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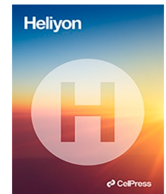

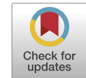

Molecular analysis Polish isolates of goose hemorrhagic polyomavirus from geese and free-living birds

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ABSTRACT

Goose haemorrhagic polyomavirus (GHPV) is the viral agent of hemorrhagic nephritis and enteritis of geese (HNEG), a lethal disease of goose. The study describes the results of a molecular analysis Polish isolates of GHPV from geese and free-living birds based on complete VP1 gene and VP2 gene sequences. The sequences were analyzed and aligned with different GHPV isolates sequences accessible in the GenBank database. This study indicates affiliation GHPV isolates from fee-living birds and GHPV isolates circulating in Polish goose flocks and around the world to the same genetic groups, which proves their evolutionary relationship and indicates the potential role of free-living birds as a source of infections for poultry.

1. Introduction

Hemorrhagic nephritis and enteritis of geese (HNEG) is a contagious and acute disease of domestic geese (*Anser*), affecting birds aged 4–12 weeks, with high morbidity and mortality.

HNEG was first described in 1969 in Hungary [1]. The disease was subsequently found in Germany [12], France [15,16] and Poland [6]. The last outbreak was also described on a Belgian goose farm, which confirms the presence of GHPV also in countries with less extensive waterfowl production [5]. Recently, the genomes of GHPV isolates detected in Hungarian duck and goose farms in 2005–2019 were submitted to phylogenetic and evolutionary analysis [10].

The first clinical case of HNEG outside Europe has been recorded in Taiwan [20]. The genomic DNA of GHPV has been detected in domestic waterfowl in China, but no clinical cases of HNEG have been reported in this country [21].

The viral agent of HNEG is goose haemorrhagic polyomavirus (GHPV), recently named *Anser anser* polyomavirus 1, small dsDNA virus belonging to the genus *Gammapolyomavirus* of the *Polyomaviridae* family.

The genome of GHPV is 5252–5256 bp and contains the genes of the VP1-VP3 structure proteins, the large and small tumour antigens (LTA and STA), and the predicted ORF-X with unknown function [4,7].

GHPV induces functional and mechanical damage in the vascular endothelial cells, resulting in the formation of subcutaneous tissues, gelatinous ascites, inflammation of the kidneys and often hemorrhagic enteritis [3]. Additionally, GHPV induces lymphoid depletion and contributes to immunodepression in birds [11].

GHPV infection has also been described in Muscovy ducks and mule ducks with no clinical symptoms and pathogenic lesions suggesting that ducks may be a potential reservoir of GHPV [2].

Recent reports have provided knowledge about new bird species susceptible to GHPV infection. The presence of GHPV genetic

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material has been confirmed in the tissues of various species of free-living birds [18]. Contact between free-living birds and domestic geese could be a possible means of transmission of the virus. GHPV infections in free-living birds are an important problem in the epidemiology of infections with these pathogens, hence the advisability of the undertaken research was justified.

The purpose of this study was the molecular analysis Polish GHPV isolates from geese and free-living birds and compared these isolates with GHPV isolates circulating in geese and ducks flocks around the world.

2. Material and methods

2.1. Standard and field isolates

Thirty five field isolates of GHPV were collected during the years 2014–2017 from internal organs (heart, lungs, liver, spleen, intestines, kidneys) from 19 dead white storks (C*iconia*), 6 western jackdaws (*Corvus monedula),* 3 common buzzards (*Buteo),* 3 European herring gulls (*Larus argentatus*), 2 rock dove (*Columba livia*), 1 feral pigeon (*Columba livia domestica*), 1 grey heron (*Ardea cinerea*). The dead free-living birds were obtained for diagnostic tests as part of the cooperation conducted by Department Poultry of Diseases, National Veterinary Research Institute (NVRI) with the Wild Birds Rehabilitation Center in Bukwałd (Albatross Foundation) and the Bird Migration Research Station of the University of Gdańsk. Additionally, six GHPV isolates from the intestines of naturally infected geese were included in the study. The birds were submitted to laboratory between 2013 and 2017 for detection of possible GHPV infection. GHPV strain 50/08 was used as a positive control. The strain was primarily isolated in goose embryo kidneys cells (GEKs) and originated from 9 weeks old geese showing anatomopathological changes characteristic of HNEG.

3. DNA extraction

About 20 mg of section of internal organs from the examined birds were suspended in Eagle's (MEM, Sigma Aldrich, USA) enriched with a 1% mixture of antibiotics (Antibiotic-Antimycotic, Gibco, U.K.) in a ratio to obtain a 20% (w/v) suspension and then centrifuged at 3000 g for 15 min. Total DNA was extracted from the virus stocks and homogenates of internal organs collected from geese or free-living birds using QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) following the manufacturer's instructions. The extracted DNA templates were stored below −20 °C for PCR analysis.

3.1. Molecular analysis and phylogeny

Each DNA was checked for the presence of GHPV with two sets of primer pairs designed by Ref. [2]. Using PCR method. In order to obtain more precise information for the phylogenetic analysis, primers comprising the full-length VP1 and VP2 coding regions of GHPV were chosen. The VP1F-VP1R and VP2F-VP2R primer sets were used to the amplification of PCR product of 1232 bp and 1290, respectively. The optimized PCR reactions were prepared at final reaction volume of 25 μl, which consisted 12,5 μl Taq PCR Master Mix (EURx, Poland) containing 1,25 U Taq DNA polymerase, 1*x* Reaction Buffer, 0,2 mM of each dNTP, 0,5 μl of VP1F-VP1R or VP2F-VP2R (10 mM for each), 0,5 μl $MgCl_2$, 2,5 μl 10 *x* Color Load, 6,5 μl of nuclease-free water and 2 μl of the isolated template DNA (0,25μg/25 μl). The PCR reaction conditions were 1 cycle of 95 °C for 15 min, 40 cycles of 94 °C for 15 s, 55 °C for 20 s, 72 °C for 45 s, and 1 cycle of 72 °C for 5 min for final extension. Amplification was conducted in basic gradient thermocycler (Biometra, Germany).

PCR products were separated in 2% agarose gel with addition of GelRed Nucleic Acid Gel Stain (Biotum, USA) for 50 min at 120 V. After the electrophoresis, the size of amplification products was compared with the GeneRuler DNA Ladder Mix molecular marker (Thermo-Scientific, USA). The results were visualised using transilluminator UV (Vilber-Lourmat, Germany) and photographed.

Amplicons were purified and sequenced at the commercial service (Genomed, Poland). Using Bioedit program, the forward and reverse nucleotide sequences were aligned as one consensus sequence. The sequences were analyzed with MEGA 4.0 [19] using the neighbor-joing method with the maximum-likelihood model. Bootsrap scores were generated from 1000 replicates.

3.2. Results PCR

All 35 GHPV isolates from free-living birds and 6 GHPV isolates from geese were subjected to PCR aimed at amplification of complete VP1 gene and VP2 gene of GHPV. The presence of a 1232 bp product indicating the amplification of the VP1 coding region

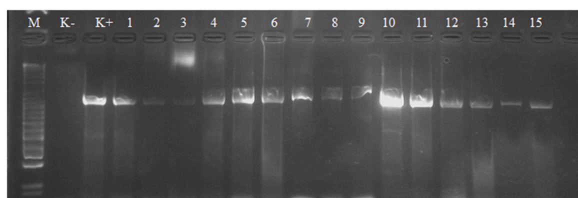

Fig. 1. Amplification reaction detecting the VP1 gene. M − DNA fragment size marker (80–10000 bp), K- negative control; K+ positive control; lanes 1–15 indicate GHPV field isolates: 1–18/13n; 2–20/13s; 3–183/17w; 4–67/14j1; 5–67/14sl3; 6–67/14p2; 7–57/15j4; 8–57/15j7; 9–57/15j9; 10–57/15j10; 11–61/15j7; 12–61/15z1; 13–147/15z7; 14–147/15w4; 15–262/17w1.

was confirmed for all GHPV isolates tested. The 1290 bp product encoding the entire VP2 gene was obtained for only 13 GHPV isolates from free-living birds and 6 GHPV isolates from geese. An example of the electrophoretic separation of PCR products obtained after testing with primers VP1 and VP2 is shown in Fig. 1 and Fig. 2.

3.3. Molecular analysis of VP1 gene and phylogeny

In order to further characterize the tested isolates, the obtained PCR products were sequenced for the VP1 gene. The primers allowed the sequencing of a 1232 bp fragment covering the entire VP1 gene and fragments of the VP2 regions and the large T antigen. Due to errors in the sequencing process, the final sequence fragments were removed. The 1062 bp long VP1 coding region was used for the final nucleotide analysis. The nucleotide position numbering was referenced to the whole genome sequence of the GHPV reference strain (GenBank Accession No: NC_004800).

From among 35 identified GHPV isolates, 18 GHPV isolates from free-living birds and 6 GHPV isolates from geese were used for the phylogenetic analysis of the VP1 gene. The choice was mainly determined by the quality of the sequences and their origin. The sequences of GHPV isolates detected in free-living birds in which they have not been identified so far and those characterized by the presence of new point mutations have been submitted to the GenBank database. The list of isolates used in the phylogenetic analysis along with the assigned GenBank accession numbers is provided in Table 1.

The sequences of Polish strains were compared with those available in the GenBank NCBI database: the sequence of the GHPV reference strain and 40 sequences of GHPV strains from France, Hungary, Germany, China and Taiwan.

Point mutations (transitions, transversions) were observed within the nucleotide sequences obtained for the VP1 gene. These mutations were not present in the GHPV strains sequences deposited in GenBank. They concerned 1 GHPV isolate 39/14j from goose and 8 GHPV isolates from free-living birds.

The analyzed strains formed 5 genetic groups on the phylogenetic tree (Fig. 3). The most numerous of them, i.e. the first group, was formed by two subgroups (1.1 and 1.2). The first one was divided into subgroups 1.1.1 and 1.1.2. Subgroup 1.1.1 was composed mainly of strains originating from Hungary, China, Taiwan, one French strain and 4 Polish isolates (3 isolates from geese and one from European herring gulls). Subgroup 1.1.2 included 13 Polish isolates from free-living birds, 2 goose isolates and two French strains identified in ducks and geese in 2008. The least numerous in the first group, i.e. subgroup 1.2, was formed by the Polish strain from white stork and the French strain of the ducklings.

Group 2 was composed of 2 Hungarian strains and a Polish isolate identified in flock of geese in 2017. The next 3 groups were formed by single GHPV isolates identified in 2015 in a pigeon and 2 white storks.

The nucleotide similarity of 97.9–100% was demonstrated between Polish GHPV isolates. The nucleotide homology of the VP1 capsid protein gene of all Polish strains to the reference strain (GenBank Accession No: NC_004800) was 98.4–100%.

The next step was to analyze the amino acid sequence. The nucleotide chain of 1062 bp in length corresponded to the amino acid sequence of the VP1 protein of 353 amino acids. The numbering of the amino acid positions in the protein chain is referenced to the VP1 sequence of the GHPV reference strain (GenBank Accession No: NP_849,169). Mutations characteristic for the tested strains are presented in Table 2. The changes detected in the nucleotide sequences in most of the analyzed GHPV isolates translated into a change in amino acids in the VP1 protein. The exceptions were the strains 61/15s1, 57/14j4, 147/15w5, in which the identified single nucleotide mutations were synonymous. The strain with the highest number of changes in the amino acid sequence of the VP1 protein (8 substitutions) was the 147/15w4 strain identified in the white stork (Table 2).

The amino acid similarity between the Polish GHPV isolates was 96.6–100%, while the homology to the reference strain (NP_849,169) was 97.7–100%.

3.4. Molecular analysis of VP2 gene and phylogeny

In order to obtain additional information about the possible variability of GHPV isolates in a different fragment of the genome, the analysis of the nucleotide and amino acid sequences obtained for the VP2 gene was performed. Five geese isolates and nine isolates identified in free-living birds were used for the analysis. The primers used allowed for the sequencing of a 1290 bp fragment covering the entire VP2 gene. Due to errors during the sequencing of long fragments, the final sections of the sequence were removed. The 981 bp long VP2 coding region was used for the final nucleotide analysis. The list of Polish isolates used in the phylogenetic analysis together with the assigned GenBank accession numbers is presented in Table 3. The sequences of the Polish strains were compared with

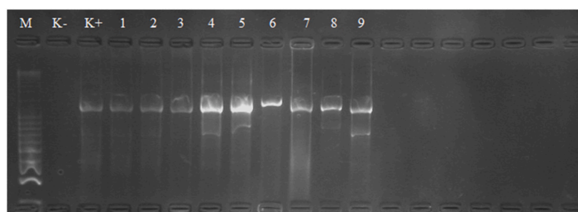

Fig. 2. Amplification reaction detecting the VP2 gene. M – DNA fragment size marker (80–10000 bp), K- negative control; K+ positive control; lanes 1–9 indicate GHPV field isolates: 1–39/14; 2–50/15; 3–262/17; 4–67/14j1; 5–67/14p2; 6–67/14p4; 7–57/15j5; 8–57/15j9; 9–57/15p3.

Table 1
List of Polish GHPV field isolates included in the sequence analysis of the VP1 gene.

Lp.	Isolate designation Test number/year of isolation, internal organ, sample number	Bird species	GenBank accession number
1.	18/13n	Goose (*Anser*)	MG844143
2.	20/13s	Goose (*Anser*)	MG655226
3.	39/14j	Goose (*Anser*)	MG605670
4.	50/15s	Goose (*Anser*)	MG655228
5.	183/17w	Goose (*Anser*)	MG655227
6.	262/17w1	Goose (*Anser*)	MG655224
7.	67/14j1	White stork (*Ciconia*)	MG673522
8.	67/14j2	White stork (*Ciconia*)	MG673523
9.	67/14sl3	White stork (*Ciconia*)	MG844139
10.	67/14p2	White stork (*Ciconia*)	MG673524
11.	57/15j1	White stork (*Ciconia*)	MG655219
12.	57/15j3	White stork (*Ciconia*)	MG655220
13.	57/15j4	European herring gulls (*Larus argentatus*)	MG655220
14.	57/15j5	European herring gulls (*Larus argentatus*)	MG649074
15.	57/15j7	Rock dove (*Columba livia*)	MG655221
16.	57/15j9	Grey heron (*Ardea cinerea*)	MG655222
17.	57/15j10	Common buzzards (*Buteo*)	MG655223
18.	61/15j7	Feral pigeon (*Columba livia domestica*)	MG649073
19.	61/15w7	Kawka (*Corvus monedula*)	MG655225
20.	61/15s1	Rock dove (*Columba livia*)	MG844137
21.	61/15z1	Western jackdaws (*Corvus monedula*)	MG844138
22.	147/15z1	White stork (*Ciconia*)	MG844140
23.	147/15z7	White stork (*Ciconia*)	MG844141
24.	147/15w4	White stork (*Ciconia*)	MG844142

the reference strain (GenBank Accession No: NC_004800) and the 35 GHPV strains for which the VP2 gene sequence was available in the GenBank database.

Analysis of the nucleotide sequence of Polish GHPV isolates in relation to the reference strain revealed the presence of three point mutations. Two of them were characteristic only for Polish isolates. Transition of guanine into thymine at position 1205 was observed in the case of 39/14j goose isolate. However, isolates identified in free-living birds, except 57/15j_1 isolate, were characterized by the transition of thymine into adenine at position 1609. The third of the detected mutations, i.e. position 1590 characterizing 39/14j_1 isolate and 8 isolates from free-living birds was previously recorded in 2 French strains: Toulouse 2008, Toulouse Muscovy Duck 2008 and 3 Hungarian strains.

Based on the nucleotide sequence of the VP2 gene, a phylogenetic tree was drawn (Fig. 4) showing the relationship of the analyzed GHPV isolates. Based on the observed topology, Polish isolates identified in free-living birds and geese were classified into 2 genetic groups. Group 1 included all Polish GHPV isolates from free-living birds and one goose strain (subgroup 1.1) showing close relationship to two French strains (subgroup 1.2). The group 1 also included Hungarian strains, two Chinese strains and two German strains. The group 2 consisted of three Polish goose strains and isolates from Taiwan, Hungary, one strain from France and one strain from China. The nucleotide similarity at the level of 99.8–100% was demonstrated between the Polish GHPV isolates. The nucleotide homology of the VP2 capsid protein gene of all Polish isolates to the reference strain (GenBank Accession No: NC_004800) was 99.7–99.9%.

The next step was to analyze the amino acid sequence Polish GHPV isolates. The nucleotide chain of 981 bp in length corresponded to the amino acid sequence of the VP2 protein consisting of 326 aa. The numbering of the amino acid positions in the protein chain is referenced to the VP2 sequence of the GHPV reference strain (GenBank Accession No: NP_849,167).

Two of the detected mutations in the nucleotide sequence caused an amino acid change in the VP2 protein. In the case of the 39/14j goose strain, a mutation in the nucleotide sequence translated into a mutation in the amino acid sequence (substitution of valine into isoline at position 41). The nucleotide mutation at position 1069, which characterized the isolates identified in free-living birds, except for isolate 57/15j1, was a synonymous mutation. Isolate 39/14j and 8 isolates from free-living birds were characterized by the substitution of threonine into serine at position 169. This mutation distinguished the French Toulouse 2008 strain previously included in the GenBank database and three Hungarian strains.

The amino acid homology of Polish GHPV isolates to each other and the reference strain (NP_849,167) was 99.4–100%. The lowest homology in relation to the reference strain and Polish GHPV isolates (99.4%) was shown by goose isolate 39/14j.

4. Discussion

The pathogenicity of GHPV has been confirmed in one susceptible species, domestic geese, and the pathogen is thought to have a limited host range similar of mammalian polyomaviruses [14]. Later studies confirmed that GHPV can effectively propagate in the cells of other species of waterfowl, including Muscovy ducks and mule ducks, which are considered an epidemiological virus reservoir [2].

Fig. 3. Phylogenetic tree based on the nucleotide sequence of the VP1 capsid protein gene of Polish and foreign GHPV isolates and a reference strain. Polish GHPV isolates identified in free-living birds are marked with green triangles, while those identified in goose herds with red dots.

The necessity to investigate the presence of GHPV infections in other bird species, especially in water birds, has previously been suggested in the scientific literature. It was emphasized that polyomaviruses are a group of poorly understood pathogens that may be of great importance in poultry and other bird species [11].

Little is known about the risk that migratory birds can pose in the spread of GHPV. Therefore, the research on the molecular characteristics of GHPV in free-living birds and geese undertaken in this paper has a clear scientific justification. The obtained results provide new knowledge in the field of GHPV pathogenesis, because molecular methods confirm the possibility of infections not only in geese and ducks, but also in other species of free-living birds.

The analysis of the nucleotide and amino acid sequence of the VP1 gene and the VP2 gene of GHPV isolates from free-living birds

Table 2
Amino acid changes within the VP1 protein of Polish GHPV isolates in relation to the reference strain.

Position aa	Amino acid		Strains with amino acid substitution
	Reference strain	Polish GHPV strains	
2	A	L	147/15w4
4	K	R	147/15z7
7	R	T	147/15w4
8	P	Q	67/14j2
8	P	A	61/15s1
9	R	K	57/15j10, 147/15z7
10	N	I	61/15s1
10	N	T	147/15z7, 147/15w4
12	P	S	147/15w4
14	P	Q	147/15w4
19	L	R	147/15z7, 147/15w4
21	K	I	61/15s1
28	G	R	57/15j3
29	V	I	57/15j1
33	P	R	147/15w4
44	N	S	39/14j
67	V	I	20/13s, 39/14j, 67/14j1, 67/14j2, 67/14sl3, 67/14p2, 57/15j1, 57/15j4, 57/15j9, 57/15j10,61/15w7, 61/15z1, 57/15j7, 147/15z1
282	K	I	57/15j4
331	P	L	61/15j7,147/15w4
335	D	G	61/15s1
339	F	L	61/15s1,147/15z7
353	N	T	147/15z7

Table 3
List of Polish GHPV field isolates included in the sequence analysis of the VP2 gene.

Lp.	Isolate designation Test number/year of isolation, internal organ, sample number	Bird species	GenBank accession number
1.	39/14j	Goose (*Anser*)	MG869736
2.	50/15s	Goose (*Anser*)	MG869737
3.	183/17w	Goose (*Anser*)	MG869738
4.	262/17w1	Goose (*Anser*)	MG869739
5.	67/14j1	White strok (*Ciconia*)	MG869742
6.	67/14sl3	White strok (*Ciconia*)	MG888057
7.	67/14p2	White strok (*Ciconia*)	MG888055
8.	67/14p4	White strok (*Ciconia ciconia*)	MG888056
9.	57/15j3	White strok (*Ciconia*)	MG888058
10.	57/15j5	European herring gulls (*Larus argentatus*)	MG869740
11.	57/15j9	Grey heron (*Ardea cinerea*)	MG869741
12.	57/15p3	European herring gulls (*Larus argentatus*)	MG869743

and Polish geese was to help assess free-living birds as a source of new GHPV isolates for geese flocks in Poland. Best to our knowledge, it was the first time to describe molecular characterization GHPV isolates from free living birds.

Molecular studies were also important in assessing the role of ducks as an epidemiological viral reservoir for geese. They showed that there is one common virus genotype among ducks and geese [2].

Two pairs of primers amplifying a fragment of the genome including the sequence coding the entire VP1 protein and the entire VP2 protein developed by Ref. [2] were used in order to obtain more precise data, for phylogenetic analyses. The applied primers did not allow the detection of all GHPV isolates from free-living birds, unlike the geese isolates, which may suggest their greater variability in the regions surrounding the VP1 and VP2 genes. This variability may occur in the VP3 regions and in the sequence encoding the large T antigen as the primers used in the study were complementary to these genome fragments. In confirming this thesis, sequencing of the full genome of GHPV isolates identified in free-living birds, which has already been done for geese and ducklings, may be helpful in the future [2,4,6]) and new species of avian polyomaviruses [8].

The next step in the research was the sequencing of the VP1 and VP2 genes of the obtained GHPV isolates. The analysis of the nucleotide and amino acid sequence allowed the assessment of genetic variability within the analyzed region of the genome of GHPV isolates circulating in free-living birds and geese in Poland.

The analysis of the nucleotide sequence of the VP1 gene of 18 GHPV isolates of free-living birds and 6 geese isolates showed the presence of new mutations in comparison with the reference strain [9] and duck and goose strains from the GenBank database. Polish

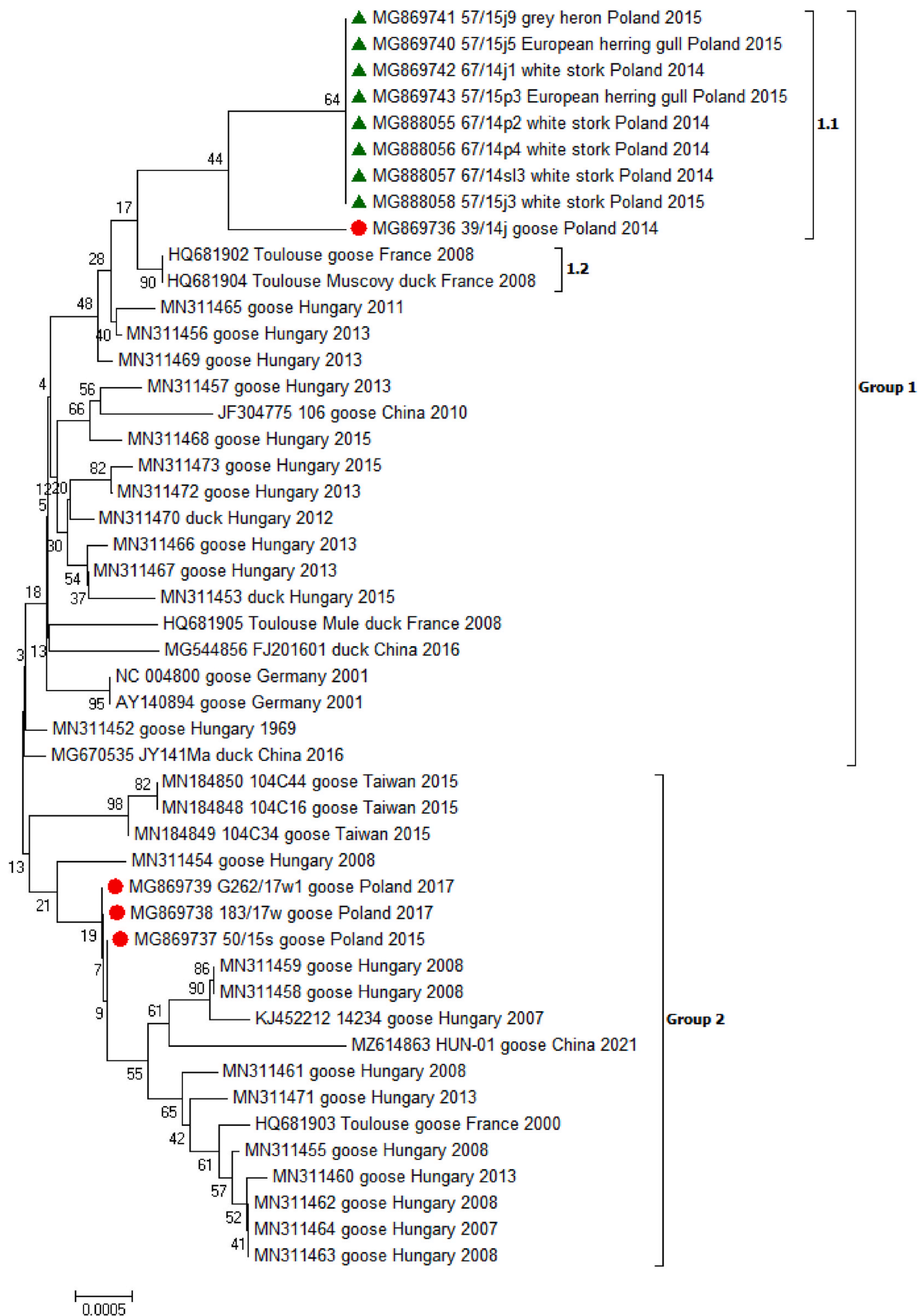

Fig. 4. Phylogenetic tree based on the nucleotide sequence of the VP2 capsid protein gene of Polish and foreign GHPV strains. Polish GHPV isolates identified in free-living birds are marked with green triangles, while those identified in goose herds with red dots.

goose isolates and GHPV isolates from free-living birds had 3 common mutations, i.e. the G→A transition at position 2048 and 2163, t G→T transition at position 2507 with previously characterized strains from GenBank database [2]. Most of the detected nucleotide mutations translated into an amino acid change in the VP1 capsid protein of the analyzed isolates. In the case of such small DNA viruses as polyomaviruses, even a single change of amino acid can drastically affect the replication and virulence of a given virus strain [17]. Studies on the effect of mutations in the GHPV genome on changing the pathogenic properties of the virus have not been studied so far, but cannot be ruled out. The analysis of the full sequence of the VP1 gene of strains isolated during the HNEG epidemic in Hungarian geese flocks in the years 2000–2002 was also conducted. The Hungarian strains were characterized by a high degree of similarity of the nucleotide and amino acid sequences to each other and to the French and German strains. A total of 9 nucleotide mutations were detected between the strains analyzed at that time, while no changes at the amino acid sequence level were detected [13]. Comparison of the VP1 nucleotide sequence between the tested isolates showed a similarity at the level of 97.9–100%. On the other hand, the similarity of the Polish strains identified in geese and free-living birds with the reference strain was 98.4–100%. Genetic analysis of the full genome sequence of the GHPV strain from the first clinical case of HNEG in Poland also showed a very high similarity to the reference strain (99.81%), while it was 100% compatible with French strains [6].

Sequence comparison at the amino acid level revealed lower homology between the tested strains with respect to the results of the analysis at the nucleotide level. Between GHPV isolates identified in geese and free-living birds in Poland, amino acid similarity was found at the level of 96.6–100%, while the homology to the reference strain was 97.7–100%.

Based on the genome sequences of GHPV isolates from France, Germany, China, Taiwan and Hungary published so far, it appears that 9 nucleotide mutations in the VP2 gene have been identified in relation to the reference strain. In order to assess the possibility of new point mutations in this fragment of the genome among Polish isolates from free-living birds and geese, the VP2 gene was sequenced.

The analysis of the nucleotide sequence of the VP2 gene showed the presence of two new mutations among Polish isolates and one mutation common to other strains. The first one distinguished the goose strain 39/14j and caused the substitution of valine for isoleucine in the VP2 protein. The second mutation was characteristic of only 8 out of 9 isolates from free-living birds, but it did not change the amino acid in the protein. The third of the detected mutations was a mutation common to the goose isolate - 39/14j, 8 isolates from free-living birds, 2 French strains, and 3 Hungarian strains and translated into an amino acid change in the protein [2]. The detection of only single mutations in the VP2 gene indicates that this gene is characterized by a high degree of conservation among GHPV isolates circulating in geese and duck flocks in different countries and in the population of free-living birds. The nucleotide homology of the tested isolates to each other and to the reference strain was 99.8–100%, while the amino acid homology was 99.4–100%.

The high similarity between GHPV isolates from free-living birds and geese, demonstrated at the molecular level, confirms the role of wild birds as a potential source of GHPV for geese flocks in Poland. Recent studies indicate that various GHPV strains circulate on Hungarian goose farms, the source of which may be permanently infected wild domesticated and migrating birds [10].

The detection of new mutations that translate into changes of amino acids in the protein, mainly among isolates from free-living birds, allows us to conclude that they may constitute a source of new isolates with altered pathogenicity. This hypothesis should be confirmed by further studies involving experimental infection of geese with isolates from free-living birds. Amino acid changes in VP1 and VP2 proteins may also result from the accumulation of mutations during the virus adaptation processes to new host species. Further studies involving whole genomic sequencing of GHPV isolates from wild birds may reveal the importance of infection of this virus in different bird species.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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