *Parvovirinae*, which infect vertebrates; *Densovirinae*, which infect invertebrates; and *Hamaparvovirinae*, which infect both vertebrates and invertebrates [3]. The *Hamaparvovirinae* subfamily includes five genera: *Hepanhamaparvovirus*, *Penstylhamaparvovirus*, *Brevihamaparvovirus*, *Ichthamaparvovirus*, and *Chaphamaparvovirus* [3]. The name "CHAPHAMA" is derived from the host groups where its members were first discovered—*ch*iropteran, *a*vian, and *p*orcine—as well as from its subfamily, *Hama*parvovirinae [3].

Chaphamaparvovirus (ChpV) was first identified through metagenomic analysis of oropharyngeal swabs from the fruit bat species *Eidolon helvum* in Ghana [4]. *Chaphamaparvovirus* can also be detected in fecal samples, with diarrhea being a common sign observed in infected pigs, dogs, cats, and chickens [5–7]. Currently, the genus *Chaphamaparvovirus* includes 36 species, with 21 identified in avian hosts, many of which have been recently discovered [8]. Specifically, *Chaphamaparvovirus galliform 1* (GaChpV-1) has been identified in turkeys [9], while *Chaphamaparvovirus galliform 2* to *7* (GaChpV2-7) have been identified in chickens [10]. A recent study in laying hens proposed 14 additional species of GaChpV [11]. High-throughput sequencing and advanced metagenomic analytical methods have significantly increased the number of novel parvoviruses discovered in animals in recent years [12, 13]. Many of these newly discovered viruses may be part of the complex virome of their host species and may be present without causing any disease, while others could be pathogens responsible for diseases for which no etiological agent has previously been identified [14].

GaChpV-2 was first identified in chicken fecal samples collected in Brazil in 2019 [15]. In 2023, an epidemiological study in China demonstrated a statistically significant correlation between GaChpV infection and diarrheal signs [5]. In 2021, GaChpV-4 was reported in the bile of a free-range laying chicken clinically diagnosed with spotty liver disease (SLD) in Australia [11]. Between 2017 and 2021, *Phasianus chaphamaparvovirus 1* was detected in several outbreaks of hepatitis among flocks of young pheasants in France, with parvovirus-like virions confirmed via electron microscopy [16]. Most recently, GaChpV-2 was detected in chickens with hepatitis in Japan [17]. Based on current information, GaChpV-2 is the most frequently detected species in broilers and chickens with enteritis signs [5, 13, 15]. Although reports suggest that avian ChpV frequently affects the gastrointestinal tract and liver, research on this topic remains scarce [18]. The pathogenicity and tropism of these viruses have yet to be definitively proven, necessitating further investigation.

No research or evidence exists regarding GaChpV in poultry in Türkiye. To address this gap, the presence of GaChpV-1 and GaChpV-2 was investigated in fecal samples from healthy and enteritis-signed animals collected from broiler and turkey farms across different regions.

Results

Detection of GaChpV and sequencing

Using the nested PCR assay developed by Cui et al. [5] with the CkChpV-OF/CkChpV-OR and CkChpV-IF/CkChpV-IR primer sets, amplicons of approximately 380 bp were successfully detected in 36 out of 76 broiler flocks, including 4 out of 10 healthy flocks. This resulted in a 47.3% positivity rate. The distribution of positive samples by region was as follows: Sakarya (9/18), Ankara (3/9), Bolu (4/12), Adana (6/8), Düzce (8/14), Mersin (1/3), and Kocaeli (3/7). Additionally, positive results were obtained in 2 out of 20 (10%) turkey flocks with enteritis, specifically in Izmir and Bolu. In addition to this information, the PCR results for parvovirus and GaChpV are presented in detail in Table S1 [19]. The study revealed that 25 out of 36 GaChpV-positive flocks (69.4%; 25/36) tested positive for both viruses. Of the 25 mixed-infected flocks, 2 originated from turkeys, while the remainder were associated with broilers.

Subsequently, four PCR products (two from broilers and two from turkeys) were subjected to sequencing using the CkChpV-IF/CkChpV-IR primers. The sequence data were then compared using BLASTn against GenBank, and the results identified GaChpV from the clinical samples. The aligned sequence data were submitted to GenBank with accession numbers PQ058514, PQ058515, PQ058516, and PQ058517. In this study, the partial capsid gene nucleotide sequences (nt) of the CkChPV/2023/BR5-TR and CkChPV/2023/UN2-TR strains, both isolated from broilers, were found to be 100% identical. Similarly, these two strains exhibited over 99% identity at the nucleotide level and 100% identity at the amino acid level in comparison to the partial capsid gene sequences of the strains identified in turkeys (CkChPV/2023/HB10-TR and CkChPV/2023/HB2-TR). These partial sequences shared 95–97% identity with GaChpV-2 strains from Europe, including Switzerland (ChPV/PB4-HII34/x1/2019, ChPV/PB32-SII33/x1/2019) and the Netherlands (Environment/NLD/2019/VE_9, Chicken/NLD/2019/V_M_051). High identity (>98%) was also observed with strains from China (CkChpV-CHN210917) and Brazil (RS/BR/15/5S).

The partial capsid gene sequences of GaChpV strains identified from turkeys in this study (CkChPV/2023/HB10-TR and CkChPV/2023/HB2-TR) exhibited over 99% identity with each other and with a chicken GaChpV strain from Switzerland (ChPV/PB4-HII34/x1/Switzerland/2019). Additionally, these turkey GaChpV strains showed 98% nucleotide identity with chicken GaChpV strains from Switzerland (ChPV/PB32-SII33/x1/Switzerland/2019), Brazil (RS/BR/15/5S), and China (CkChpV-CHN220216, CkChpV-CHN220124, CkChpV-CHN210917, CkChpV-CHN210619). A 95% identity was observed with strains from the Netherlands (Environment/NLD/2019/VE_9_parvo_152, Chicken/NLD/2019/V_M_051_parvo_8).

Complete genome sequencing of GaChpV

A sample each from a chicken and a turkey was randomly selected for complete genome sequencing. The primers listed in the Table 1 successfully amplified the targeted regions, producing amplicons of the expected sizes at the end of the PCR. These amplicons were sequenced, and the overlapping sequences were aligned using MEGA-X software. Subsequent BLASTn analysis and overlapping assembly led to the construction of viral genomes. This process identified a GaChpV strain with a genome consisting of 4,229 nucleotides (NS: 2,021 nt, 672 aa; VP: 1,640 nt, 546 aa). The identified strain was named CkChPV/2023/UN-2-TR, and its sequence was submitted to GenBank under accession number PQ058514. However, the primers did not yield amplification for the complete genome in the GaChpV strain obtained from the turkey.

In this study, we conducted a comparative analysis using nearly all available complete genome sequences of avian *Chaphamaparvovirus* (132 sequences) from GenBank. The 4,229 nt sequence of CkChPV/2023/UN-2-TR showed nucleotide identities ranging from 78.93 to 98.82% with other GaChpV strains. Its capsid gene amino acid identity, when compared to ChPV strains from GenBank, ranged from 73.2 to 98.4% with Swiss strains, 73.3–97.6% with Dutch strains, 97.8–98.4% with Chinese strains, and 96.5–98.3% with Brazilian strains. For the NS1 gene, amino acid identity varied between 27.3% and 98.7% when compared with GaChpV strains from 16 different animal species.

Phylogenesis and amino acid substitutions

In this study, a phylogenetic tree was constructed using the NS gene of the CkChPV/2023/UN-2-TR strain, alongside sequences from various avian and mammalian *Chaphamaparvovirus* strains stored in GenBank. The analysis revealed that the nucleotide (nt) sequence of the NS gene of the CkChPV/2023/UN-2-TR strain clustered together with the GaChpV-2 strain, specifically the RS/BR/15/5S strain, which was isolated from fecal samples collected in 2015 from *Gallus gallus* in Brazil (Fig. 1).

However, within the same branch, GaChpV-3, GaChpV-4, GaChpV-5, and *Chestnut teal chaphamaparvovirus 1* grouped into a different cluster, while GaChpV-1 and GaChpV-7 exhibited significantly distinct genetic characteristics. Furthermore, the analysis showed that GaChpV-1 shared a high degree of genetic similarity with the red-crowned crane, whereas GaChpV-7 was more closely related to *Peafowl parvovirus 1* and *2* (Fig. 1).

Phylogenetic analysis based on the complete genome sequence indicated that the CkChPV/2023/UN-2-TR strain (PQ058514) from this study, along with all other GaChpV-2 strains, resided within the same branch. In

Table 1 Primer sets and temperature conditions used in the PCR stage

Primer labels	5′- 3′nucleotide sequences	*Position for (OP172530.1)	PCR product (bp)	94 ◦ C (sec)	PCR annealing (temp. – sec)	72 ◦ C (sec)	References
CkChappo 1 F	CTAGGGTATAAGTATGAGTAAGTAC	1–25	1275	40	55–30s	100	In this
CkChappo 1250R	ATATTTAACAGTAACCTCTGGACC	1250–1275					study
CkChappo 1 F	CTAGGGTATAAGTATGAGTAAGTAC	1–25	1582	40	55–30s	100	In this
CkChappo 1556R	TGTTACTTGGTCCATACAAGTGGA	1556–1582	1021	40	55–30s	80	study
CkChpV-F4	CACCAACACGTTATCAATGGC	262–283	397	40	54–30s	80	[5]
CkChpV-R4	ATCTGTATCACGAGACCACGTT	1262–1283					[5]
CkChPV-NesF	CTGCTTTCAACAATTGCACGTA	782–802					
CkChPV-NesR	TTTTCCAGCTCGCAATTCACC	1159–1179					
CkChpV-F2	AAAGCACCAGTTTGGATAATGTCG	1095–1118	1073	40	55–30s	80	[5]
CkChpV-R2	CACATCCTCTGGCACTATCGG	2145–2168					
CkChpV-F3	CGTCTACTTCTGGCATCCCAAC	1960–1981	1121	40	55–30s	80	[5]
CkChpV-R3	TCCCAAAATACACCATTCGGTA	3060–3081					
CkChPV-OF	TGTATAATTCCACGTCAATGGG	2765–2786	711	40	55–30s	80	[5]
CkChPV-OR	TGTAGAATATGCAGCTAACCAA	1075–1096					
CkChappo 3993 F	ATCAGATATCGAACAGGAGGAAGAAGA	3899–3925	333	40	55–30s	100	In this
CkChappo 4300R	TTCAGGTTTTGGTTGTGATTGTTC	4206–4232					study

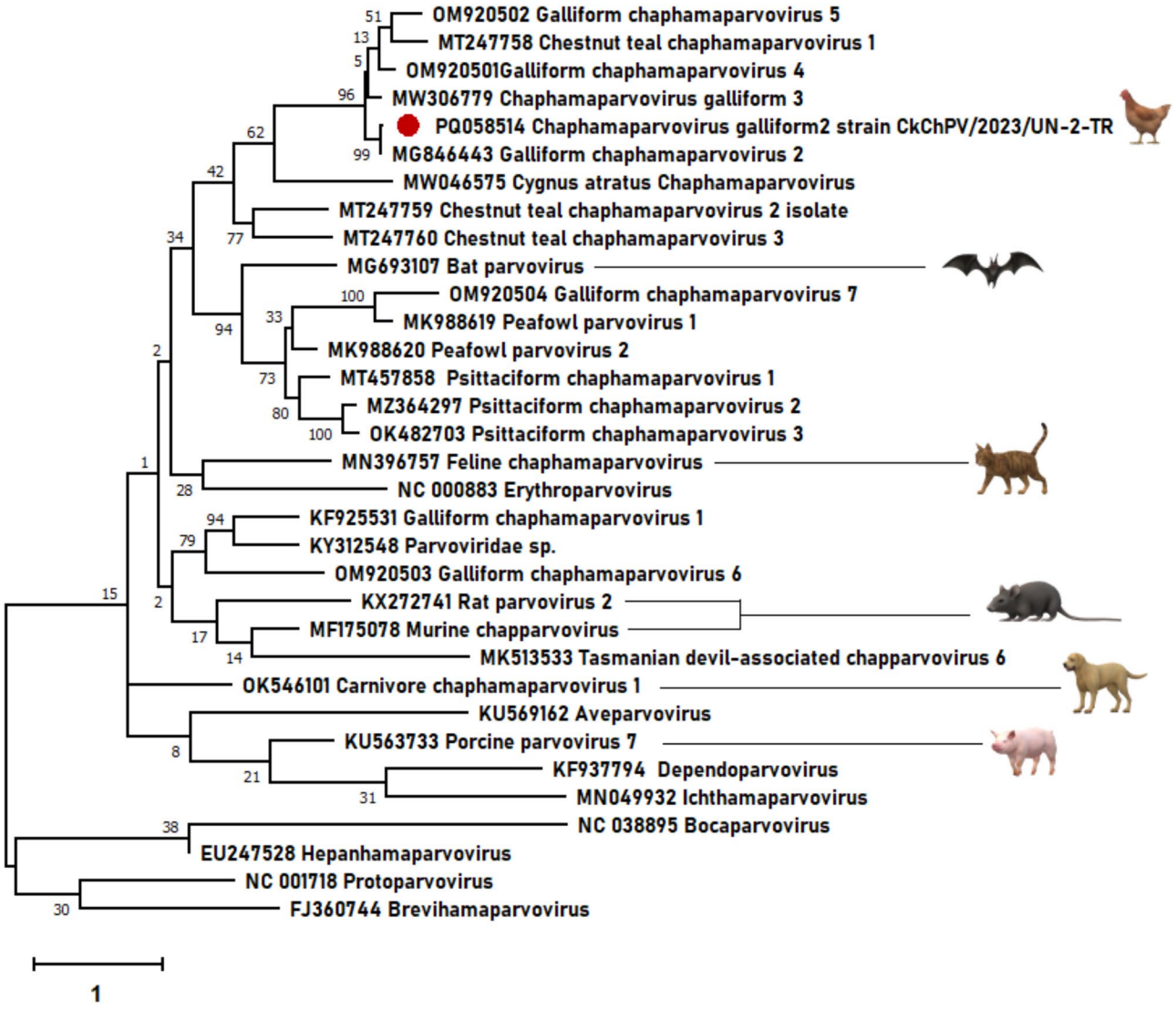

Fig. 1 Phylogenetic analysis based on the nucleotid sequence of the NS1 of CkChPV. The tree was performed with a selection of ChPV representative of each species of the genus *Chaphamaparvavovirus* strains. Also, viruses representative of the genera *Hepanhamaparvovirus*, *Brevihamaparvovirus* and *Ichthamaparvovirus* classified within the newly established subfamily *Hamapaparvovirinae*, was included in the analyses. Phylogenetic analysis was constructed using the Maximum Likelihood method, with statistical support provided by bootstrapping of 1,000 replicates, and the Tamura-Nei model was applied for nucleotide substitution. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. Red dot indicate the CkChPV strain detected in this study. Evolutionary analysis was conducted in MEGA X [29]

contrast, GaChpV-3 strains were distributed across two distinct branches (Fig. 2).

When analyzing the nucleotide sequence of the capsid gene, the GaChpV-1 and GaChpV-2 strains isolated from turkey and broiler in this study grouped within the same branch as other GaChpV-2 strains, while GaChpV-3 to GaChpV-7 clustered separately. Consistent with the complete genome sequence analysis, GaChpV-3 strains formed two different lineages. Both the capsid gene and whole-genome analyses revealed that the Turkish GaChpV strains exhibited a remarkable genetic similarity to the Brazilian GaChpV-2 strains (Fig. 3). When the capsid protein sequences of CkChPV/2023/UN-2-TR and the Brazilian strain RS/BR/15/5S were compared, amino acid substitutions were identified at the following positions: 17th (H to N), 182nd (S to R), 290th (S to N), 292nd (S to T), 295th (G to S), 297th (G to S), 297th (H to Q), 385th (P to T), 458th (T to A), 461st (K to R), 464th (L to I), 484th (E to D), 500th (T to S), and 508th (T to S). When compared to the Chinese strain CkChpV-CHN210619, amino acid substitutions at positions 17, 295, 297, 385, and 464 in the capsid protein were shared with those mentioned above, whereas differences were observed at positions 207 (S-A), 296 (T-S), 456 (N-S), 480–482 (ASI-TNV), 506 (S-T), and 534 (E-K). In addition to these, the capsid protein amino acid variations

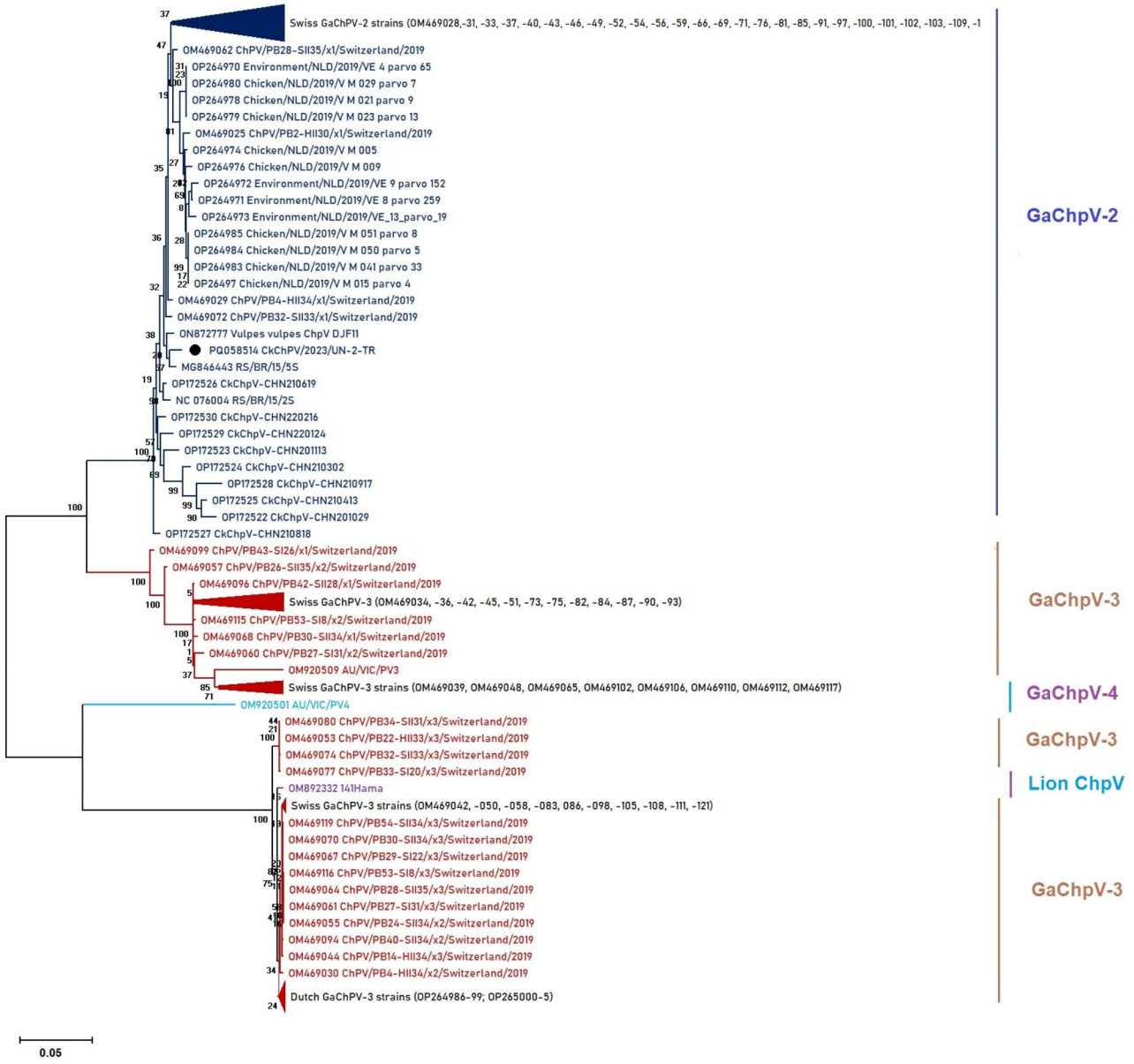

Fig. 2 Phylogenetic analysis based on the nucleotid sequence of the complete genome of CkChPV. Phylogenetic analysis was constructed using the Maximum Likelihood method, with statistical support provided by bootstrapping of 1,000 replicates, and the Tamura-Nei model was applied for nucleotide substitution. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. Black dot indicate the CkChPV strain detected in this study. Evolutionary analysis was conducted in MEGA X [29]

of CkChPV/2023/UN-2-TR and certain other strains from countries such as Brazil, China, Switzerland, and the Netherlands are presented in the Fig. 4. Based on the capsid protein amino acid comparisons, the H at position 17 and 297, as well as the P at position 385, were unique to CkChPV/2023/UN-2-TR.

Genetic recombination

CkChPV/2023/UN-2-TR was examined for genetic recombination signals along with 132 selected complete genome sequences. Although recombination signals and breakpoints between CkChPV/2023/UN-2-TR and several other strains were detected using seven methods in RDP4.0, these observations did not match those obtained from SimPlot analysis (Fig. S1-2).

B cell linear epitope of capsid protein

Bepipred Linear Epitope Prediction 3.0 identified potential B cell linear epitopes within the capsid protein sequence of the chicken chaphamaparvovirus (CkChPV/2023/UN-2-TR). For CkChPV/2023/UN-2-TR, the potential epitopes within the capsid protein

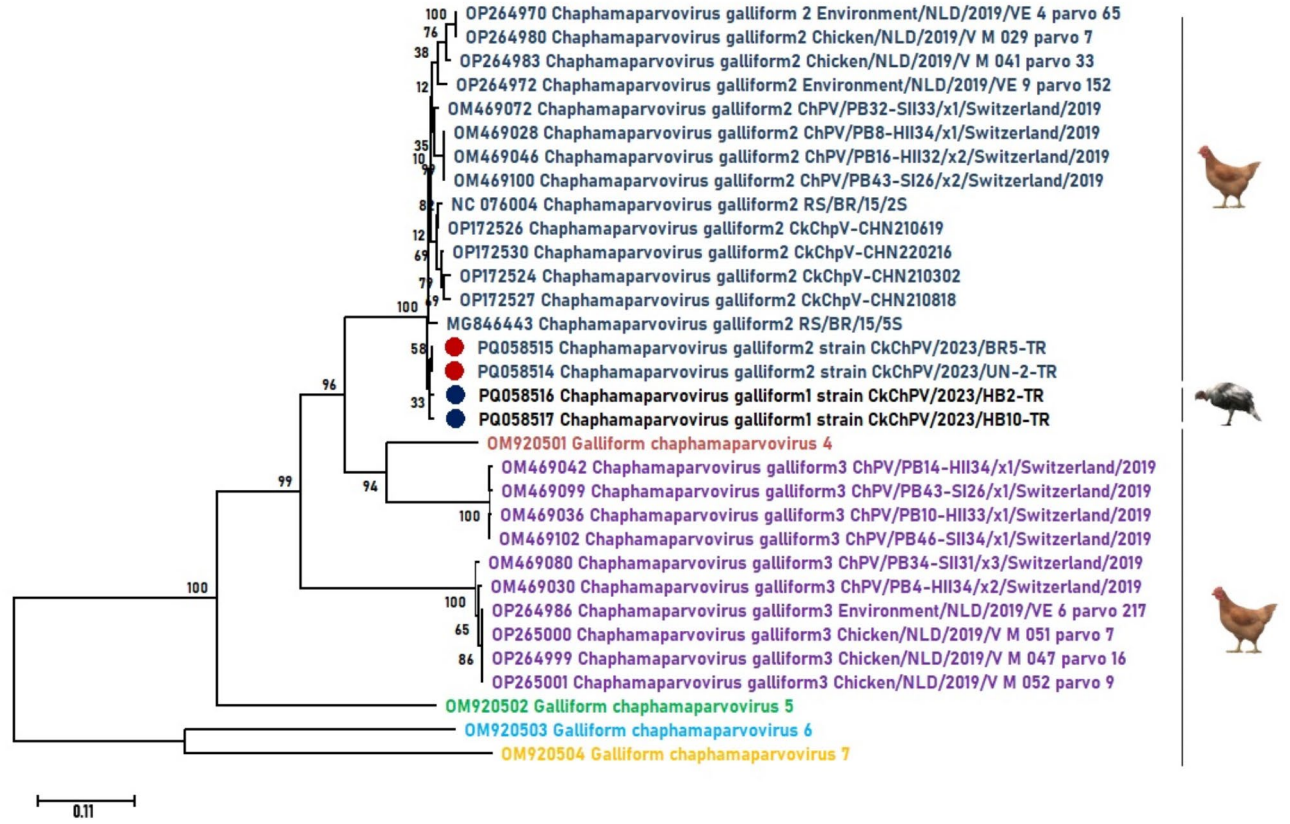

Fig. 3 Phylogenetic analysis based on the nucleotid sequence of the Capsid protein of CkChPV. Phylogenetic analysis was constructed using the Maximum Likelihood method, with statistical support provided by bootstrapping of 1,000 replicates, and the Tamura-Nei model was applied for nucleotide substitution. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. Black dots indicate CkChPV strains of chickens detected in this study, while red dots indicate CkChPV strains of turkeys detected in this study. Evolutionary analysis was conducted in MEGA X [29]

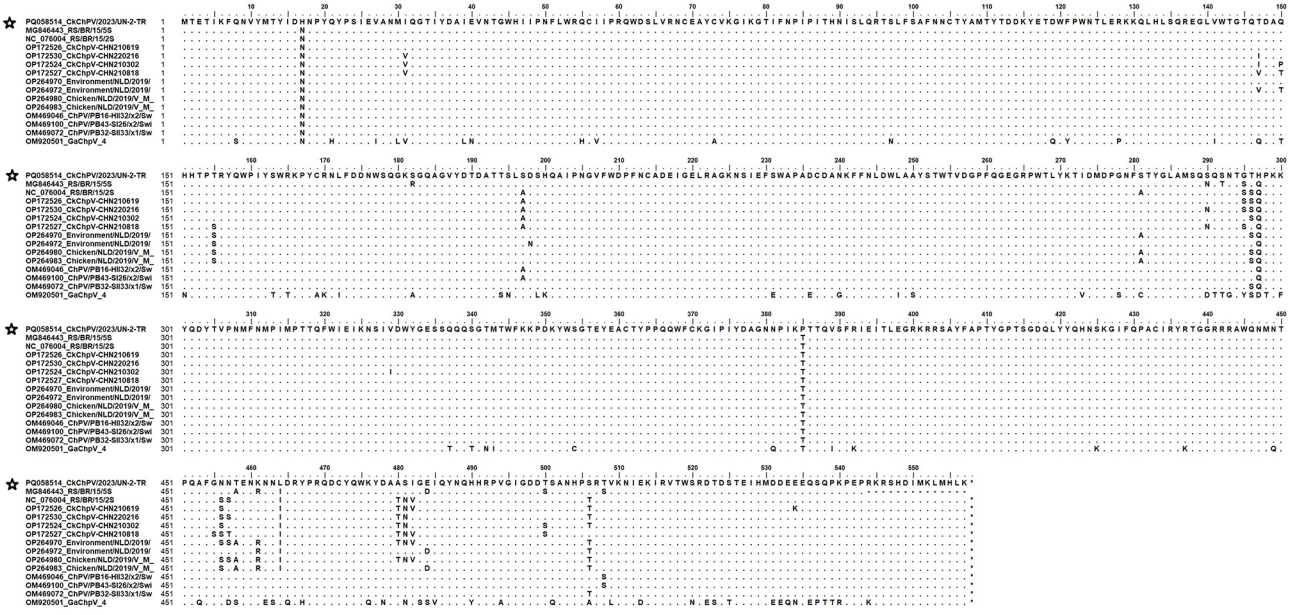

Fig. 4 Amino Acid alignment of the capsid protein of CkChPV/2023/UN-2-TR and selected ChpV strains from other countries

Table 2 Bepipred linear epitope prediction 3.0 identified potential B cell linear epitopes within the capsid protein sequence of the chicken chaphamaparvovirus (CkChPV/2023/UN-2-TR)

No	Start	End	Peptide	Length
1	90	101	NISLQRTSLFSA	12
2	132	157	LHLSQREGLVWTGTQTDAQHHTPTRY	26
3	172	201	IFDDNWSQGKSGQAGVYDTDATTSLSDSHQ	30
4	259	305	PFQGEGRPWTLYKTIDMDPGNFSTYGLAMSQSQSNTGTHPKKYQDYT	47
5	334	341	ESSQQQSG	8
6	419	430	IYYQHNSKGIFQ	12
7	435	443	RYRTGGRRR	9
8	445	525	WQNMNTPQAFGNNTENKNNLDRYPRQDCYQWKYDAASIGEIQYNQHHRPVGIGDDTSANHPSRTVKNIEKIRVTWSRDTDS	80
9	530	542	MDDEEEQSQPKPE	13

encompass positions 90 to 101, 132 to 157, 172 to 201, 259 to 305, 334 to 341, 419 to 430, 435 to 443, 445 to 525, and 530 to 542, (Table 2) (Fig. S3).

Statistical anaylsis

No statistically significant difference was observed between the GaChpV and the presence of enteritis findings ($p = 0.617$). Similarly, no statistical difference was found between mixed infection and enteritis ($p = 0.151$).

Discussion

Poultry farming plays a vital role in fulfilling the protein needs of many countries worldwide, including Turkey, while also significantly contributing to their economies [20]. In this industry, the quality of both the rearing environment and feed is paramount, but equally crucial is the animal's ability to effectively utilize the feed provided [21]. This is because feed utilization, growth, and immunity are all closely tied to maintaining a healthy digestive system.

Any disruption in digestive health is commonly referred to as enteritis, a condition that can be triggered by a variety of pathogens, including bacteria, viruses, fungi, protozoa, or parasites [22]. Often, these pathogens present as mixed infections. Among the viral agents, adenovirus, rotavirus, coronavirus, enterovirus, astrovirus, reovirus, and parvovirus are of particular importance [23, 24]. Extensive global research has focused on viral-induced enteritis. With advancements in viral metagenomics and next-generation sequencing technology, there has been a significant increase in the identification of novel viral species in recent years [7, 9, 15, 25]. One such novel virus is GaChpV, a member of the *Parvoviridae* family.

Recent studies have frequently detected *Chaphamaparvovirus* (ChPV) in various biological environments [1, 5, 26–28]. In this context, our study investigated the presence of GaChpV in broiler and turkey flocks from commercial farms across different regions of Türkiye. The results showed that GaChpV was detected in both chicken (36/76; 47.3%) and turkey flocks (2/30; 6.6%), with positive cases observed in broiler flocks with enteritis (31/66; 46,9%) and turkey flocks with enteritis (2/20; 10%). Despite these findings, no statistically significant association was observed between GaChpV and cases of enteritis ($p = 0.617$). This is in line with most previous studies [13, 15, 27], such as a 2015 study in Rio Grande do Sul, Brazil, which found no significant difference between healthy chickens and those affected by malabsorption syndrome [15]. Similarly, Kubacki et al. [13] detected GaChpV sequences in all broiler flocks in Switzerland, regardless of whether they were healthy or affected by runting-stunting syndrome (RSS). Fernandez-Cassi et al. [25] also identified 34 GaChpV sequences through metagenomic analysis of farm dust and chicken feces collected from broiler farms in the Netherlands at 4–5 different time points.

However, contrasting findings have emerged. Cui et al. [5] reported a stronger association between GaChpV and chickens exhibiting diarrhea compared to healthy ones. In their study in China, GaChpV was detected in 32% of 478 animals, both healthy and diarrheal. Notably, GaChpV had the highest detection rate among all viruses examined, with the majority of positive cases found in diarrheal animals (94.3%, 116/123; $p < 0.05$).

Our previous study investigated the presence of chicken and turkey parvoviruses [19], while the current study further examined GaChpV in the same samples. This enabled an evaluation of co-infection patterns, demonstrating that the rate of co-infected samples (25/38, 65.8%) was higher than that of those positive for a single pathogen (13/38, 34.2%). In this study, the analysis assessing whether mixed infections increased susceptibility to enteritis compared to single infections or the absence of infection was not statistically significant ($p = 0.151$). Furthermore, the absence of investigation into other microbial agents, coupled with the potential impact of factors such as stress and immunosuppression in the birds, complicates the determination of whether these conditions predispose to additional infections. Additionally, we lacked sufficient data to compare birds exhibiting enteritis-related symptoms (e.g., blood, foam, odor,

mucus) within their respective groups. These limitations should be considered when interpreting the findings of this study.

This study also presents the first report of *Chaphamaparvovirus* in poultry from Turkey, contributing to the growing body of research on this virus. To the best of our knowledge, only one other study has reported the detection of ChPV in turkeys [9]. In this study, we aimed to elucidate the complete genome sequences of GaChpV strains obtained from both broiler and turkeys by assembling partial overlapping sequences for more detailed molecular analysis. To achieve this, we selected one strain from each animal species and applied the same primer sets and PCR conditions as in the study by Cui et al. [5]. While PCR was largely successful for the broiler samples, it failed in the two turkey samples except for the partial capsid gene sequence. Interestingly, the partial capsid gene sequences from both chicken and turkey strains exhibited a high degree of identity (>99%), and phylogenetic analysis revealed that the GaChpV-1 strain from the turkey clustered with GaChpV-2 strains from chickens, supporting this finding. The failure of PCR amplification in turkey samples may be due to oversight in designing turkey-specific ChPV primers, the limited availability of turkey sequences in GenBank, or sequence variability over time.

Although Parvoviruses are DNA viruses, they are known to mutate and evolve rapidly, much like RNA viruses. In addition to mutations, genetic recombination has been shown to play a significant role in the genetic diversity and evolution of Parvoviruses [29]. In our study, we analyzed genetic recombination signals using the RDP-4.0 program. Although some breakpoint signals were detected at specific positions across seven different methods, these findings could not be confirmed by SimPlot analysis.

Considering the extensive genetic diversity and rapid evolution of Parvoviruses, we analyzed the linear B-cell epitope regions of the CkChPV/2023/UN-2-TR strain and compared them with selected Chinese (CHN210619), Brazilian (RS_BR_15_5S), and Swiss strain (PB32-SII-33_x1). Despite significant conservation of the B-cell epitope boundaries across the strains, a detailed analysis of capsid protein amino acid substitutions revealed that changes within the regions 259–305 and 445–525 significantly altered the epitope profiles. These findings suggest that amino acid substitutions in these regions may substantially influence the antigenic properties of the virus.

The phylogenetic analysis of the CkChPV/2023/UN-2-TR strain, fully assembled in this study, revealed genetic diversity with heterogeneous distributions among 132 GaChpV strains with complete genome sequences available in GenBank. Our results showed that CkChPV/2023/UN-2-TR and some Chinese, Swiss, and Dutch strains clustered under GaChpV-2, while the majority of the remaining strains clustered under GaChpV-3. This outcome is largely consistent with the findings of Kubacki et al. [13]. Furthermore, Turkish GaChpV strains, as revealed by complete-genome, NS, and VP phylogenetic analyses, exhibited close genetic similarity to the Brazilian GaChpV-2 strain RS/BR/15/S (MG846443) (Figs. 1, 2 and 3). Interestingly, the strains CkChPV/2023/UN-2-TR and RS/BR/15/S exhibited genetic proximity to the DJF11 strain (ON872777), which was previously identified in the intestinal virome of red foxes in China. This intriguing result may suggest either cross-species adaptation of a GaChpV-like virus in foxes or the incidental detection of GaChpV-like viral DNA in the fecal virome of foxes, possibly due to their consumption of a meat-based diet.

Although next-generation sequencing technologies have enabled the discovery of viral genomes in fecal viromes, the question of whether newly identified viruses like GaChpV cause disease in their hosts remains a subject of debate. To definitively establish the role of GaChpV in disease causation, experimental infections are required. This would involve isolating GaChpV from chickens or turkeys exhibiting clinical signs, reinfecting healthy chickens or turkeys, and subsequently re-isolating GaChpV, following Koch's postulates.

Conclusions

This comprehensive study marks the first detection of GaChpV in turkey and chicken flocks in Türkiye, and the findings will enhance our understanding of the molecular epidemiology and evolutionary dynamics of GaChpV.

Materials and methods

The geographical distribution and origin of clinical samples

The clinical samples utilized in this study were obtained from a previous study conducted by Abayli et al. [19], in which chicken and turkey parvoviruses were investigated. The original materials were 1060 fecal samples collected from 76 broiler and 30 turkey farms in various regions of Turkey. Samples were systematically collected from each flock, with 10 samples obtained per flock, including either fecal swabs or fresh feces. Among these, 200 samples were derived from healthy poultry (comprising 100 samples from turkeys and 100 from broilers), while the remaining samples were sourced from poultry exhibiting clinical signs of enteritis. (Table S1).

DNA isolation and PCR

After thawing the samples at 4 °C, they were diluted 1:10 with sterile phosphate-buffered saline (PBS). Following a 5-minute centrifugation at 10,000 ×g at 4 °C, 500

µL of the supernatant was collected from the centrifuged samples. DNA isolation was performed using a lysis buffer containing proteinase K (10 mM Tris-HCl, 10 mM EDTA, 100 mM NaCl, 40 mM DTT, 250 µg/mL proteinase K). The 500 µL supernatant was mixed with an equal volume of lysis buffer and vortexed. After incubation at 56 °C for 30 min, 500 µL of phenol-chloroform-isoamyl alcohol (saturated, 25:24:1) was added and vortexed. After a 15-minute incubation at room temperature, the mixture was centrifuged at 12,000 ×g for 30 min at + 4 °C. After centrifugation, The upper liquid phase was carefully transferred to a new tube, and 1 volume of ethanol and 1/10 volume of 5 M sodium acetate (pH: 5.5) were added to the tube containing the mixture, which was then inverted to mix. The tube was kept at -80 °C for 1 h before being centrifuged at 12,000 ×g for 15 min. The resulting pellet was washed twice with 1 mL of 75% (v/v) ethanol by centrifugation at 8,000 ×g for 5 min each time, and the supernatant was removed. After completely removing the ethanol, the DNA was diluted with DNase/RNase-free water to a final concentration of 100 ng/µL as measured by a NanoDrop 2000 spectrophotometer (Thermo Fisher, USA). The diluted DNA was stored at -20 °C.

For the investigation of GaChpV, nested PCR analysis was performed using primers reported [5]. In the first round, 5 µL of DNA, 25 µL of PCR master mix, and 4 µL of CkChPV-OF/CkChPV-OR primer mix (10 pmol) were combined and the total volume was adjusted to 50 µL with ultrapure water. The PCR conditions for the first round were as follows: initial denaturation at 94 °C for 5 min, followed by 35 cycles of denaturation at 94 °C for 35 s, annealing at 50 °C for 30 s, extension at 72 °C for 40 s, and final extension at 72 °C for 5 min. After the first PCR round, a second PCR round was performed. For the second round, 1 µL of the first-step PCR product was added to 25 µL of master mix along with 4 µL of CkChpV-IF/CkChpV-IR primer mix (10 pmol), and the total volume was adjusted to 50 µL with ultrapure water. The PCR conditions were as follows: initial denaturation at 94 °C for 5 min, followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 54 °C for 30 s, extension at 72 °C for 40 s, and final extension at 72 °C for 5 min. After the reaction was completed, the PCR products were loaded onto 1.5% agarose gels containing ethidium bromide and subjected to electrophoresis at 120 V for 35 min in 1X TAE buffer. At the end of the process, the agarose gels were visualized under UV light.

Complete genome amplification

For the complete genomic PCR amplification of GaChpV, previously published protocols were followed as references [5]. When initial amplification attempts were unsuccessful, new primer sets were designed using Primer-BLAST, based on reference strains available in GenBank and partial sequences obtained during this study. For the complete genome amplification, 5 µL of template DNA and 4 µL of primer mix were added to 25 µL of PCR master mix, and the reaction volume was brought to 50 µL with ultrapure water. After the reaction, PCR products were subjected to electrophoresis on a 1.5% agarose gel containing ethidium bromide at 120 volts for 35 min. The resulting amplicons were visualized under UV light. The primer sets used for GaChpV detection and complete-genome amplification are presented in the Table 1.

Sequencing and phylogenetic analysis

Sequencing analysis of the PCR-positive samples was carried out by a commercial laboratory (BMlab, Ankara). The resulting sequences were compared both internally and with other sequences available in GenBank using the MEGA X software [30]. To assess the genetic relationships among the sequences, a phylogenetic tree was constructed using MEGA X. During the construction of the phylogenetic tree, bootstrap analysis with 1000 replicates was performed using the Maximum Likelihood method and the Tamura-Nei.

model [31]. The Sequence Identity And Similarity (SIAS) tool was used to analyze the similarity of strain CkChPV/2023/UN2-TR with strains identified in references and in this study based on NS1 and capsid gene and amino acids [32].

Genetic recombination

Complete genome sequences were analyzed using the Recombination Detection Program 4 (RDP 4) [33] with detection methods including RDP, Bootscan, GENECONV, MaxChi, Chimaera, SiScan, and Phylpro. Results for strains exhibiting genetic recombination signals were also evaluated using SimPlot v3.5.1 [34] (Fig. S1.

B cell linear epitope of capsid protein

To evaluate potential B cell epitopes in the full-length capsid protein of GaChpV, Bepipred Linear Epitope Prediction 3.0 [35] was used. The detection threshold for the assay was set at 0.151.

Statistical anaylsis

The data were analyzed using the SPSS 21 package program. A chi-square test was used for comparison of the data, and a value of $p \leq 0.05$ was accepted as statistically significant.

Abbreviations

aa	Amino acid
GaChpV	*Chaphamaparvovirus galliform*
ChpV	*Chaphamaparvovirus*
nt	Nucleotide
PBS	Phosphate-buffered saline
RDP 4	Recombination detection program 4

SIAS The sequence identity and similarity

Supplementary Information

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Supplementary Material 1

Author contributions

All authors contributed to the concept and design of the study. Collection of study samples was carried out by Mehmet AKAN and Akın Ünal. Material preparation, data collection and analysis were carried out by Hasan ABAYLI, Ahsen Nisa ASLAN, Şükrü TONBAK, Hasan Öngör and Ertuğ Yalçınkaya. Hasan ABAYLI and Ahsen Nisa ASLAN wrote the first draft of the manuscript. All authors provided feedback on earlier versions of the manuscript. The final manuscript was reviewed and approved by all authors.

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Data availability

Sequence data that support the findings of this study have been deposited in the GenBank (National Center for Biotechnology Information) with the accession numbers PQ058514-17.

Declarations

Ethical approval

This study was approved by Fırat University Animal Experiments Local Ethics Committee (Ethical approve number 2022/07 – 02). No attempt was made to adversely affect animal health or disrupt the tissue integrity. All procedures performed in animal studies were in compliance with the local and international ethical standards.

Consent to participate

All the authors consented to participate in this study.

Informed consent

All the authors consent to publish. The human participants and their personal data are not included in this article. The consent of animal owners was obtained at the time of sampling.

Consent for publication

All the authors consent to publication of this article.

Competing interests

The authors declare no competing interests.

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