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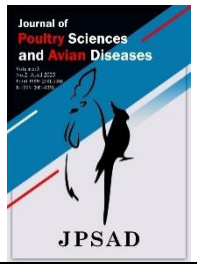

Detection, identification, Clinical and Histopathological Features of Novel Ostrich parvovirus in Iran

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A B S T R A C T

Recently, a novel disease affecting ostriches has emerged in several regions of Iran. This disease is characterized by sudden death, diarrhea, and paralysis. This outbreak has significantly impacted the Iranian ostrich farming industry, leading to substantial economic losses. This study aims to characterize and identify a novel ostrich parvovirus (OsPV) originating from chicks that exhibited paralysis and diarrhea in ostrich flocks across three provinces in Iran. Samples were collected from paralyzed ostriches and evaluated through clinical examination, histopathology, molecular techniques, and phylogenetic analysis. Before necropsy, radiographs were taken to assess for trauma. Affected tissues were evaluated histopathologically. PCR was performed to amplify the NSP-VP1 gene, and two positive samples, named SANA/OsPV/001/Ostrich/IR/2024 and SANA/OsPV/002/Ostrich/IR/2024, were sent for sequencing using both forward and reverse primers. A comprehensive analysis of the NSP-VP1 gene of OsPV was conducted to elucidate its genetic characteristics. Throughout 2024, four distinct isolates of OsPV were detected in three provinces: Tehran, Razavi Khorasan, and Isfahan. A 90 bp fragment of the NSP-VP1 gene of OsPV was detected in multiple organs, indicating that the new OsPV variant has multiple organ tropism. Radiographic findings revealed severe enteropathy and maldigestion/absorption alongside cloacal distention. Histopathological analysis showed metaplastic changes in the sciatic nerve, extensive necrosis of the intestinal mucosa, acute tubular necrosis, glomerulonephritis in the kidneys, and signs of pneumonia. Comparative sequence analysis of the NSP-VP1 gene revealed a high degree of homology between the SANA/ OsPV / 001/ Ostrich/ IR/ 2024 isolate and strains from Turkey, the United Kingdom, and Russia.

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Notably, the SANA/OsPV isolates were found to be closely related to goose parvovirus and other ostrich parvovirus strains. This study represents the first documented molecular detection and characterization and a histopathological assessment of ostrich parvovirus in Iran. Our findings contribute to a better understanding of the virus's epidemiology and impact on the ostrich farming sector, underscoring the need for ongoing surveillance and control measures in affected regions.

Keywords: *Ostrich parvovirus (OsPV), Phylogenetic, Molecular Detection, Iran, Histopathology, NSP-VP1 gene*

1 Introduction

The ostrich (*Struthio camelus*) is the largest living bird and belongs to the ratite family of birds (1, 2). Ostrich farming is an economic activity worldwide, and several commercial ostrich farms exist in Iran (1, 3). Several viral, bacterial, and fungal diseases with clinical manifestations of diarrhea, reluctance to move, and sternal recumbency with high morbidity and mortality rates have been reported in ostriches (4, 5).

Parvoviruses are small, non-enveloped viruses with linear, single-stranded DNA (ss DNA) genomes that belong to the *Parvoviridae* family (6, 7). Parvoviruses can infect both invertebrates and vertebrates (6, 7). According to the International Committee of Taxonomy of Viruses (ICTV) classification, all parvoviruses that infect vertebrates belong to the *Parvovirinae* subfamily (6). Based on the newest classification, the *Parvovirinae* subfamily is divided into eight genera: *Amdoparvovirus*, *Aveparvovirus*, *Bocaparvovirus*, *Copiparvovirus*, *Dependoparvovirus*, *Erythroparvovirus*, *Protoparvovirus*, and *Tetraparvovirus* (6, 7). The variety of these viruses also had widely different effects on their hosts, ranging from severe diseases to subclinical or nonpathogenic infections (8). Recent studies have shown that the diversity of some known parvovirus species has expanded due to biological adaptation, and the host range of parvoviruses may include many animals (7, 8).

Parvovirus infection has been reported in several avian species, especially waterfowl (9). For the first time, goose parvovirus (GPV) was discovered in China in 1956 and was isolated from goose embryos in 1961 (10). During the 1960s, a prevalent disease of goslings and Muscovy ducklings with a high mortality rate was reported in Europe, and GPV was identified as the causative agent in these cases (9, 10). Also, another type of parvovirus, with similar symptoms to those of GPV, was isolated from Muscovy ducks and was named Muscovy duck parvovirus (MDPV) in 1989 (8, 11). Although GPV infection has been reported in goslings, Muscovy ducklings, swans, and *Anser cygnoides*, MDPV infection has only been detected in Muscovy ducklings (12, 13). The reported clinical manifestations of parvovirus-infected geese and Muscovy ducks were significant weight loss, back and neck feather shedding, diarrhea, and high mortality (14, 15). The presence of parvovirus in turkeys has been reported with the symptoms of stunting, enteritis, and a high mortality rate (16). Furthermore, parvovirus has been isolated in chickens with running-stunting syndrome (RSS) (17, 18). Also, in recent studies, a novel duck parvovirus (NDPV) was reported as a new variant of GPV that can infect ducks and geese (19, 20).

In 2020, for the first time, the presence of parvoviruses was reported in farmed ostriches, with paralysis as the primary clinical manifestation. This novel ostrich parvovirus (OsPV) was reported as another new variant of GPV by doing Genetic distance analysis (21). Also, in another study, parvovirus has been isolated in farmed ostriches with the symptoms of paralysis and diarrhea (22).

2 Materials and Methods

2.1 Sample Collection and preparation

Muscles, sciatic nerve, kidneys, spleen, thymus, heart, pancreas, and gut samples were collected from 12 commercial broiler flocks (aged 1-3 months, which did show paralysis, diarrhea, and weight loss) from different areas of Iran, including Razavi Khorasan, Isfahan, and Tehran provinces during 2024 and processed for viral detection and isolation.

2.2 Clinical Anatomy and Histopathology

Before dissecting diseased ostriches, the animal's legs were evaluated for trauma by taking dorsoventral and lateral view radiographs. Then, tissues from their Sciatic nerve, articular content, liver, Bursa of Fabricius, intestine, spleen, and thymus were collected and examined. All tissues were stored at -80° C for further study. Parts of the tissues were

fixed in 4% neutral formalin at room temperature, and blocks were sent to Prof. Hess, the Department for Farm Animals and Veterinary Public Health at the University of Veterinary Medicine, Vienna, Austria, for histopathological evaluation.

2.3 Detection of the Potential Pathogen Using Polymerase Chain Reaction

Tissues were homogenated separately, and DNA and RNA were extracted using a commercial DNA Extraction kit (MBST DNA extraction kit, Cat No. DNA Ex-BT-1388) and RNA Extraction kit (MBST DNA extraction kit, Cat No. RNA Ex-Liquid-1399) according to the manufacturer's instructions. All samples were stored at - 20°C.

In cases of viral RNA detection, a cDNA synthesis kit (Yekta Tajhiz Azma, Tehran, Iran) was used. Then, conventional PCR, Reverse transcriptase PCR, and quantitative PCR were performed using the primers listed in Table 1.

2.4 Partial NSP-VP1 gene Amplification of SANA/OsPV/Ostrich/IR/2024 isolate and Sequencing

Two of the positive samples were randomly selected and named SANA /OsPV /001 /Ostrich /IR /2024 and SANA /OsPV /002 / Ostrich /IR /2024 isolates. They were then sent for sequencing with T3-Forward and T3-Reverse primers (Gene Fanavaran, Tehran, Iran).

2.5 Comparison of the sensitivity of conventional PCR and Quantitative PCR methods

The diagnostic method's sensitivity was evaluated by preparing Ten-fold serial dilutions of intestine tissue and performing conventional PCR and q-PCR for the NSP1 and VP protein genes, respectively.

2.6 Phylogenetic Analysis

Genome sequences of the NSP-VP1 coding region of OsPV were retrieved from GenBank. The sequences were aligned using the MEGA-7 software (23). Sequence analysis was conducted using the MEGA-7 software. Phylogenetic analysis was conducted using the Neighbor-Joining method, Kimura 2-parameter method (24), and JTT matrix-based model, respectively, and bootstrapping up to 500 replicates by MEGA-7 software(23).

3 Results

3.1 Clinical Syndrome and Histopathologic Changes

In recent years, a disease outbreak characterized by paralysis as the main symptom occurred in farmed ostriches in different regions of Iran. The disease causes major damage to the ostrich breeding industry in Iran. Between July 2023 and June 2024, 12 commercial ostrich flocks with this problem were referred to. The incidence rate ranged from 20 to 91 percent, and the mortality rate was between 12 to 79 percent. The affected ostriches were mostly 1-3 months old; however, there were several other cases between 15 days and 9 months old. Adult and yearling ostriches appeared resistant. The first and the most important observed clinical symptoms were the inability to stand fully, movement on the intertarsal joint, paralysis of pelvic limbs, and sternal recumbency with the head and neck up (Figure 1, a).

Figure 1. Clinical (a) and postmortem (b) signs of disease; a. inability to stand and paralysis. b. Enteritis, in some cases

In a minority of cases, clinical signs of incoordination were seen 1-2 days before recumbency, and at this level, some birds could stand and move with difficulty if given external support. High rectal temperature (39.6- 42.1 ºC) in the first two days of commencement of symptoms before recumbency was the other clinical finding in most cases. Although the appetite of paralyzed ostriches had not changed during the first days of recumbency, progressive weight loss was observed due to possible secondary infections and pressure sores resulting from recumbency. Although foul-smelling diarrhea was observed in some cases, this finding was not common in all the affected chicks, and in some cases, severe constipation was observed. In the physical examination of the affected chicks, no abnormal finding was seen. The affected ostriches progressively became thin and weak, with the disease lasting for up to 56 days from onset to death. About 5 percent of the affected ostriches aged between 50 and 75 days old recovered if they were given intensive supportive care.

In necropsy findings, severe enteritis with severe mucosal and intestinal villi necrosis was seen in ostriches with more than ten days of recumbency (Figure 1, b & Figure 2, a). In some cases, with long-term paralysis, a moderate to severe inflammation of the intertarsal joint was observed. Also, degeneration of pelvic limb muscles accompanied by minor bleeding points was another finding in most long-term recumbent ostriches. Several samples containing whole blood, heart, lung, liver, spleen, kidney, small and large intestines, colon, sciatic nerve, spinal cord, and brain were collected for further laboratory and histopathology evaluations (Figure 2).

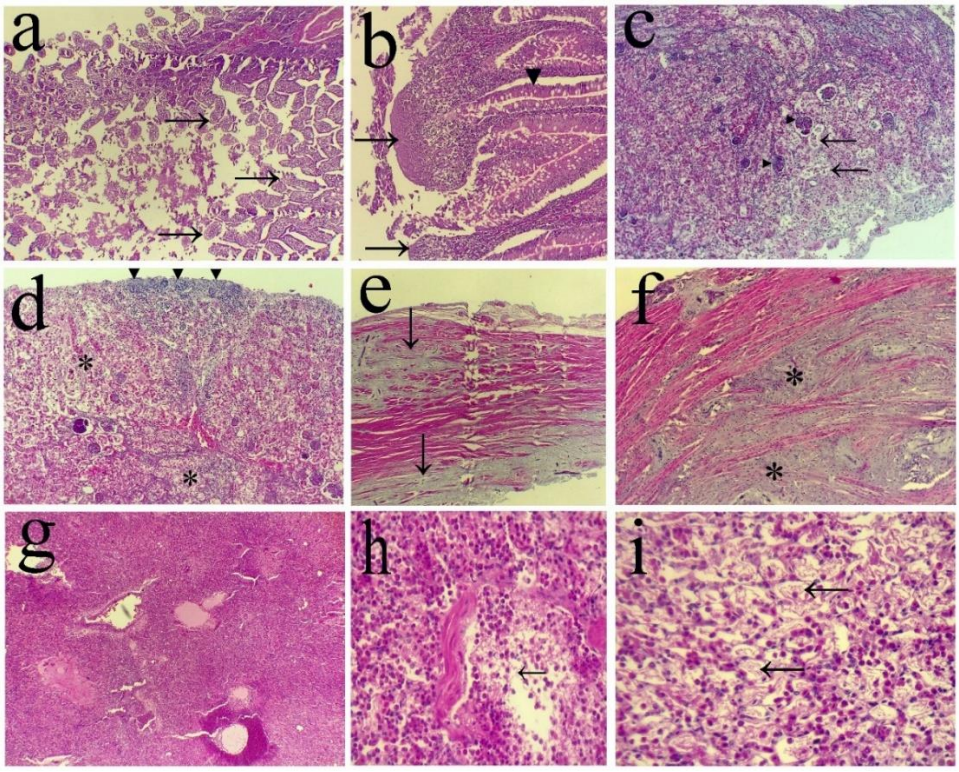

Figure 2. Some of the histopathological findings in different organs. a, b; Intestine: Extensive necrosis of the mucosa and intestinal villi, which have lost their surface layer of epithelial cells and collapsed (arrows) (a). Necrosis is confined to one-third of proximal intestinal villi with large numbers of inflammatory cell infiltration (arrows). However, the crypts are still lined by intact glandular epithelial cells (arrowhead) (b). c, d; Kidney: Acute tubular necrosis and glomerulonephritis. The epithelium lining of renal tubules is sloughed into the lumen (arrows). Other tubules are showing degeneration. Most of the glomeruli are invaded by inflammatory cells, which fill the urinary spaces (arrowheads) (c). A sheet of inflammatory cells is broadened at the outermost part of the cortex (arrowheads). Numerous renal tubules are degenerated or died and desquamated into their lumens (asterisks) (d). e, f; Peripheral neuropathy: The extensive surface of the sciatic nerve is converted to the myxoid-like structure at the peripheral and central parts of the nerve (arrows) (e). The mucinous blue-staining stroma resembles the myxoid or cartilaginous structure, probably indicating metaplastic changes (asterisks)(f). g-i; Lung: Pneumonia Intensive inflammation of pulmonary parenchyma, in which nearly all air ducts and air capillaries are filled with a large population of leukocytes. Follicular structures are being made (g). Inflammatory cells with the largest population of heterophils with eosinophilic cytoplasm associated with other mononuclear cells. The pale pink fibrin threads are also present among leukocytes. A prominent venule with a thick, deeply eosinophilic hyalinized wall is located at the center of the picture (arrow) (h). Air capillaries are filled with diffused fine fibrin threads and leukocytes, and dominantly heterophils are settled around the capillaries or inside them (arrows) (i).

Figure 2 also characterized some pathologic changes in the sciatic nerve; however, the cerebral tissues had no obvious changes in almost all samples. Mild cerebral edema was observed in two cases (a two-month-old and a five-month-old). The histopathologic evaluations of the collected embryos showed no abnormal changes. The collected whole blood was evaluated for CBC and biochemistry tests, and the values were within normal limits.

The obtained radiographs also showed some abnormal findings, summarized in Figure 3. Severe enteropathy and maldigestion/malabsorption, along with cloacal distention, can be seen.

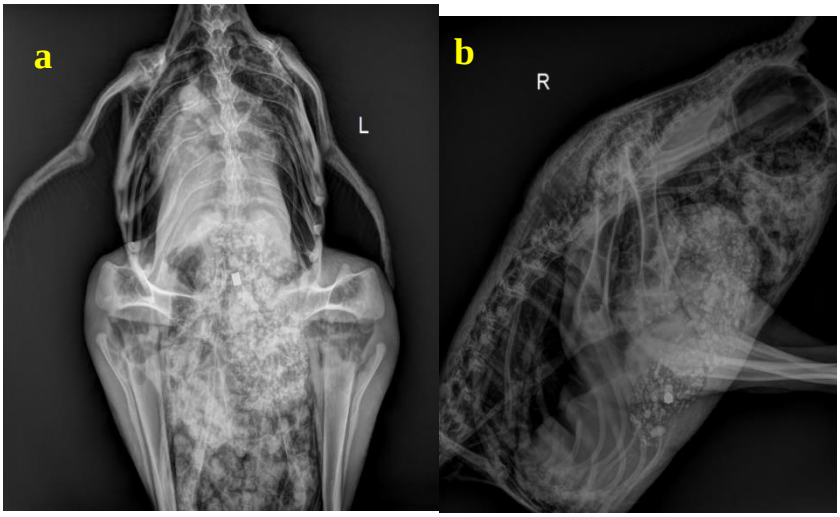

Figure 3. Sample radiograph of affected birds in ventrodorsal (a) and lateral (b) views.

3.2 Detection of the OsPV and partial Genome Sequencing

Several viral screening tests containing Newcastle disease (ND), Avian influenza (AI), Borna disease, Avian encephalomyelitis (AE), Infectious Bronchitis (IB), Avian reovirus, and Marek disease (MD) were evaluated, and the results were negative. However, in the last four referred paralyzed cases from four different ostrich farms, the ostrich parvovirus was detected in visceral organs like the spleen, liver, and heart. For this purpose, conventional and real-time PCR assays were performed to detect parvovirus infection using the primers mentioned (Table 1).

Table 1. *The primers and probe used in this study.*

Target	Name	Gene	Sequence	Length (bp)	References
Ostrich Parvovirus	T3-Forward	NSP, VP1	TTGTTCTCATCAGTCGCTCCA	1417	(22)
	T3-Reverse		GCGGCAGGGCATAGACAT		
Chicken Parvovirus	PV-F	nonstructural protein 1 (NSP1)	TTCTAATAACGATATCACT	695	(25)
	PV-R		TTTGCGCTTGCGGTGAAGTCTGGCTCG		
Ostrich Parvovirus	OsPV-Forward		CAAATTCCATCCTTCTCCGAATCT	90	(22)
	OsPV-Reverse	VP Protein	TCTGCAGGCACTGGTGTATTCTTGA		
	OSPV-Probe		6-Fam-CTGCACAATCCACCACCGCAGGTGTTC-BHQ-1	53	
Newcastle Disease Virus (NDV)	Alle (B-)	Fusion Protein	GGAGGATGTTGGCAGCATT	363	(26)
	Alls (A+)		TTGATGGCAGGCCTCTTGC		
Avian Influenza Virus (AIV)	H9-151F	Haemagglutinin Protein	CTYCACACAGARCACAATGG	488	(27)
	H9-638R		GTCACACTTGTTGTTGTRTC		
Avian Encephalomyelitis Virus	AE-F	VP1 Gene	GAATTAGCTCCTGGTAAACCTCG	288	(28)
	AE-R		TATTATCGCAACACCCTCAGG		
REO Virus	P2	sigma B (S3)	CAAGCATTACAGGGCCAGC	540	(29)
	P3		ATTACGGGACTCTGCCCGC		
Marek's Disease Virus	pp38-F	Phosphoprotein 38	GTGATGGGAAGGCGATAGAA	226	(30)
	pp38-R		TCCGCATATGTTCCTCCTTC		
Bornavirus	PDD-N-F	N Protein	CATGAGGCTATWGATTGGATTA	389	(31)
	PDD-N-R		TAGCCNGCCMKTGTWGGRTTYT		

Then, agar gel electrophoresis with 0.8% agarose gel was performed for the conventional PCR product, which showed the expected product size of 1417 bp (Figure 4).

Figure 5 also shows agar gel electrophoresis with 1.5% agarose gel for 90 bp PCR product in the liver, brain, spleen, sciatic nerve, bursa of fabricius, thymus, articular content, and intestine tissue separately.

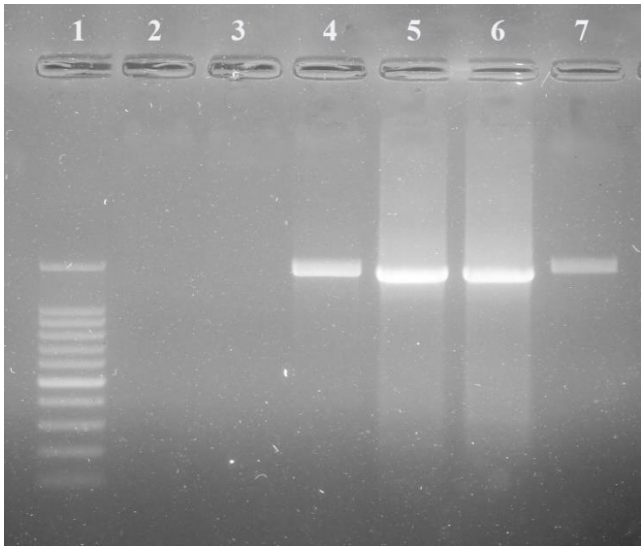

Figure 4. Agar gel electrophoresis for conventional PCR product with expected 1417 bp product size. Lane 1: 100 bp DNA ladder (SINACLON, Tehran, Iran); Lane 2: Negative control for DNA extraction; Lane 3: Negative control for water; Lane 4: Farm 1; Lane 5: Farm 2, Lane 6: Farm 3, Lane 7: Farm 4

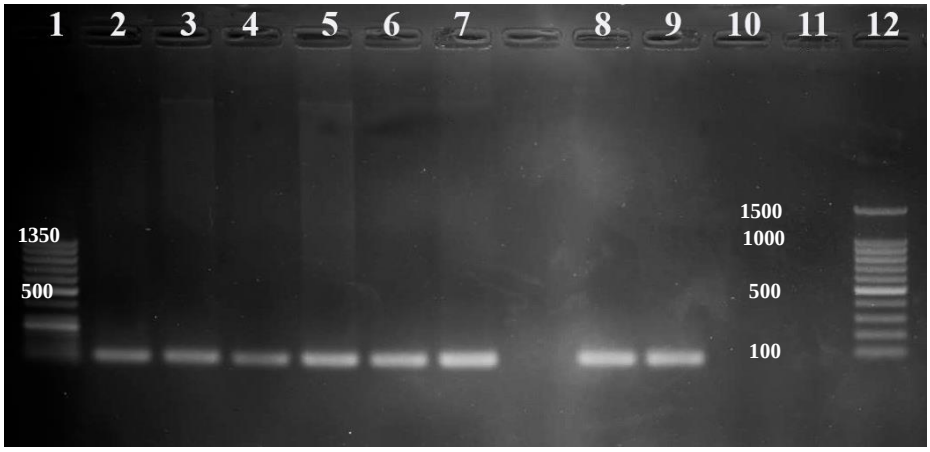

Figure 5. Agar gel electrophoresis for conventional PCR product with expected 90 bp product size. Lane 1: 50 bp DNA ladder (SINACLON, Tehran, Iran); Lane 2:Liver, Lane 3:Thymus, Lane 4: Bursa of Fabricius, Lane 5: Spleen, Lane 6: Articular content, Lane 7: Brain, Lane 8:Intestine, Lane 9: sciatic nerve, Lane 10: Negative control for DNA extraction, Lane 11: Negative control for water, Lane 12: 1000 bp DNA ladder (SINACLON, Tehran, Iran).

The results for detecting ostrich parvovirus in collected embryos of affected ostrich farms were negative.

In the bacterial culture of collected samples from 17 cases, *E. coli* was isolated from brain tissue in one case. Also, *Clostridium perfringens* was isolated from the culture of abdominal cavity contents in another chick. In the other cases, there was no bacterial growth. The results of *Aspergillus niger* and *Salmonella* Pullorum tests in different tissue samples were all negative, and there were no fungal and bacterial growth. Also, the screening tests for *Mycoplasma gallisepticum* (MG) and *Mycoplasma synoviae* (MS) were negative.

Preparing serial dilutions of intestine-positive samples and comparing them in PCR and qPCR methods in Figure 6

showed that the qPCR method detects the virus up to a dilution of 10^{-4}, while the Conventional PCR method can only detect it up to a dilution of 10^{-3}.

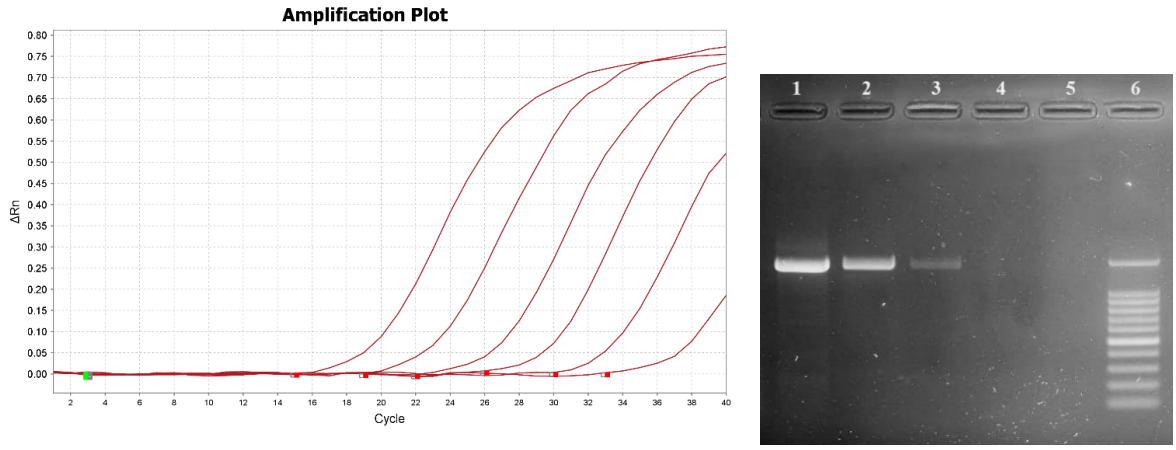

Figure 6. A: Diagram of real-time PCR from intestine tissue serial dilation; B: Agar gel electrophoresis for conventional PCR product with expected 1417 bp product size. Lane 1: INTESTINE N/10; Lane 2: INTESTINE N/100; Lane 3: INTESTINE N/1000; Lane 4: INTESTINE N/10000; Lane 5: Control Negative; Lane 6: 100 bp DNA ladder (SINACLON, Tehran, Iran)

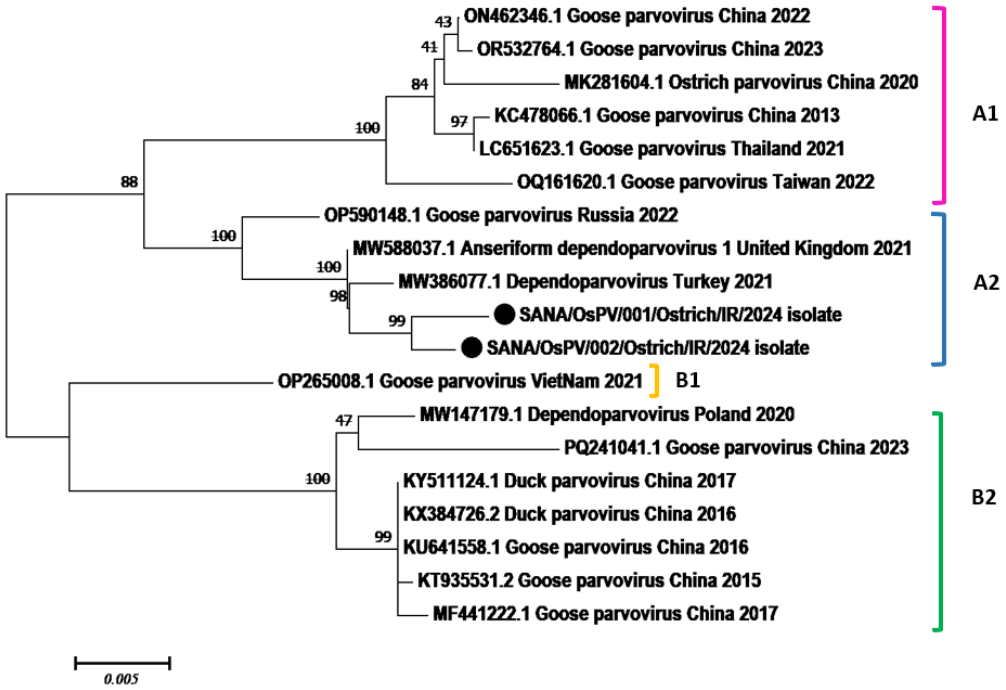

Figure 7. Avian parvoviruses belong to branches designated as branches A and B. SANA isolated belong to branch A and clade A2.

Table 2. Estimates of evolutionary divergence for the NSP-VP1 gene of SANA isolates. The number of base differences per site is shown between sequences. The analysis involved 19 nucleotide sequences, which is the closest result derived from BLAST. Codon positions were 1st+2nd+3rd. All ambiguous positions were removed for each sequence pair. Evolutionary analyses were conducted in MEGA-7 (23)

NO.		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
1	SANA- No.2_IR_2024																			
2	SANA- No.1_IR_2024	99.99																		
3	Turkey_2021 (MW386077)	99.99	99.99																	
4	Russia_2022 (OP590148)	99.98	99.98	99.99																
5	_ UK_2021 (MW588037)	99.99	99.99	100.00	99.99															
6	China_2013 (KC478066)	99.97	99.96	99.97	99.97	99.97														
7	China_2015 (KT935531)	99.95	99.95	99.96	99.96	99.96	99.95													
8	China_2016 (KU641558)	99.95	99.95	99.96	99.96	99.96	99.95	100.00												
9	China_2016 (KX384726)	99.95	99.95	99.96	99.96	99.96	99.95	100.00	100.00											
10	China_2017 (KY511124)	99.95	99.95	99.96	99.96	99.96	99.95	100.00	100.00	100.00										
11	Thailand_2021 (LC651623)	99.97	99.96	99.97	99.98	99.97	100.00	99.95	99.95	99.95	99.95									
12	China_2017 (MF441222)	99.95	99.95	99.96	99.96	99.96	99.95	100.00	100.00	100.00	100.00	99.95								
13	China_2020 (MK281604)	99.96	99.96	99.97	99.97	99.97	99.99	99.95	99.95	99.95	99.95	99.99	99.94							
14	Poland_2020 (MW147179)	99.96	99.95	99.96	99.96	99.96	99.95	99.99	99.99	99.99	99.99	99.95	99.99	99.95						
15	China_2022(ON462346)	99.97	99.96	99.97	99.98	99.97	100.00	99.95	99.95	99.95	99.95	100.00	99.95	99.99	99.95					
16	VietNam_2021(OP265008)	99.95	99.95	99.96	99.96	99.96	99.97	99.97	99.97	99.97	99.97	99.97	99.97	99.97	99.97	99.97				
17	Taiwan_2022(OQ161620)	99.96	99.95	99.96	99.97	99.97	99.99	99.95	99.95	99.95	99.95	99.99	99.95	99.98	99.95	99.99	99.97			
18	China_2023(OR532764)	99.97	99.96	99.97	99.97	99.97	100.00	99.95	99.95	99.95	99.95	100.00	99.95	99.99	99.95	100.00	99.97	99.99		
19	China_2023(PQ241041)	99.95	99.95	99.96	99.96	99.96	99.94	99.98	99.98	99.98	99.98	99.95	99.98	99.94	99.99	99.95	99.96	99.94	99.95	

3.3 Genomic Characterization and Phylogenetic Analysis

Based on NSP-VP1 gene sequence analysis, SANA isolates, and the previously reported Avian parvoviruses were placed into the phylogenetic tree (Figure 3). Also, the isolates from Iran are included within A2 and show the highest similarity to strains from Turkey, the UK, and Russia. Furthermore, they form a distinct clade separate from OsPV strains from China and GPV strains from China and Thailand.

It shared the most similarity of 99.99%, 99.98%, and 99.99%, with the previous Avian parvovirus Turkey_2021 (MW386077), Russia_2022 (OP590148), UK_2021 (MW588037), respectively (Table 2). However, based on partial genome sequencing, Avian parvovirus isolates were classified in a phylogenetic tree with a high identity of 99.95% to 99.99%.

4 Discussion

As mentioned earlier, since 2016, there have been several outbreaks of a disease characterized by paralysis as the main symptom in ostrich farms in Iran. In the current study, we investigated the probable pathogen that caused this disease in farmed ostriches and detected ostrich parvovirus (OsPV) in collected samples of the affected ostriches in different ostrich farms that indicates this virus is widely distributed in ostrich flocks of Iran. Although in the absence of experimental animal infection models, whether ostrich parvovirus is the responsible pathogen of ostrich paralysis remains questionable, the fairly positive prevention and treatment methods against ostrich parvovirus using inactivated GPV vaccines in other studies and similarity of clinical manifestation of the disease, it can be hypothesized that OsPV is the pathogen causing paralysis and death in ostriches (22). Although Newcastle disease and avian influenza have been reported with the clinical symptoms of anorexia, lethargy, weakness, diarrhea, and terminal recumbency in ostriches (32, 33), molecular and serological tests for these viral diseases were negative in the current study. Post-mortem findings were not the same as observed in ND or AI infection, which agrees with the results of others (21, 22). A Borna-like disease has been reported in ostriches, with limb paresis and gastroenteritis as the main clinical signs (34). Although the clinical symptoms of the affected ostriches in the current study were close to the clinical manifestations of the Borna-like virus infection, this virus was not isolated from evaluated samples (34). The morbidity and mortality rates in the current study were significantly higher compared to the reported Borna-like virus infection (34). Also, it has been reported that in Borna-like disease in ostriches, several cases died without showing paretic condition. Still, in the current study, all the affected birds showed paralysis in their hind limbs (34). Ataxia and recumbency have been reported as terminal clinical signs in eastern equine encephalomyelitis virus infection among emu flocks in the United States. However, the current study did not detect this virus. Also, filariid nematode *chandlerella quiscali* has been reported to cause torticollis, ataxia, and abnormal gate, followed by recumbency and death in emus.

Still, in the current study, this parasite was not observed in pathologic evaluations of the cerebral and spinal cord tissues (35). Laboratory investigations for the other viral, bacterial, and fungal diseases in the present study of collected samples were negative. All these findings were consistent with the results of others (21, 22). Although Yuan *et al.* reported the prevalence of the OsPV in ostrich chicks up to four months old and the affected ostriches in another study were aged between 1-3 months old (21, 22), in our study, the symptoms observed in ostriches aged from 15 days to 9 months old that could be the result of different maternal antibody levels of ostrich chicks. Also, Zhao *et al.* reported that parvovirus can be detected in ostrich eggs, indicating that the virus has the characteristics of vertical transmission (22). Still, in the current study, ostrich parvovirus was not detected in fertile eggs of affected farms, with positive results in ostrich chicks. It has been reported that ostrich parvovirus, despite other waterfowl parvoviruses that mainly infect the spleen and intestines after infection, has a wide range of tissue tropism and can be detected in different organs that are in agreement with the results of the present study (22).

In contrast to our histopathological findings, Zhao *et al.* reported that there were hemorrhages in the muscle of the leg as well as the thymus and bursa during their investigation. Necrosis and degeneration were described in renal tubules associated with interstitial nephritis and intensive hepatitis. Moreover, gross examination revealed that stifle joints were effusive and swollen. However, exceptionally to the common point of renal tissue in both studies, a large segregation of differences was observed without rational explanation. One of the probabilities might be the discrepancy of infected strains (22).

The isolation of ***Escherichia coli*** in the present study might be accidental. Based on the extent of the clinical signs

in different affected ostriches of various farms, it might not be the main pathogen. It has been reported that clostridial infection can lead to sternal recumbency and paralysis in the ostriches. In the current study, ***Clostridium perfringens*** was isolated from one case without recumbency, which can also be accidental (36, 37).

Molecular detection indicated that the isolated virus had extensive tissue tropism and could be detected in various tissues, including the liver, heart, kidney, intestine, thymus, spleen, and bursa. According to the data obtained from comparing the two conventional PCR and qPCR methods by serial dilutions of intestine samples, the qPCR method has higher sensitivity. It can identify low infection levels that conventional PCR can not detect.

Phylogenetic analysis has shown that Iranian isolates have the highest similarity to isolates from Türkiye, the United Kingdom, and Russia, with 99.99% and 99.98%, respectively.

In the present study, in some cases, antibacterial and antiviral drugs have a mild supportive effect on treating the disease. However, based on the observed consequences, no effective solution has been reported, and these results agree with the others (21, 22). Although it has been reported that the inactivating GPV vaccine had a fairly positive effect in preventing and treating OsPV, we did not examine it (22). In the current study, in two affected farms, the fertile ostrich eggs were transferred to another farm after disinfection and incubation. The hatched chicks were raised, and the results of parvovirus evaluation on two-month-old chicks showed no parvovirus involvement. This experiment needs more investigations to be trusted, but due to extensive commercial losses of the disease in ostrich farms, it might be a temporary solution to control the disease.

5 Conclusion

In summary, our study highlights the significant challenges the ostrich parvovirus poses in Iranian ostrich farms, suggesting it may be a key pathogen associated with paralysis and mortality. While we have not definitively established OsPV as the sole cause, the evidence supports its prominent role in affecting ostrich health. The study underscores the necessity for ongoing research to clarify the dynamics of this disease, including the exploration of effective treatment options and preventive measures. The findings also call for increased awareness among ostrich farmers about the potential implications of OsPV and the importance of implementing biosecurity measures to control its spread. By fostering collaboration between veterinarians, researchers, and the farming community, we can work towards understanding and ultimately managing this disease to protect the health of ostrich populations and the livelihoods of those who depend on them.

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Conflict of Interest

The authors declared no conflicts of interest.

Author Contributions

SM and **MA**: They collaborated closely to develop the final draft of the manuscript, ensuring that the ideas flowed smoothly and that the text accurately reflected the collective input of all authors.

MHNSh: Conducted the real-time PCR experiments with great attention to detail. His expertise was crucial in generating reliable data that bolstered our findings, further enriching the study's contributions.

SAP: Played a pivotal role by performing conventional PCR assays. His meticulous work provided valuable complementary data, reinforcing the overall results of our research.

SCh and **MD:** Together, they spearheaded the clinical evaluation and sample collection. Their dedication to interacting with affected ostriches and carefully gathering samples was key to the integrity of our study.

SB: Authored the initial draft of our manuscript. His hard work in outlining the study’s aims and methodologies laid a strong foundation for the ensuing discussions and revisions.

OD: Brought his pathology expertise to the team, examining tissue samples and ensuring accurate histological interpretations that were essential for our findings.

JR: Guided the project design with a clear vision and coordinated different study components seamlessly. He played a critical role in finalizing the manuscript, making sure it met the journal's standards for publication.

Data Availability Statement

Data are available from the first author upon reasonable request.

Ethical Considerations

In compliance with ethical standards for research involving human participants, this study will seek ethical approval from research ethics committee under the grant number EC-104-1403.

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References

1. Ritchie BW, Harrison GJ, Harrison LR. Avian medicine: principles and application1994.
2. Ballard B, Cheek R. Exotic animal medicine for the veterinary technician: John Wiley & Sons; 2016.
3. Araghi M, Ghaniei A, Heidari T. Aspergillosis outbreaks in ostrich flocks of eastern Iran during 2010-2012. 2014.
4. Cooper RG, Horbanczuk JO, Fujihara N. Viral diseases of the ostrich (Struthio camelus var. domesticus). Animal Science Journal. 2004;75(2):89-95. [DOI]
5. Verwoerd D. Ostrich diseases. Revue scientifique et technique-Office international des épizooties. 2000;19(2):638-52. [PMID: 10935285] [DOI]
6. Cotmore SF, Agbandje-McKenna M, Canuti M, Chiorini JA, Eis-Hubinger A-M, Hughes J, et al. ICTV virus taxonomy profile: Parvoviridae. Journal of General Virology. 2019;100(3):367-8. [PMID: 30672729] [PMCID: PMC6537627]
7. Pénzes JJ, Söderlund-Venermo M, Canuti M, Eis-Hübinger AM, Hughes J, Cotmore SF, et al. Reorganizing the family Parvoviridae: a revised taxonomy independent of the canonical approach based on host association. Archives of Virology. 2020;165:2133-46. [PMID: 32533329] [DOI]
8. Kapgate SS, Kumanan K, Vijayarani K, Barbuddhe SB. Avian parvovirus: classification, phylogeny, pathogenesis and diagnosis. Avian pathology. 2018;47(6):536-45. [PMID: 30246559] [DOI]
9. Tarasiuk K, Woźniakowski G, Samorek-Salamonowicz E. Occurrence of chicken parvovirus infection in Poland. The open virology journal. 2012;6:7. [PMID: 22393336] [PMCID: PMC3282887] [DOI]
10. Niu Y, Zhao L, Liu B, Liu J, Yang F, Yin H, et al. Comparative genetic analysis and pathological characteristics of goose parvovirus isolated in Heilongjiang, China. Virology journal. 2018;15:1-8. [PMID: 29391035] [PMCID: PMC5795831] [DOI]
11. Glávits R, Zolnai A, Szabó É, Ivanics É, Zarka P, Mató T, et al. Comparative pathological studies on domestic geese (Anser anser domestica) and Muscovy ducks (Cairina moschata) experimentally infected with parvovirus strains of goose and Muscovy duck origin. Acta Veterinaria Hungarica. 2005;53(1):73-89. [PMID: 15782661] [DOI]
12. Liu W-j, Yang Y-t, Zou H-y, Chen S-j, Yang C, Tian Y-b, et al. Identification of recombination in novel goose parvovirus isolated from domesticated Jing-Xi partridge ducks in South China. Virus genes. 2020;56:600-9. [PMID: 32676956] [DOI]
13. Wang J, Ling J, Wang Z, Huang Y, Zhu J, Zhu G. Molecular characterization of a novel Muscovy duck parvovirus isolate: evidence of recombination between classical MDPV and goose parvovirus strains. BMC veterinary research. 2017;13:1-10. [PMID: 29121936] [PMCID: PMC5680767] [DOI]
14. Derzsy D. A viral disease of goslings. 1967.
15. Ting C-H, Lin C-Y, Huang Y-C, Liu S-S, Peng S-Y, Wang C-W, et al. Correlation between goose circovirus and goose parvovirus with gosling feather loss disease and goose broke feather disease in southern Taiwan. Journal of Veterinary Science. 2021;22(1). [PMID: 33522153] [PMCID: PMC7850790] [DOI]
16. Zhang Y, Feng B, Xie Z, Deng X, Zhang M, Xie Z, et al. Epidemiological surveillance of parvoviruses in commercial chicken and Turkey farms in Guangxi, Southern China, during 2014–2019. Frontiers in Veterinary Science. 2020;7:561371. [PMID: 33134348] [PMCID: PMC7579407] [DOI]
17. Day JM, Zsak L. Determination and analysis of the full-length chicken parvovirus genome. Virology. 2010;399(1):59-64. [PMID: 20097398] [PMCID: PMC7173076]
18. Zsak L, Cha RM, Day JM. Chicken parvovirus–induced runting-stunting syndrome in young broilers. Avian diseases. 2013;57(1):123-7. [PMID: 23678740] [DOI]
19. Ning K, Liang T, Wang M, Dong Y, Qu S, Zhang D. Pathogenicity of a variant goose parvovirus, from short beak and dwarfism syndrome of Pekin ducks, in goose embryos and goslings. Avian Pathology. 2018;47(4):391-9. [PMID: 29630396] [DOI]
20. Fan W, Sun Z, Shen T, Xu D, Huang K, Zhou J, et al. Analysis of evolutionary processes of species jump in waterfowl parvovirus. Frontiers in microbiology. 2017;8:421. [PMID: 28352261] [PMCID: PMC5349109] [DOI]
21. Yuan K, Wang D, Luan Q, Sun J, Gao Q, Jiang Z, et al. Whole genome characterization and genetic evolution analysis of a new ostrich parvovirus. Viruses. 2020;12(3):334. [PMID: 32204363] [PMCID: PMC7150892] [DOI]
22. Zhao K, Hao X, Lei B, Dong S, Wang J, Zhang W, et al. Emergence and genomic analysis of a novel ostrich-origin GPV-related parvovirus in China. Poultry Science. 2022;101(7):101929. [PMID: 35691050] [PMCID: PMC9194870] [DOI]
23. Kumar S, Stecher G, Tamura K. MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. Molecular biology and evolution. 2016;33(7):1870-4. [PMID: 27004904] [PMCID: PMC8210823] [DOI]
24. Kimura M. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. Journal of molecular evolution. 1980;16:111-20. [PMID: 7463489] [DOI]
25. Zsak L, Strother KO, Day JM. Development of a polymerase chain reaction procedure for detection of chicken and turkey parvoviruses. Avian diseases. 2009;53(1):83-8. [PMID: 19432008] [DOI]
26. Kant A, Koch G, Van Roozelaar D, Balk F, Huurne AT. Differentiation of virulent and non-virulent strains of Newcastle disease virus within 24 hours by polymerase chain reaction. Avian pathology. 1997;26(4):837-49. [PMID: 18483949] [DOI]
27. Farzin H, Ajam G, Asgharzadeh M, Motlagh ZZ, Ghasemitabas S, Tabatabaeizadeh S, et al. The role of the major viral pathogens in a respiratory disease outbreak of broiler flocks in Eastern Iran. Iranian Journal of Veterinary Research. 2023;24(3):205.
28. Zhang F, Luo Y, Wei Q, Xiong L, Xie Q, Tan J, et al. Research Note: Pathogenetic characteristics of avian encephalomyelitis virus in Guangdong and Jiangxi Provinces, China. Poultry Science. 2024;103(2):103264. [PMID: 38035474] [PMCID: PMC10697995] [DOI]
29. Kort YH, Bourogâa H, Gribaa L, Hassen J, Ghram A. Genotyping and classification of Tunisian strains of avian reovirus

using RT-PCR and RFLP analysis. Avian diseases. 2015;59(1):14-9. [PMID: 26292528] [DOI]
30. Cao W, Mays J, Dunn J, Fulton R, Silva R, Fadly A. Use of polymerase chain reaction in detection of Marek's disease and reticuloendotheliosis viruses in formalin-fixed, paraffin-embedded tumorous tissues. Avian Diseases. 2013;57(4):785-9. [PMID: 24597122] [DOI]
31. Weissenböck H, Bakonyi T, Sekulin K, Ehrensperger F, Doneley RJ, Dürrwald R, et al. Avian bornaviruses in psittacine birds from Europe and Australia with proventricular dilatation disease. Emerging infectious diseases. 2009;15(9):1453. [PMID: 19788814] [PMCID: PMC2819881] [DOI]
32. Ghiamirad M, Pourbakhsh A, Keyvanfar H, Momayaz R, Charkhkar S, Ashtari A. Isolation and characterization of Newcastle disease virus from ostriches in Iran. African journal of microbiology research. 2010;4(23):2492-7.
33. Huchzermeyer FW. Diseases of farmed crocodiles and ostriches. Revue Scientifique et Technique-Office International Des Épizooties. 2002;21(1):265-76. [PMID: 11974614] [DOI]
34. Malkinson M, Weisman Y, Perl S, Ashash E. A Borna-like disease of ostriches in Israel. Borna disease: Springer; 1995. p. 31-8.[PMID: 7789149] [DOI]
35. Suedmeyer WK, Bermudez A, Kazacos KR. Cerebellar nematodiasis in an emu (Dromaius novaehollandiae). Journal of Zoo and Wildlife Medicine. 1996:544-9.
36. Lublin A, Mechani S, Horowitz H, Weisman Y. A paralytic-like disease of the ostrich (Struthio camelus masaicus) associated with Clostridium chauvoei infection. The Veterinary Record. 1993;132(11):273-5. [PMID: 8465505] [DOI]
37. Allwright D, Wilson M, vanRensburg W. Botulism in ostriches (Struthio camelus). Avian Pathology. 1994;23(1):183-6. [PMID: 18671084] [DOI]