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Goose parvovirus, goose hemorrhagic polyomavirus and goose circovirus infections are prevalent in commercial geese flocks in Poland and contribute to overall health and production outcomes: a two-year observational study

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Abstract

Background The intensification of poultry production and the constantly growing number of geese flocks in Poland increases the risk of infectious diseases. The study aimed to determine the health status of commercial geese, with particular emphasis on infections with goose parvovirus (GPV), goose circovirus and goose hemorrhagic polyomavirus. The study monitored 27 geese flocks, ranging in size from 3,000 to 13,000 birds, over a two-year period.

Results The results showed the presence of genetic material GPV in all flocks tested, whereas GoCV and GHPV were detected in some flocks, 44.4% and 59.3% respectively. A significant number of flocks were found to be co-infected with two (74.1%) and three viruses (22.2%). Additionally, a phylogenetic analysis of GPV and GHPV was conducted based on a fragment of the virus genome, while for GoCV the phylogenetic analysis was performed on whole genome. Analysis of the *vp1* gene revealed that 30.8% of the sequences obtained belonged to a variant of the virus known as novel GPV, so far attributed to short beak and dwarfism syndrome in Pekin ducks. The majority of the GoCV genomic sequences exhibited high homology to the Polish sequence, which was previously isolated from domestic geese. Only one sequence was found to be closely related to sequences from wild birds.

Conclusions Our research indicates that viral and bacterial co-infections are a significant problem in flocks of geese. Rarely did a single factor have a clear impact on the health status of the flock. Typically, mixed viral infections, as well as bacterial complications (mainly *Escherichia coli*, less frequently *Ehrysipelotrix rhusiopathiae*, *Gallibacterium anatis*, and *Salmonella* Typhimurium), or fungal complications lead to an increase in mortality in the flock, growth diversification of birds, and thus a reduction in production rates.

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Introduction

Poland, together with Hungary, is one of the largest producers of geese in the European Union and the third largest in the world, just behind China and Egypt (Food and Agriculture Organization of the United Nations, FAO, 2021). Since 2018, domestic production has been relatively stable and amounts to about 1 million geese, which yields approximately 31,000 tons of meat per year; which is mainly exported to European Union countries. In Poland, the dominant goose breed is the White Kołudzka Goose, which accounts for more than 95% of the national goose population and is the result of the work of the Experimental Centre of the Research Institute of Animal Production in Kołuda Wielka. This breed is characterised by very good weight gain, high disease resistance, low fat content and excellent taste [1]. Geese produced in Poland are mainly intended for consumption purposes. Goose downs and feathers, which are used as filling materials with excellent thermal properties, are also of great economic importance. The intensification of goose production and the worldwide trade in breeding material constantly have led to an increase in the incidence of this epidemiological threat and, as a consequence, the potential for outbreaks of infectious diseases has increased.

The most frequently reported viral disease in fattening geese in Poland is Derzsy's disease caused by goose parvovirus (GPV). Infections with goose hemorhagic polyomavirus (GHPV) and goose circovirus (GoCV) in geese have also been reported [2, 3].

Since it was first described in the early 1960s, Derzsy's disease has caused severe losses in the poultry industry worldwide [4]. Recent outbreaks of parvovirus in goose flocks have been reported in Turkey and China [5, 6].

Waterfowl parvoviruses constitute a serious epizootic and economic problem in large-scale poultry production. There are three variants of GPV: classic GPV (CGPV), which causes Derzsy's disease in geese; novel GPV (NGPV), which is specific for Pekin ducks and mule ducks and is the etiological agent of short beak and dwarfism syndrome (SBDS); and Muscovy duck parvovirus (MDPV), which infects Muscovy and mule ducks. CGPV causes infection not only in geese, but also in Muscovy ducks, swans, and swan geese (*Anser cygnoies*), whereas MDPV causes disease only in Muscovy and mule ducks [7, 8]. Despite having similar pathologies and host ranges, all of the abovementioned parvoviruses belong to the same species, *Anseriform dependoparvovirus 1*, from the genus *Dependoparovirus*, the subfamily *Parvovirinae*, and the family *Parvoviridae* [9]. The viruses of this species have a linear, single-stranded DNA genome of approximately 5 kb in length, the genome encodes capsid proteins (VP1, VP2 and VP3), which play an important role in virus tropism and pathogenicity [10].

The CGPV spreads both vertically through infected eggs and horizontally: directly through infected feces, and indirectly through contaminated equipment, litter, and even through people moving between farms [10, 11]. The disease is characterized by high mortality, apathy, watery diarrhea, anorexia, wheezing, locomotor dysfunction and feather loss. Specific autopsy findings include ascites, skeletal muscle myopathy, hepatitis, myocarditis, sciatic neuritis and polioencephalomyelitis. Atrophy of lymphatic organs (bursa of the Fabricius, spleen and thymus) is also frequently observed [12]. The course of the disease varies depending on the immune status of the chicks and the age of the birds. In the case of several-day-old chicks, fully susceptible to infection, mortality can reach 100% [13]. However, infections in older birds are often asymptomatic except for ascites or feather loss [10].

There are numerous outbreaks of short beak and dwarfism syndrome (SBDS) in Pekin and mule ducks, mainly in China, caused by a new genetic variant of GPV (NGPV) [14–16]. Analysis of the viral genome identified in 2015 revealed that genome pairwise identity of NGPV and CGPV and NGPV and MDPV was 90.8–94.6% and 78.6–81.6% respectively [14].

Another serious disease of importance in the industrial waterfowl breeding and fattening is hemorrhagic nephritis and enteritis of geese (HNEG), initially called "young geese disease" or "late form of Derzsy's disease" [17]. The etiological agent of the infection is goose hemorrhagic polyomavirus (GHPV), recently named *Anser anser polyomavirus*, belonging to the *Polyomaviridae* family, Gammapolyomavirus genus. The viral genome consist of double-stranded circular DNA of approximately 5,250 base pairs containing genes encoding the structural proteins VP1-VP3, the large and small tumor antigens (LTA and STA), and the predicted ORF-X of unknown function [18, 19].

Hemorrhagic nephritis and enteritis in geese was first diagnosed in 1969 in Hungary, and subsequently in other European countries, such as Germany, France, Poland and Belgium, and its occurrence has recently been confirmed in Taiwan [20–24]. GHPV has also been shown to occur in duck flocks, which become asymptomatic carriers of the virus [25, 26].

The pathogenesis of GHPV infection is related to the tropism of the virus in the endothelial cells of blood vessels, particularly in the gastrointestinal and urogenital tracts. This results in anaerobic necrosis of the renal

tubules located around the large veins and then circulatory disorders causing swelling, congestion, and necrosis of the renal parenchyma. In the case of goslings infection, ascites and oedema associated with vascular changes are observed. If infection occurs in older birds, the clinical course is slower and is associated with urate deposition due to kidney damage [27]. Hemorrhagic enteritis and nephritis occur most frequently in birds between 3 and 6 weeks of age, although there are cases of illness in geese and adult birds [28].

In addition to GPV and GHPV, goose circovirus (GoCV) also poses an epidemic threat to goose production. Goose circovirus (GoCV) belongs to the family *Circoviridae* and the genus *Circovirus*. It has single-stranded circular DNA (ssDNA) about 2 kb in length and encodes genes for proteins Rep (replicase), Cap (capsid) and putative ORF3.

The disease was first described in geese in Germany in 1999 [29]. Reports from Europe and Asia have confirmed the occurrence of GoCV [3, 30, 31]. Geese infected with GoCV show symptoms related to growth inhibition, diarrhea and plumage abnormalities. Autopsy examinations may reveal enlargement of the spleen, thymus and liver, as well as petechiae in the epicardium, endocardium, lungs and thymus. It should be emphasized that these are immunosuppressive viruses that reduce the overall immunity of the bird. Based on experimental infections, factors such as virus dose, virus strain, inoculation route, presence or absence of co-infection, and breed of geese have been found to significantly influence the course of the disease [4, 32].

It should be emphasized that GPV, GHPV and GCV often coinfect goose farms resulting in the presentation of diverse and mixed clinical signs, which can lead to difficulties in disease diagnosis [4, 17, 33].

The increase in the number of geese in Poland requires the monitoring of viral infections in these birds. The aim of the present study was to monitor the main viral agents of geese, GPV, GHPV and GoCV, on geese farms over two years (4 production cycles) and to monitor secondary bacterial and fungal infections, clinical outcomes of infections and mortality rates. Phylogenetic analysis of obtained viral sequences was performed, providing insight into the origin and evolution of these viruses in domestic geese in Poland.

Results

In 2019, 13 geese flocks were tested for viral infections, of which 7 in the first round and 6 in the second round. The presence of GPV genetic material was detected in all the samples (100%) in both production rounds (I and II). Goose circovirus (GoCV) infection was confirmed in 6 flocks (46.15%), of which in 4 flocks (57.14%) in the first round (breeders A, B, E - hatchery X and breeder G - hatchery Z) and in 2 flocks (33.33%) in the second round (breeders B and C - hatchery X). For goose polyomavirus (GHPV), 10 flocks (76.92%) were positive, of which 6 flocks were in the first round (85.71%) (breeders A, B, C, E, F - hatchery X and breeder D - hatchery Y) and 4 flocks were in the second round (66.67%) (breeders A, C, E and F - hatchery X).

The presence of GPV + GHPV co-infection was confirmed in 8 flocks (61.54%), of which in 4 flocks in the first round (57.14%) (breeders A, B, C, D, F - hatchery X, breeder D - hatchery Y) and in 4 flocks in the second round (66.66%) (breeders A, C, E and F - hatchery X). In the case of GPV + GoCV co-infection, a positive result was confirmed in 3 flocks (23.08%), of which 1 was positive in the first round (14.29%) (breeder G - hatchery Z) and 2 were positive in the second round (33.33%) (breeders B and C - hatchery X). The presence of co-infections caused by three viruses GPV + GHPV + GoCV was also analysed; these co-infections were confirmed in 4 flocks (30.77%) in 2019, of which in 3 flocks (42.86%) (breeders A, B and E - hatchery X) in the first round and in 1 flock (16.66%) (breeder C– hatchery X) in the second round (Table 1).

In 2020, 14 geese flocks were tested (7 flocks in each round). GPV infection was confirmed in all samples tested (100%), both in season I and II. GoCV infection was found in 1 flock (14.29%) (breeder A - hatchery X) in the first round and in 5 flocks (71.43%) (breeders A, C - hatchery X; breeders D, E, H, hatchery Y) in the second round. A positive result for GHPV infection was confirmed in 6 flocks (42.86%), of which 1 flock (14.28%) was positive in the first round (breeder B - hatchery Y) and 5 flocks (71, 43%) in the second round (breeders A, C, hatchery X; breeders B, D, I, hatchery Y) (Table 2).

Among the mixed infections in 2020, GPV + GHPV co-infection was predominant, confirmed in 5 flocks (35.71%), including 1 flock (14.28%) (breeder B - hatchery Y) in the first round and in 4 flocks (breeder C - hatchery X, breeders B, D, I - hatchery Y) in the second round (57.14%). Similarly, mixed GPV + GoCV infection was also confirmed in 5 flocks (35.71%), of which in 1 flock (14.28%) (breeder A - hatchery X) was infected in the first round and 4 flocks (57.14%) (breeder A - hatchery X, breeders D, E, H - hatchery Y) were infected in the second round. Mixed infections of GPV + GHPV + GoCV were less frequent than in 2019. Only 2 flocks (28.57%) (breeders A and C - hatchery X) had a positive result in the second round, representing for 14.29% of the total number of flocks in 2020 (Table 2).

The research material was obtained from a total of 27 flocks of fattening geese. Some of them were tested by PCR only once ($n = 11$), twice ($n = 13$), or three times ($n = 3$). The mean testing time was 62.26 days (earliest sampling on day 14 and latest sampling on day 126)

Table 1 Detailed results of GPV, GoCV and GHPV infections obtained from Goose samples in 2019

Hatching season	Hatchery	Farm	Isolate	Age [days]	Detection PCR/Deposited sequence							Mixed infections		
					GPV	VP1 gene accession no./ variant	GoCV	Entire genome accession no.	GHPV	VP1 gene accession no.	VP2 gene accession no.	GPV + GHPV	GPV + GoCV	GPV + GHPV + GoCV
Round I	X	A	M.18.19	35	+	OK085817/CGPV and NGPV?	-	NA	+	ND	ND	+		
			M.23.19	70	+	ND	+	OK070807	+	ND	ND			+
	X	B	M.4.19	56	+	OK85816/NGPV	+	ND	+	ND	ND			+
			M.19.19	105	+	ND	-	NA	-	NA	NA			
	X	C	M.6.19	56	+	ND	-	NA	+	ND	ND	+		
			M.8.19	28	+	ND	-	NA	+	ND	ND	+		
	Y	D	M.5.19	21	+	ND	-	NA	-	NA	NA			
			M.20.19	91	+	OK085819/CGPV	-	NA	+	ND	ND	+		
	X	E	M.10.19	63	+	OK085813/CGPV	+	OK070804	+	ND	ND			+
	X	F	M.7.19	56	+	ND	-	NA	-	NA	NA			
			M.14.19	98	+	OK085823/NGPV	-	NA	+	OR730861	OR730867	+		
	Z	G	M.13.19	15	+	ND	-	NA	-	NA	NA			
			M.3.19	35	+	ND	+	OK070801	-	NA	NA		+	
Round II	X	A	M.9.19	63	+	ND	-	NA	+	ND	ND	+		
			M.12.19	35	+	ND	-	NA	-	NA	NA			
	X	B	M.1.19	49	+	ND	+	OK070800	-	NA	NA		+	
	X	C	M.15.19	98	+	ND	+	OK070806	-	NA	NA		+	
			M.22.19	112	+	OK085820/CGPV	+	OR552538	+	OR730862	OR730868			+
			M.25.19	14	+	ND	-	NA	+	ND	ND	+		
	X	E	M.2.19	98	+	ND	-	NA	-	NA	NA			
			M.21.19	105	+	ND	-	NA	+	ND	ND	+		
	X	F	M.24.19	98	+	OK085821/NGPV	-	NA	+	OR730863	OR730869	+		
	Z	G	M.11.19	77	+	OK085815/CGPV	-	NA	-	NA	NA			

Legend: GPV– goose parvovirus (Derzsy's disease virus); CGPV– classical GPV; NGPV– novel GPV; ND– not determined; NA– not applicable; + positive result; -– negative result; Accession no.– accession number in GenBank database

(Table 3). In the case of a clinical form of Derzsy's disease, the genetic material of viruses obtained from the same flock at different stages of rearing was sequenced twice to verify the identity of the strains. This situation occurred in the flock of breeder C in the second season of 2020. The sequences of both Derzsy's disease viruses tested (collected from on the 21st and 35th day of the birds' life) belonged to the classical GPV variant (OK085812, OK085811) (Table 2).

The incidence of GoCV infection was slightly higher in 2019 than in 2020 (46.15% and 42.86%, respectively). The whole genome sequence of GoCV was obtained twice from the same goose flock. In 2019, in round II, farm C, the GoCV DNA was detected in samples from day 98 and day 112. This flock was negative for GoCV on day 14 (Table 1). The sequences were identical, indicating that the same GoCV strain was detected. In the case of the year 2020 in round II on farm A, the same virus sequence was detected on days 63 and 98, but no virus was detected on day 112. The mean isolation time for GoCV was 65.69 days (the earliest isolation occurred on day 21, and the latest isolation occurred one on day 112) (Tables 1 and 2). The presence of GoCV genetic material in the tested birds was not always associated with feathering disorders, although it occurred in 4 cases (breeders E and G, season I of 2019; breeder A and C, season II of 2020). GPV and GHPV co-infection, as well as bacterial (*E. coli*, *E.rhusiopathiae*) and fungal infections, were also confirmed in the mentioned flocks (Table 3).

In general, polyomavirus infections occurred more frequently in 2019 than in 2020 (76.92% and 42.86%, respectively). The mean isolation time for GHPV was 77 days (the earliest isolation on day 35, and the latest isolation occurred on day 112). Despite the high frequency of detection of GHPV genetic material in the flocks studied, the clinical form of HNEG was only detected in one of them (breeder C - season II of 2019). In this case, co-infection with GoCV and GPV was also confirmed, and the flock was treated twice for colibacillosis during fattening. In the same flock, aspergillosis was also diagnosed during the goose rearing stage (Table 3).

Mixed infections of GPV + GHPV and GPV + GHPV + GoCV were also detected more frequently in goose flocks in 2019 than in 2020 (61.54%-35.71% and 30.77%-14.29%, respectively). Viral infections were also often accompanied by bacterial complications which occurred in 11 out of 13 flocks in 2019 and in 12 out of 14 flocks in 2020. Mortality of the studied geese flocks ranged from 3 to 11%, with an average mortality of 5.84%. A higher mortality rate of 10–11% was recorded in flocks affected by bacterial infections and clinical forms of viral diseases. No significant relationship was observed between mortality and the length of the fattening period.

In addition, in flocks infected with erysipelas (breeders B and C, second round of 2020), a significant increase in falls was observed in older birds, aged over 13 weeks. In turn, salmonellosis did not have a significant impact on the increase in mortality in the flock. Aspergillosis was also the cause of increased mortality in the stages of rearing and was found more frequently in geese in 2019. However, it cannot be clearly stated whether *Gallibacterium anatis* infection, was the direct cause of the increase in mortality in the geese flocks (breeder A - first round of 2019 and breeder B second round of 2020), as additional complicating factors such as *E. rhusiopathiae*, GoCV, or GHPV were confirmed in the mentioned flocks (Table 3).

A phylogenetic analysis of GPV was performed on the basis of the 1037 bp fragment, compraising a part of the viral capsid gene (*cap*) (a coding nucleotide sequence of 371 aa out of 732 aa of the VP1 protein) (Fig. 1). The sequences obtained were compared with those available in the GenBank database and classified into 2 distinct phylogenetic groups: 8 sequences belonging to the group of the classic strain of GPV (CGPV), namely, OK085819, OK085812, OK085818, OK085811, OK085822, OK085813, OK085815 and OK085820; and the remaining 4 to the group of the strain called as novel GPV (NGPV), namely, OK085821, OK085823, OK085816 and OK085814. The CGPV group was divided into two subgroups. Sequences from the first subgroup are similar to the Polish sequences previously obtained from geese and ornamental ducks. The second subgroup included sequences closely related to sequences from Polish and Turkish geese and one from the British mute swan. The separate phylogenetic group formed by NGPV strains also included sequences from Pekin ducks flocks previously studied in Poland and China. The NGPV virus sequences from this study share nucleotide similarity with the strains from China ranging from 98.50 to 99.81% (Fig. 1).

When analysing the multiple alignment of the OK085817 GPV sequence, we noticed that the unrecognised nucleotides were located at single nucleotide polymorphism sites that distinguish NGPV from CGPV. After detailed re-analysis of the sequencing chromatograms from forward and reverse sequencing, we noticed overlapping peaks of high intensity among the peaks of perfect quality. This looks like the sequencing result obtained when two mutated sequences are present in the PCR product in equal amounts (Additional file 1, the chromatograms can be sent upon request). It is possible that in the case of sample M18.2019 from farm A, two different viral DNAs were present, that of CGPV and NGPV, but to prove this, further sequencing attempts or cloning of the PCR products into the plasmids should have been performed, but this was not possible due to the lack of DNA template used for other PCR reactions.

Table 2 Detailed results of GPV, GoCV and GHPV infections obtained from Goose samples in 2020

Hatching season	Hatchery	Farm	Isolate	Age [days]	Detection PCR/Deposited sequence							Mixed infections		
					GPV	VP1 gene accession no./variant	GoCV	Entire genome accession no.	GHPV	VP1 gene accession no.	VP2 gene accession no.	GPV + GHPV	GPV + GoCV	GPV + GHPV + GoCV
Round I	X	A	M.4.20	98	+	ND	+	OK070808	-	NA	NA		+	
	Y	B	M.18.20	21	+	OK085818/CGPV	-	NA	-	NA	NA			
			M.19.20	35	+	ND	-	NA	+	OR730865	OR730871	+		
	X	C	M.5.20	35	+	ND	-	NA	-	NA	NA			
			M.20.20	105	+	ND	-	NA	-	NA	NA			
	Y	D	M.17.20	21	+	ND	-	NA	-	NA	NA			
			M.14.20	84	+	ND	-	NA	-	NA	NA			
	X	E	M.16.20	35	+	ND	-	NA	-	NA	NA			
	Y	H	M.10.20	28	+	OK085814/NGPV	-	NA	-	NA	NA			
			M.12.20	91	+	ND	-	NA	-	NA	NA			
	Y	I	M.3.20	56	+	ND	-	NA	-	NA	NA			
Round II	X	A	M.13.20	63	+	ND	+	OK070805	+	ND	ND			+
			M.7.20	98	+	ND	+	OK070809	-	NA	NA		+	
			M.11.20	112	+	ND	-	NA	-	NA	NA			
	Y	B	M.15.20	42	+	ND	-	NA	+	OR730864	OR730870	+		
	X	C	M.2.20	21	+	OK085812/CGPV	-	NA	+	ND	ND	+		
			M.1.20	35	+	OK085811/CGPV	+	OK070803	+	ND	ND			+
			M.23.20	126	+	ND	-	NA	-	NA	NA			
	Y	D	M.9.20	63	+	ND	+	OK070811	-	NA	NA		+	
			M.22.20	119	+	ND	-	NA	+	ND	ND	+		
	Y	E	M.24.20	49	+	OK085822/CGPV	+	OK070802	-	NA	NA		+	
	Y	H	M.8.20	21	+	ND	+	OK070810	-	NA	NA		+	
	Y	I	M.6.20	28	+	ND	-	NA	+	ND	ND	+		

Legend: GPV– goose parvovirus (Derzsy's disease virus); CGPV– classical GPV; NGPV– novel GPV; ND– not determined; NA– not applicable; + positive result; -– negative result; Accession no.– accession number in GenBank database

Table 3 Summary of results of GPV, GoCV and GHPV infections and flock sizes, fattening periods and health condition of geese flocks in 2019 and 2020

Year	Hatching season	Hatchery	Farm	Flock size	Fattening period [days]	Mortality [%]	GPV/ variant	GoCV	GHPV	Health condition of geese flocks
2019	Round I	X	A	6700	112	6	+/CGPV and NGPV?	+	+	2 occurrences of colibacillosis, *Gallibacterium anatis* infection
		X	B	13,000	114	8	+/NGPV	+	+	colibacillosis, aspergillosis
		X	C	4500	98	3	+	-	+	-
		Y	D	6900	99	4	+/CGPV	-	+	2 occurrences of colibacillosis, aspergillosis
		X	E	7777	110	6	+/CGPV	+	+	colibacillosis, aspergillosis, abnormal feathering
		X	F	8000	112	5	+/NGPV	-	+	colibacillosis
		Z	G	4500	100	6	+	+	-	colibacillosis, abnormal feathering
	Round II	X	A	7500	114	4	+	-	+	aspergillosis
		X	B	11,300	116	8	+	+	-	colibacillosis, aspergillosis
		X	C	4500	120	10	+/CGPV	+	+	2 occurrences of colibacillosis, aspergillosis, clinical form of HNEG
		X	E	7500	98	4	+	-	+	aspergillosis,
		X	F	7500	120	5	+/NGPV	-	+	colibacillosis
		Z	G	4500	116	3	+/CGPV	-	-	-
2020	Round I	X	A	7500	114	4	+	+	-	aspergillosis
		Y	B	13,000	100	7	+/CGPV	-	+	2 occurrences of colibacillosis
		X	C	3000	120	3	+	-	-	-
		Y	D	7500	135	5	+	-	-	colibacillosis, aspergillosis
		X	E	7000	122	3	+	-	-	-
		Y	H	3700	118	4	+/NGPV	-	-	colibacillosis
		Y	I	7200	120	5	+	-	-	colibacillosis
	Round II	X	A	8000	140	7	+	+	+	colibacillosis, aspergillosis, abnormal feathering
		Y	B	8000	135	10	+	-	+	colibacillosis, erysipelas, *Gallibacterium anatis* infection
		X	C	3500	140	11	+/CGPV	+	+	colibacillosis, erysipelas, abnormal feathering, clinical form of Derzsy's disease
		Y	D	7000	139	6	+	+	+	colibacillosis
		Y	E	4500	123	4	+/CGPV	+	-	colibacillosis
		Y	H	4000	133	5	+	+	-	2 occurrences of colibacillosis
		Y	I	7000	125	6	+	-	+	colibacillosis, salmonellosis

Legend: GPV– goose parvovirus (Derzsy's disease virus); CGPV– classical GPV; NGPV– novel GPV; GoCV– goose circovirus; GHPV– goose hemorrhagic polyomavirus

A phylogenetic tree of GoCV was constructed, based on the alignment of whole-genome sequences of 35 goose circovirus strains obtained from the GenBank database and 11 sequences obtained in this study (Fig. 2). All but two of the sequences clustered together and were similar to the sequence obtained from domestic geese in Poland in 2014 [34]. The sequences (OK070806, OR552538), obtained in this study from one flock in 2019, clustered with sequences originating from wild geese from Poland and from Hungary in 2014 and 2013, respectively. The amino acid sequence analysis of the two main GoCV proteins (Cap and Rep) reveals that, in the OR552538 strain, the Rep sequence shows 98.14–100% identity with other GoCV sequences, while the Cap shows between 80% and 99.6% identity. In the OK070800 strain, the Rep exhibits 97.77–99.66% identity, and the Cap ranges from 75.6 to 99.6% amino-acid identity. These results align with the typical conservation patterns observed in circovirus proteins [35].

For the phylogenetic analysis of GHPV, complete genes encoding the Vp1 and Vp2 proteins were obtained from 5 samples after virus isolation on goose embryonic kidney cells. The sequences for both Vp1 and Vp2 were identical to each other. These sequences were closely related to goose strains previously isolated in Poland and Hungary and to strains from wild mallard ducks (Fig. 3).

Discussion

In this study, an attempt was made to determine the health status of the fattening geese in Poland, with particular emphasis on viral infections.

The most dangerous disease affecting geese is Derzsy's disease. Despite the availability of immunoprophylaxis, both in Poland and worldwide, new cases of this disease are reported every year. In this study, the presence

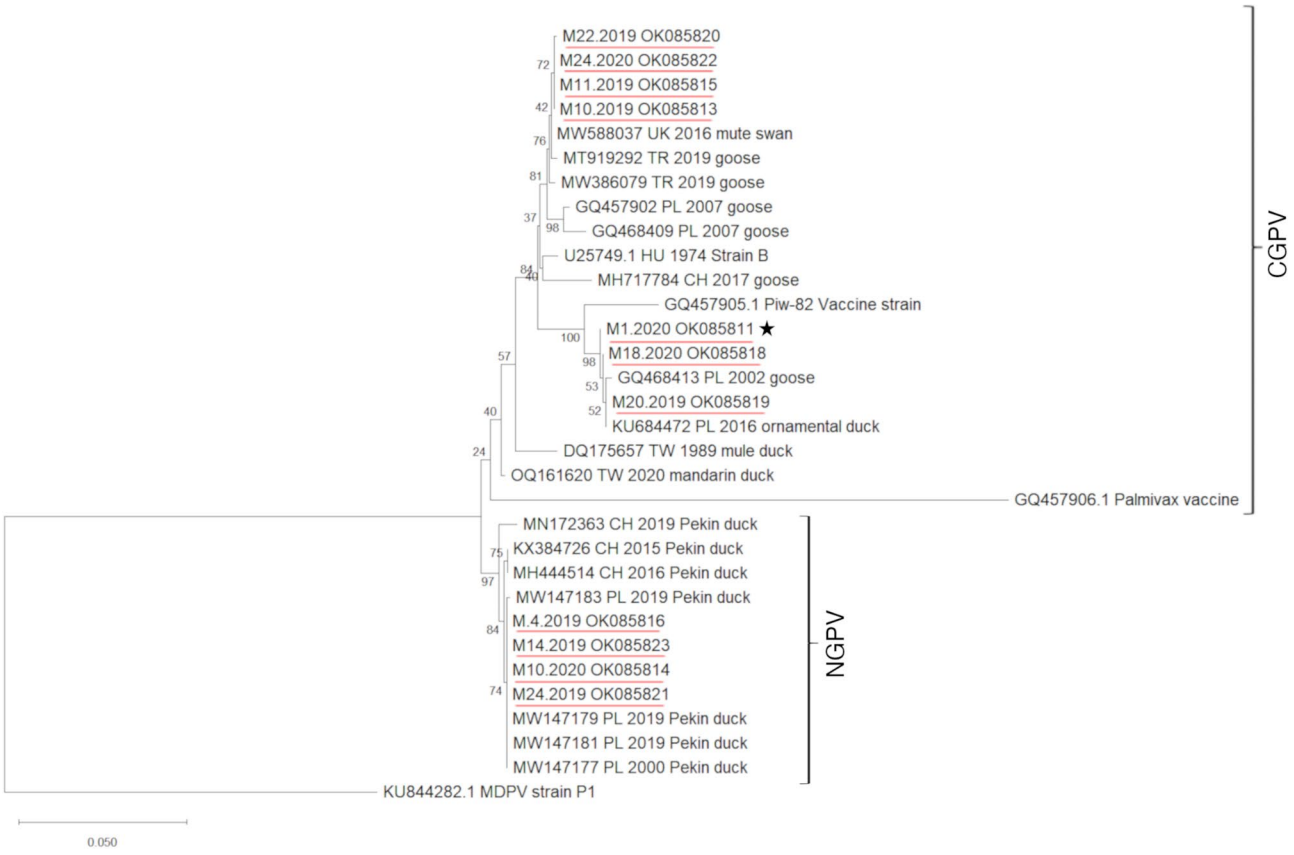

Fig. 1 Phylogenetic tree based on partial GPV sequences with the sequences of other waterfowl parvovirus (*Anseriform dependoparvovirus 1*) isolates available in the GenBank database. The Polish sequences obtained in this study are underlined in red. The asterisk indicates the sequence obtained from the flock with clinical Derzsy disease. MDPV, Muscovy duck parvovirus; CGPV, classical goose parvovirus; NGPV, novel goose parvovirus. The phylogenetic tree was constructed using the Maximum Likelihood (ML) algorithm with 1000 bootstrap replicates and HKY+I nucleotide substitution model. The OK085812 sequence is not shown on the tree because it was identical to OK085811. The OK085817 sequence is not shown on the tree because it had overlapping sequencing reads of NGPV and CGPV. The tree was rooted to MDPV sequence. PL- Poland; Ru- Russia; Hu- Hungary; TR- Turkey; CH- China; TW- Taiwan, UK- United Kingdom

genetic material of the GPV virus was detected in all flocks (100%), in both 2019 and 2020. An older study of samples originating from sick and symptomatic geese flocks in Poland, showed a lower GPV incidence of 29,50% [3]. However, it should be emphasized that the real-time PCR used in this study is more sensitive than the conventional multiplex PCR used in previous studies and cannot distinguish between wild-type and vaccine strains. This may mean that the prevalence of GPV in Poland was underestimated, especially because we were not able to obtain the PCR products for sequencing purposes by conventional PCR from each sample. The identification of GPV genetic material in the studied flock was not always associated with the occurrence of a clinical form of Derzsy's disease. However, there were cases of feather loss, increased falls and cachexia in the flock, especially if co-infection with goose circovirus was confirmed in the tested birds. In contrast, the clinical signs of Derzsy's disease were only observed in one flock in 2020 (round II, flock C); this flock was co-infected with GoCV, GHPV and erysipelas, which may have contributed to increased mortality.

In our study, the earliest GPV infection was detected on day 14. In all cases, GPV genetic material was detected as late as day 119. We conclude that the birds are infected during the fattening process. It is not known whether the birds are re-infected or chronically infected with GPV, as most baseline infection studies in geese have been performed over limited time periods [4].

Interestingly, in addition to classical GPV, the novel GPV sequence, a genetic variant of the virus that is an etiological agent of short beak and dwarfing syndrome in Pekin ducks, was detected in 5 flocks. To our knowledge, NGPV has not been shown experimentally to infect geese. The Chinese strain of NGPV SD15 was successfully grown on both duck embryonated eggs and duck embryo fibroblasts, but goose embryo fibroblasts were not permissive [36]. However, the vaccine strain used in the Palmivax vaccine is based on the Hoechstra strain, which clusters with NGPV strains in the phylogenetic

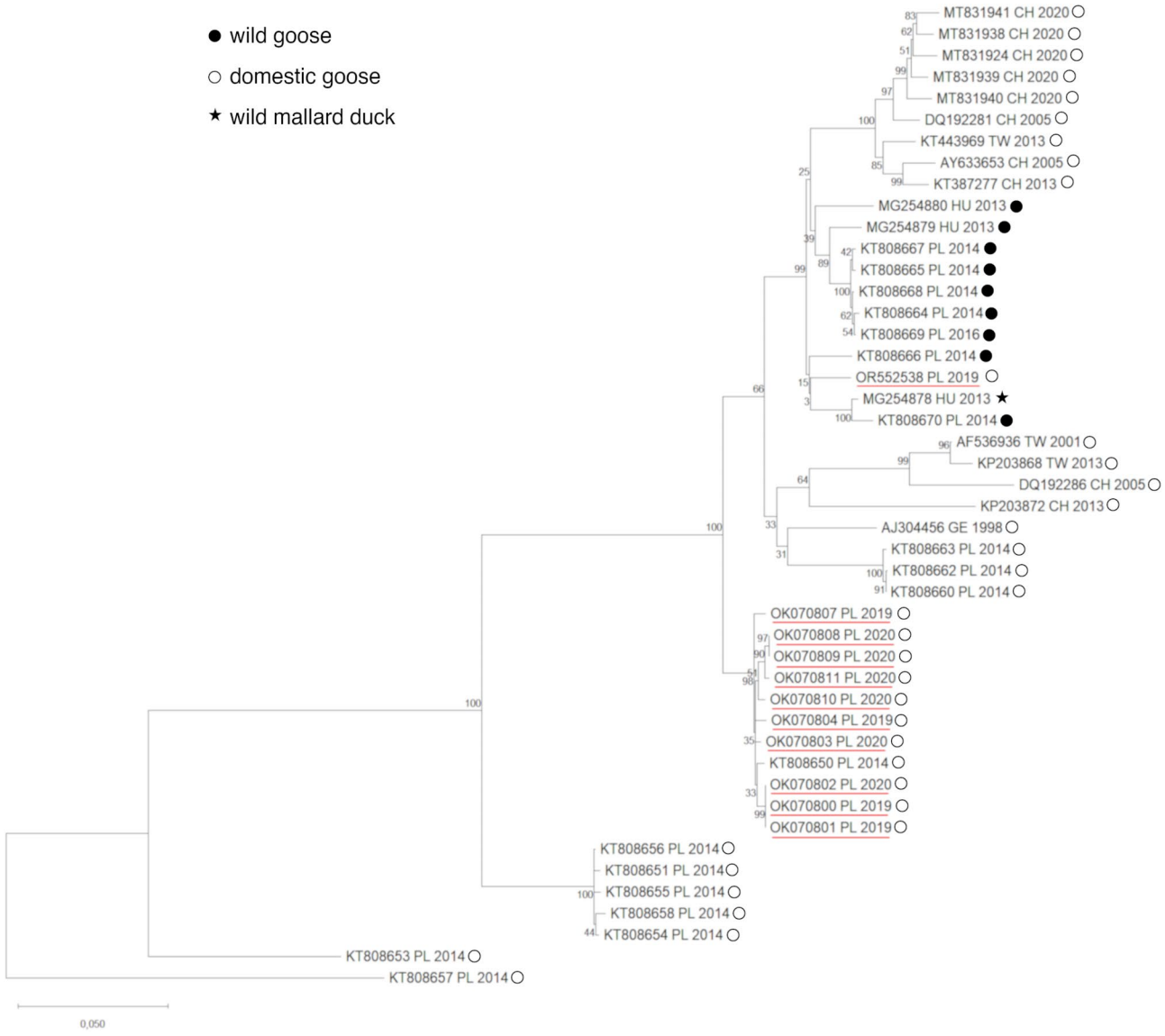

Fig. 2 Phylogenetic tree based on whole-genome GoCV sequences and sequences of other goose circovirus isolates available in the GenBank database. The Polish sequences obtained in this study are underlined in red. The phylogenetic tree was constructed using the Maximum Likelihood (ML) algorithm with 1000 bootstrap replicates and TN93 + G + I nucleotide substitution model. The tree was rooted to KT808657 sequence. The OK070806 sequence is not shown on the tree because it was identical to OR552538. The OK070805 sequence is not shown on the tree because it was identical to OK070809. PL- Poland; GE- Germany; HU- Hungary; CH- China; TW- Taiwan

tree (Fig. 1). It is likely that NGPV can infect geese, but further studies are needed to assess the pathogenicity of NGPV in this species. To our knowledge, this is the first identification of the NGPV sequence in a goose species. From sequencing of one of the samples in 2019, we obtained interesting sequencing result. Because the PCR were made on pooled organ samples, this indicate that either some geese were co-infected with NGPV and CGPV or that some birds were infected with NGPV and some with CGPV. Nevertheless, this strongly suggest the simultaneous presence of NGPV and CGPV in this particular flock. In 2019, our group described the outbreaks of SBDS in Pekin ducks on farms in the same region of Poland [37]. This could mean that either the virus jumped from the Pekin ducks to the goose flocks investigated in this study, or it was present in the goose flocks and was subsequently introduced into the Pekin ducks. The most recent GPV sequences available in GenBank were obtained from domestic geese in 2007, and were derived from clinical cases of Derzsy's disease in geese [38]. However, asymptomatic birds are rarely tested and may therefore harbor undetected viruses of low virulence. In 2020, the infection with recombinant MDPV, a virus that is

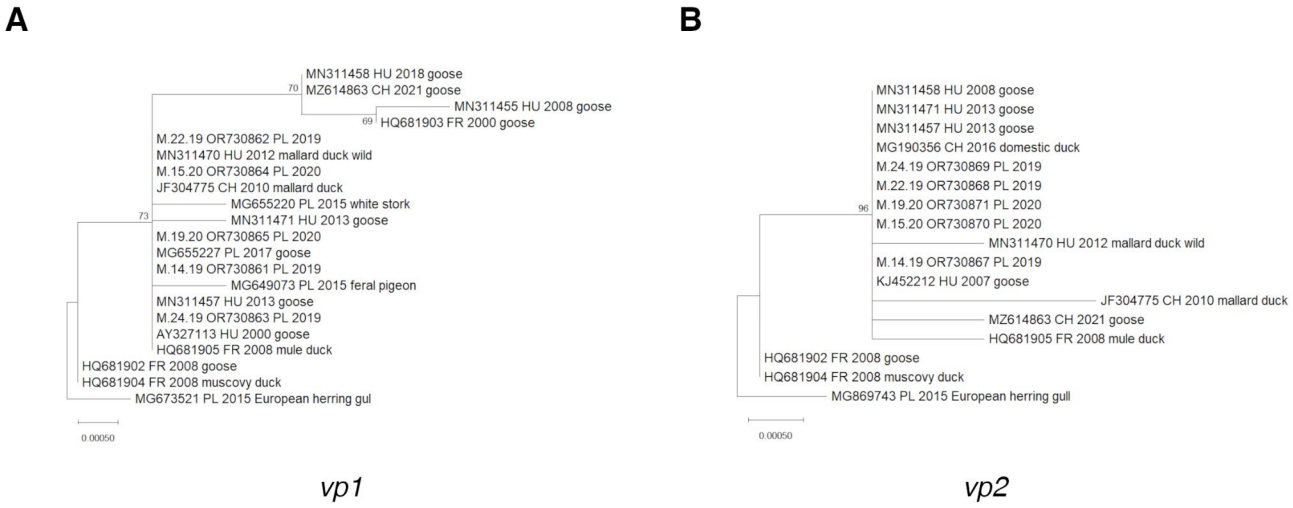

Fig. 3 Phylogenetic tree based on partial GHPV sequence analysis of the *vp1* gene (**A**) and *vp2* gene (**B**) with the sequences of other goose haemorrhagic polyomaviruses (*Anser anser polyomavirus 1*). The phylogenetic tree was constructed using the Maximum Likelihood (ML) algorithm with 1000 bootstrap replicates and nucleotide substitution models, such as K2 for *vp1* gene and JC for *vp2* gene. The tree was rooted to outlier sequences: MG673521 and MG869743 for A and B trees, respectively. PL- Poland; FR- France; HU- Hungary; CH- China

genetically more distant from classical GPV than BGPV, was detected in goose flock in Taiwan [39].

In our study, one flock infected only with NGPV (negative for GoCV and GHPV) did not show increased mortality or other symptoms (year 2020, farm H, mortality 4%), whereas two other flocks with detected NGPV sequences were also coinfected with GHPV (year 2019, farm F, mortality 5%) or with GoCV and GPV (year 2019, farm B, mortality 8%). Nevertheless, it would be wise to monitor these flocks in the future and to investigate the possible adaptation of NGPV to geese using next-generation sequencing.

In this study, the clinical form of Derzsy's disease was observed in one flock in 2020 in round II (farm C). This rather small flock had a mixed infection with all three viruses tested, colibacillosis, erysipelas and abnormal feathering. The mortality rate was 11%. The same farm in round I (first fattening period from February to April) was not co-infected with GoCV or GHPV and was healthy with a very low mortality rate of 3%. The poor health status in round II (May-July) may be attributed to lower maternal antibody levels, in goslings hatched later in breeding season [40].

There is a spoken knowledge among farmers that geese reared in first round (months) are healthier because of higher maternal antibody titres against GPV and because the farm has long autumn and winter rest period (no animals on the farm), which reduces the virus load in the farm environment. In this study, we observed two different outcomes at two years. However, this pattern was only detected in 2020, when we detected only one infection of GoCV and one infection of GHPV in round I, while the detection of GoCV and GHPV was much higher in round II. In 2019, virus detection rates for GoCV and GHPV were higher in round I than in round II, but sampling was less frequent in the second round.

The incidence of co-infection with two or three viruses studied here was very high, which is not surprising as we have shown that the prevalence of all these viruses is very high. In addition, the use of real-time PCR to detect GPV is likely to be more sensitive than the standard PCR techniques used in older studies. In Poland, only 4.9% of flocks were co-infected with GPV and GoCV in the 1998–2010 study. GHPV was only detected in two flocks in 2007. Certainly, the number of GoCV and GHPV infections has increased significantly over the last decade. In this study, the prevalence of GoCV in flocks was maintained at similar levels – 46.15% and 42.86% in 2019 and 2020 respectively, while the incidence of GHPV was 76.92% and 42.86% in 2019 and 2020 respectively. This high prevalence could also be attributed to the multiple testing of most herds. Unfortunately, there are no recent published prevalence data on GPV, GoCV or GHPV infection in commercial goose flocks. Therefore, this study provides new data, but additional flocks should be tested to determine prevalence data for the whole country.

In goose production, the acceptable mortality rate is higher than in chicken broilers and is estimated at 5%. The 6 flocks not infected with GoCV or GHPV had a low mortality rate of 3–5%; as a secondary infection there were some cases of colibacillosis and in one flock aspergillosis was detected. In contrast, flocks with triple viral co-infections had higher mortality rates and secondary infections were more common. Similar secondary

infections were found in flocks of geese co-infected with GPV and GHPV in a recent study in Taiwan [41].

Abnormal feathering was observed only in flocks infected with GoCV infection, which is consistent with disease progression, and experimental studies have shown that even after experimental infection, only 10% of infected birds may develop abnormal plumage [32, 42].

Phylogenetic analysis of whole genome sequences of GoCV obtained in this study revealed that all but one of these sequences were similar to each other and closely related to sequences obtained from commercial goose farms in 2014 Poland [34]. This may indicate that the endemic GoCV strain was maintained in the goose population at this particular location. Only one sequence obtained in 2019 differed and clustered on the phylogenetic tree with sequences isolated only from wild geese and one from wild ducks in Poland and Hungary [34, 43]. These results indicate that most of the GoCV infections circulating in geese are endemic to domestic farmed geese in this area of Poland, but here, we show that introduction of the GoCV from wild animals is possible. The nature of the semi-intensive goose production system in Poland facilitates contact with wild birds and the introduction of new viruses into this population, as has been shown in recent outbreaks of avian influenza [44].

The stability of the GHPV was confirmed in this study, as neither the *vp1* nor the *vp2* gene sequences differed from sequences obtained in the past in Europe. The sequences are very similar to those obtained from domestic geese and duck flocks in Poland, as well as to sequences obtained in other parts of Europe and to sequences obtained from wild birds of several species [45]. Our study supports the knowledge of the slow evolution of this virus species, but also highlights the possibility of virus introduction from the wild birds, which can be asymptomatic carriers of this virus [46].

Conclusions

In this study we have shown a high prevalence of three viruses that are common and important in domestic goose flocks. We showed that the incidence of secondary bacterial infections was higher in flocks co-infected with GHPV and GoCV. This led to higher mortality rates in these flocks. The incidence of viral and bacterial infections was not always repeated during the second round of fattening on a particular farm, indicating that the control measures used were appropriate. For the first time, the novel GPV sequence (the causative agent of SBDS in Pekin ducks) was isolated from goose species, although without clinical signs. We showed that a sequence of GoCV was similar to sequences previously isolated from wild birds, suggesting the possible introduction of a new strain into domestic geese.

Materials and methods

Animals and sampling

The study was carried out over a two-year observation cycle during 2019–2020. The commercial geese farms were located in the southwestern and central parts of Poland (Lower Silesia, Greater Poland, and Lodz Voivodeships). Goslings of the White Kołudzka breed were obtained from 3 different hatcheries (X, Y, Z). The herd monitoring program was carried out throughout the rearing and fattening period and covered 27 flocks with a size of 3,000 to 13,000 birds (Table 3). The geese were kept in semi-intensive systems, always with access to free range. The birds were fed ready-made feed mixtures or concentrates containing cereals (wheat, corn, soya, oats). In the summer, some herds had access to pastures or fresh green fodder.

Furthermore, the flock health analysis included two periods of introduction of goslings to the farm: the first hatching season (I), which included hatchings from February to April, and the second hatching season (II) which included hatchings from May to July (Table 3).

Sampling was carried out three times during the each hatching season according to the following schedule: (1) between 12 and 16 days of life of birds, (2) between 21 and 98 days of life of birds, and (3) between 98 days of life and the end of fattening. Samples consisted of cloacal swabs and/or internal organs (liver, spleen, kidneys, heart) from dead birds (Fig. 4).

Microbiological culture

Bacterial and mycological isolation was performed on solid media in a Petri dish (Nutrient agar, Columbia Blood Agar Base and MacConkey and Sabouraud Dextrose Agar) (Oxoid, United Kingdom), incubated under aerobic conditions in 37 degrees for 18–24 h. The bacterial colonies obtained were seeded on nutrient agar and, in the case of suspected *Gallibacterium*, *Erysipelothrix or Pasteurella* growth, on agar supplemented with 5% defibrinated sheep blood to obtain a pure bacterial culture. In the case of *Salmonella*, isolation took place according to Polish Standard PN-EN ISO 6579 Annex 3 1:2017-04/A1:2020-09 + White- Kaufmann- Le Minor scheme 2007. In the case of *Gallibacterium* and *Erysipelothrix*, species affiliation was confirmed by molecular testing (PCR) [47, 48].

Cell culture

Goose embryonic kidney (GEK) cells were prepared from six 20-day-old goose embryos as described previously, with modifications [21]. Trypsin (Biological Industries, Israel) was used for cell tissue disintegration. Cells were grown in Eagle's minimal essential medium (Gibco BRL) supplemented with 10% fetal calf serum (Biological Industries, Cromwell, USA), and antibiotics (penicillin

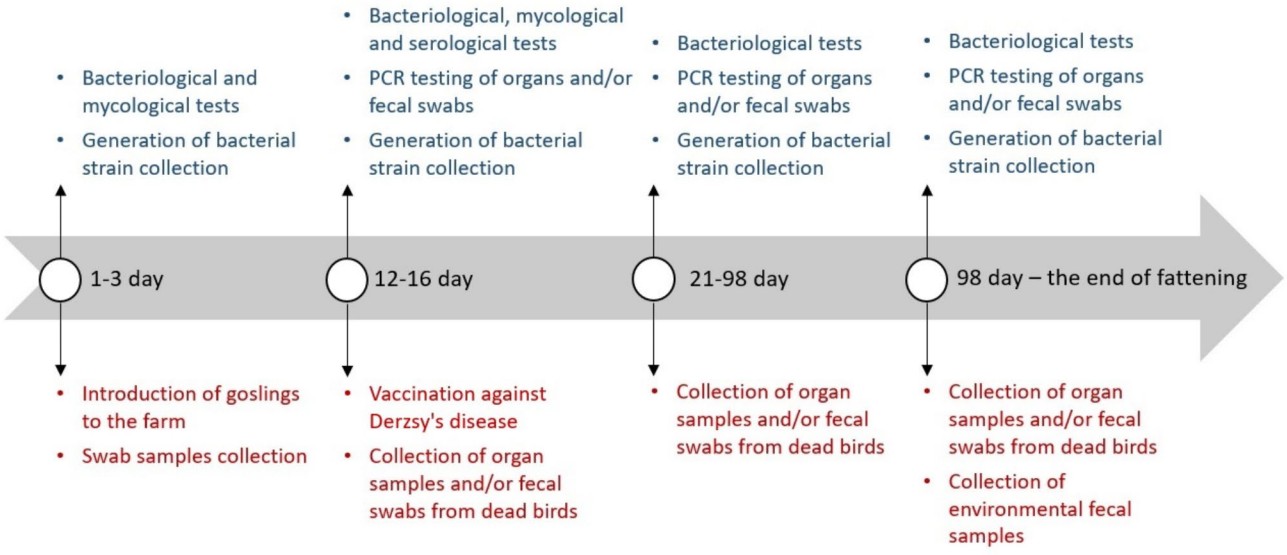

Fig. 4 Scheme of the sampling procedure. Samples for PCR screening were collected at least three times during the fattening period. The time of vaccination against Derzsy's disease is also indicated

[100 IU/ml] and streptomycin [100 µg/ml]) (Sartorius, Göttingen, Germany). Cells were trypsinised and split at a 1:2 ratio every 5–7 days.

GHPV virus cultivation

Spleen and liver tissues (0.5 cm3) were ground with sterile sand and medium in a 1:10 ratio, centrifuged and filter sterilised. Aliquots (100 µl) of this supernatant were used to inoculate GEK cultures. Early passage GEK cultures (passages 1 to 2) were seeded into 6-well plates and inoculated the following day when the cell monolayers were subconfluent. The inoculum was allowed to adsorb for 2 h, then removed and replaced with 2 ml of culture medium containing 5% fetal calf serum. Cell cultures were monitored daily for cytopathic effects (CPE). At 5 to 7 days post-inoculation (dpi), the flasks were frozen and the viral inoculum for the next passage was a crude lysate prepared by three consecutive freeze-thaw cycles and vigorous shaking. Centrifuged cells were lysed and DNA isolated as described above.

DNA isolation and PCR

Viral DNA was isolated from cloacal swabs, pooled internal organs of dead birds or from infected GEK cultures. The Syngen Viral Mini PLUS isolation kit (Syngen Biotech, Wroclaw, Poland) was used.

For the detection of goose parvovirus (GPV), real-time PCR was performed with a Taq-Man probe using Probe qPCR Master Mix (2x) (Eurx, Gdansk, Poland). The detection of circovirus (GoCV) and polyomavirus (GHPV) in geese was performed by conventional PCR using Color Taq PCR Master Mix (2x) (Eurx, Gdansk, Poland).

Conventional PCR reaction products were separated by electrophoresis in 2% (wt/vol) agarose gels stained with Midori Green DNA Stain (Nippon Genetics Europe GmbH, Düeren, Germany) and visualised under UV light. Amplicons for sequencing were excised from the gel with a scalpel and cleaned using a Geneclean kit (MP Biomedicals, Solon, USA). The PCR products was then sequenced in both directions using the Sanger method (Genomed S.A., Warsaw, Poland). All PCR reagents and purification kits were used according to the manufacturer's instructions. The thermal cycling conditions and primer sequences used are summarised in Table 4. For sequencing of whole GoCV genome two new pairs of primers were designed to cover whole circovirus circular genome, and the PCR products were sequences as mentioned above. The three fragments of GoCV genomes were assembled manually in MEGA version 12.0.9 software.

Phylogenetic analysis

The resulting PCR products sequences were edited and aligned using the ClustalW multiple alignment algorithm implemented in the MEGA version 12.0.9 software. The phylogenetic analysis for GPV was based on a partial fragment of the gene encoding the VP1 protein, for GoCV on the whole genome of 1821 base pairs, and for GHPV on a complete gene encoding the VP1 and VP2 proteins. Phylogenetic trees for mentioned above viruses were constructed using the Maximum Likelihood (ML) method under HKY + I, TN93 + G + I, K2 and JC models respectively, with 1000 bootstrap replicates. See Additional file 2 for analysis of correct model selection [50].

Table 4 Primer sequences and specific thermal cycling conditions used for the PCR reactions

Assay target	Primer sequence (5′→3′)	Thermal cycling conditions	Product size bp	Reference
GPV detection	F: ACC GGA AGT CAC GTG AC R: GTT CGT TCG TTC GAA CC Probe: 6-FAM ACC GGA AGC AyG TGA CCG GAA TAMRA(Q)	50 °C–2 min, (95 °C–10 min, 94 °C–15 s, 60 °C–1 min) x 40	123	[12]
GHPV detection	F: ACC CGT GCT TCC ATT CAC AA R: CTG CTC CCC AAA CCT GTC AA	95 °C–3 min, (94 °C–20 s, 62 °C–30 s, 72 °C–20 s) x 30, 72 °C–3 min	379	[2]
GoCV detection	F: TAA ATG CGA GTT TGA TGT GTC T R: CAT TTA ACC CCT TCC AAA GAG T	95 °C–3 min, (94 °C–20 s, 60 °C–30 s, 72 °C–20 s) x 30, 72 °C–3 min	565	[42]
GPV sequencing	F: TTG CGA TTC CCA ATG GAT G R: CCC AAA TAG GTC CCT GTA GAT A	98 °C–3 min, (98 °C–10 s, 61 °C–20 s, 72 °C–1 min) x 40, 72 °C–5 min	1171	[49]
GHPV sequencing	F1: GGA TGC TGC CTC TAA TTC TA R1: CGC AGT TAA TCA GCT TAC AA	98 °C–3 min, (98 °C–10 s, 55 °C–20 s, 72 °C–1 min) x 40, 72 °C–5 min	1232	[26]
	F2: GCC CCT ACT ATG AAG GAT CT R2: GGC TAA AGC CAT TAT CAG TG		1290	
GoCV sequencing	F1: CGT CCG ATG TGT AGC CTT CGT R1: CAT AAA CTC GGG GGC GGG TG	98 °C–3 min, (98 °C–10 s, 57 °C–20 s, 72 °C–1 min) x 40, 72 °C–5 min	1091 Nucleotide position 1-930 and 1661–1821	This study
	F2: TAA ATG CGA GTT TGA TGT GTC T R2: CAT TTA ACC CCT TCC AAA GAG T	98 °C–3 min, (98 °C–10 s, 60 °C–20 s, 72 °C–1 min) x 40, 72 °C–5 min	564 Nucleotide position 837–1400	[42]
	F3: GCC CAG TCC ATT GTC CGA ATC R3: GCC CAT CAT GCC GCT GTA TCG	98 °C–3 min, (98 °C–10 s, 57 °C–20 s, 72 °C–1 min) x 40, 72 °C–5 min	749 Nucleotide position 1020–1768	This study

Protein blast search for Rep and Cap of GoCV was performed with blast p (NIH).

Abbreviations

NGPV Novel goose parvovirus
CGPV Classical goose parvovirus
HNEG Hemorrhagic nephritis and enteritis of geese
GHPV Goose hemorrhagic polyoma virus
SBDS Short beak and dwarfism syndrome
GoCV Goose circovirus
Cap Capsid protein
Rep Replicase

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12917-025-04653-8.

Supplementary Material 1

Supplementary Material 2

Author contributions

A.W., A.K.M. and M.C-W. conceived and designed the study; M.S., AK and M.C-W conducted the research; A.K.M. and A.W. analysed and interpreted the data; A.K.M. and M.S. wrote the manuscript; A.W. revised the manuscript. All the authors read and approved the final version of the manuscript. Monika Chmielewska-Władyka and M.S. contributed equally to this manuscript as first authors.

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Data availability

All nucleotide sequences obtained in this study have been deposited in GenBank database under accesion numbers: OK085811-OK085823, OK070800-OK070811, OR730861-OR730871 and OR552538.

Declarations

Ethics approval and consent to participate

Sample collection was performed according to the Act for the Protection of Animals for Scientific or Educational Purposes of 15 January 2015 (Official Gazette 2015, No. 266, Art. 2), applicable in the Republic of Poland. Due to the fact that samples were collected during veterinary procedures (conducted by the first author– Monika Chmielewska-Władyka, PhD, MDV, veterinarian on farms from which samples were taken) these activities did not require additional agreement from the Local Ethical Committee. Dr Monika Chmielewska-Władyka who supervised the herds had the consent of the owners for all veterinary procedures, including samples collection, carried out on the farms. The study complies with all institutional, national or international guidelines, such as the Basel Declaration. According to the Polish animal law (The Act on the protection of animals used for scientific and educational purposes, which was passed in January 2015 and transposed Directive 2010/63/EU into current Polish legislation; Official Gazette 2015, No. 266) avian embryos are not considered as "live vertebrae animals", so the Approval of Local Animal Ethics Commission was not required. On the 20th day of incubation, according to the AVMA guidelines and animal welfare, goose embryos were sacrificed by decapitation.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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