

Preliminary survey of the occurrence of goose haemorrhagic polyomavirus (GHPV) in wild birds in Poland

Natalia Styś-Fijoł, Wojciech Kozdruń, Hanna Czekaj

Department of Poultry Viral Diseases,
National Veterinary Research Institute, 24-100 Pulawy, Poland
natalia.stys@piwet.pulawy.pl

Received: November 26, 2015 Accepted: May 9, 2016

Abstract

Introduction: The aim of the study was to investigate the occurrence of goose haemorrhagic polyomavirus (GHPV) in wild birds inhabiting Poland. **Material and Methods:** Samples from 508 birds of different species were obtained between 2010 and 2015. The internal organ sections were homogenised and then total cellular DNA was isolated. The study was performed by means of PCR assay using primers complementary to the VP1 gene of the GHPV. **Results:** The presence of genetic material of GHPV was detected in 22 (4.33%) samples. **Conclusion:** It was the first such study in Poland to emphasise the role of wild birds as a potential source of GHPV infection for farmed geese.

Keywords: geese, goose haemorrhagic polyomavirus, haemorrhagic nephritis and enteritis of geese, wild birds, Poland.

Introduction

Polyomaviruses of birds cause acute and chronic diseases characterised by high mortality rates in young birds. There are four known bird polyomaviruses: avian polyomavirus (APV), goose haemorrhagic polyomavirus (GHPV), finch polyomavirus (FPyV), and crow polyomavirus (CPyV). The first one, APV, was isolated from nestling budgerigars with acute fatal disease and designated as budgerigar fledgling disease virus (BFDV), but now it is called APV because of its broad host range (3, 9). GHPV was identified as the aetiological agent of haemorrhagic nephritis and enteritis of geese (HNEG) in 2000 (8). In 2006, two additional polyomaviruses were detected in the wild bird populations and characterised by genome sequencing (12).

GHPV exhibits the typical characteristics of a polyomavirus. The genome consists of circular double-stranded DNA and is divided into two regions. The early genes encode large and small T antigens, which are multifunctional regulatory proteins, and the late genes encode structural proteins: VP1-main capsid protein, VP2, and VP3 (11). All avian polyomaviruses have an additional ORF located upstream of the VP2-encoding region, which in the case of GHPV encodes protein

homologous to VP4 (designated as product of ORV-X) (10). The phylogenetic analysis carried out on the basis of the VP1 gene sequence confirmed that this virus is significantly divergent from other polyomaviruses and represents a distinct virus species in the *Polyomavirus* genus (8).

HNEG is one of the most important diseases of geese with high morbidity and mortality (7). This disease, affecting birds aged 4 to 10 weeks, was first reported in Hungary in 1969 (2). A few years later, cases of HNEG were confirmed in flocks of domestic geese in France and Germany (18, 19). A novel outbreak of HNEG with a 32% mortality rate was observed in Poland in 2013 (6). Diarrhoea, loss of locomotor activity, apathy, and nervous signs were most frequently observed in the affected geese.

The characteristic symptoms of HNEG include oedema of the subcutaneous tissues, ascites, inflammation of the kidneys, and often haemorrhagic enteritis (5, 15). These post-mortem findings are caused by the damage of endothelial cells, in the nuclei of which viral replication occurs. In subclinical infections, GHPV induces immunosuppressive effects by lymphocyte depletion in the lymphoid organs (13). GHPV spreads from geese showing clinical disease and being carriers

mostly by faecal route. Infected birds excrete significant amounts of the virus in their droppings, which constitute a source of infection for other birds settling in the contaminated environment (7).

It has been recently shown that GHPV may be detected in other waterfowl species, such as Muscovy ducks (*Carina moschata*) and mule ducks (hybrid) with no pathologic evidence of infection. High replication levels of GHPV in these birds have suggested that ducks may be a subclinical reservoir of the virus (17). The occurrence of the GHPV infection in other avian species seems to be very likely, because subclinical infections have been reported in migrating wild geese (8).

The classical methods of GHPV identification, such as virus isolation on goose kidney cell culture (GKC) or goose embryo inoculation, are time-consuming and seldom applied in routine diagnosis of HNEG (15). An accurate biological method for GHPV titration is inoculation of goose embryos onto the chorioallantoic membrane (CAM) (1). The detection of viral genetic material by PCR assays is a much more effective method of identification of the causative agent (8, 14). PCR allows to detect DNA extracted from infected tissues with primers designed on VP1 gene, which is remarkably conserved among GHPV isolates from different countries (11, 15).

The purpose of this study was to evaluate the prevalence of GHPV infections in different species of wild birds in Poland due to the fact that the significance of GHPV, the second polyomavirus in wild birds, remains unclear and because GHPV infections in migrating wild geese have been reported (8). The paper presents preliminary results of the study on the prevalence of GHPV in different species of wild birds inhabiting Polish territory. This is the first report of GHPV detection among wild birds in Poland.

Material and Methods

Birds and samples. Birds were obtained between 2010 and 2015 from zoological gardens, Wild Birds Rehabilitation Centre (Albatross Foundation), Bird Migration Research Station, Hunting Associations, and free practice veterinarians. During the post-mortem examinations, sections of the heart, lungs, liver, spleen, gizzard, intestines, and kidneys were collected. The following numbers of bird species were examined: 215 mute swans (*Cygnus olor*), 134 wild ducks (*Anas platyrhynchos*), 68 greylag geese (*Anser anser*), 32 white storks (*Ciconia ciconia*), 12 grey herons (*Ardea cinerea*), 11 seagulls (*Larus argentatus*), 10 rock pigeons (*Columba livia*), 10 western jackdaws (*Corvus monedula*), four common kestrels (*Falco tinnunculus*), three common magpies (*Pica pica*), three common buzzards (*Buteo buteo*), two brown owls (*Strix aluco*), two great spotted woodpeckers (*Dendrocopos major*), and two common swifts (*Apus apus*).

DNA isolation. The samples of internal organs were homogenised in phosphate-buffered saline (PBS) supplemented with 1% Antibiotic-Antimycotic (Gibco, U.K.), and then total cellular DNA was isolated using the commercial DNA Mini Kit (Qiagen, Germany) according to manufacturer's procedure. The obtained DNA was stored at -20°C for the next step of the study.

Primers. Oligonucleotide (primers) specific for GHPV structural protein encoding sequences (VP1) were designed using 'Primer 3' web-available software on the basis of complete sequence of strain Toulouse 2008 (Accession number: HQ681902.1) from the NCBI database of GenBank. The sequences of primers were as follows: 5' ACC CGT GCT TCC ATT CAC AA 3' (660-679) and 5' CTG CTC CCC AAA CCT GTC AA 3' (1038-1019).

Controls. The negative control was total cellular DNA isolated from non-infected goose embryo fibroblast cell culture (GEF). As a positive control, DNA extracted from 20/13 GHPV field strain from the collection of Department of Poultry Viral Diseases of the National Veterinary Research Institute in Pulawy was used.

PCR. PCR amplifications were carried out in basic gradient thermocycler (Biometra, Germany) in a total volume of 25 µL containing: 2.5 µL of PCR buffer (EurX, Poland), 0.5 µL of 25 mM magnesium chloride, 1 µL of 0.2 mM dNTP mixture, 0.5 µL of each primer, 0.5 µL of 2.5 U of DNA polymerase, 2 µL of DNA template, and 17.5 µL of deionised water. Amplification conditions were as follows: incubation at 95°C for 5 min, 35 cycles of denaturation at 94°C for 1 min, annealing at 62°C for 1 min, elongation at 72°C for 1 min, and a final extension at 72°C for 10 min.

PCR product analysis. PCR products were separated in 2% agarose gel with addition of GelRed (Biotum, USA) for 50 min at 120 V. After the electrophoresis, the size of amplification products was compared with the MassRuler Low Range DNA Ladder (Thermo-Scientific, USA). The results were visualised using transilluminator UV (Vilber-Lourmat, Germany) and photographed. The result was considered as positive when the received DNA product had a predicted size of 379 bp.

DNA sequencing. PCR products for VP1 gene were sequenced from forward and reversed primers on GS FLX/Titanium sequencer (Roche, USA) in a commercial company GENOMED (Poland). The sequences were assembled into single contigs in Bioedit ver. 7.0.9.0, and then compared with other sequences accessible in NCBI GenBank.

Results

The study was conducted on 508 wild birds belonging to 11 orders, 14 genera, and 14 species. Most of the tested birds belonged to *Anseriformes* (Table 1).

During the post-mortem examination, a significant enlargement of the liver and kidneys was observed in 30 birds. Furthermore, the kidneys were noticeably congested, with no pathological lesions in other organs of 10 birds. In the case of 10 birds, haemorrhages in the liver and intestines were observed, which suggested the possibility of infection with GHPV.

After gel electrophoresis, amplicons of 379 bp were observed in DNA samples extracted from different

internal organs from 22 (4.33%) birds. Positive samples were identified by PCR product sequencing as GHPV (data not shown). The product with the size of 379 bp was also confirmed in the positive control represented by the Polish 20/13 GHPV strain. The strain was isolated from a four-week-old goose showing haemorrhages in the intestines and kidneys. In the negative control, amplification product was not observed.

Table 1. Overview of wild birds examined and the results of the GHPV PCR test

Order	Genus	Species	Number of birds tested	Number of GHPV positive birds	Number of GHPV negative birds
<i>Anseriformes</i>	<i>Cygnus</i>	<i>Cygnus olor</i>	215	1	214
	<i>Anas</i>	<i>Anas platyrhynchos</i>	134	1	133
	<i>Anser</i>	<i>Anser anser</i>	68	0	68
<i>Ciconiiformes</i>	<i>Ciconia</i>	<i>Ciconia ciconia</i>	32	7	25
<i>Pelecaniformes</i>	<i>Ardea</i>	<i>Ardea cinirea</i>	12	1	11
<i>Charadriiformes</i>	<i>Larus</i>	<i>Larus argentatus</i>	11	3	8
<i>Columbiformes</i>	<i>Columba</i>	<i>Columba livia</i>	10	2	8
<i>Accipitriformes</i>	<i>Buteo</i>	<i>Buteo buteo</i>	3	1	2
<i>Strigiformes</i>	<i>Strix</i>	<i>Strix aluco</i>	2	0	2
<i>Falconiformes</i>	<i>Falco</i>	<i>Falco tinnunculus</i>	4	2	2
<i>Passeriformes</i>	<i>Corvus</i>	<i>Corvus monedula</i>	10	4	6
	<i>Pica</i>	<i>Pica pica</i>	3	0	3
<i>Piciformes</i>	<i>Dendrocopos</i>	<i>Dendrocopos major</i>	2	0	2
<i>Apodiformes</i>	<i>Apus</i>	<i>Apus apus</i>	2	0	2
Total number (%)			508	22 (4.33)	486 (95.66)

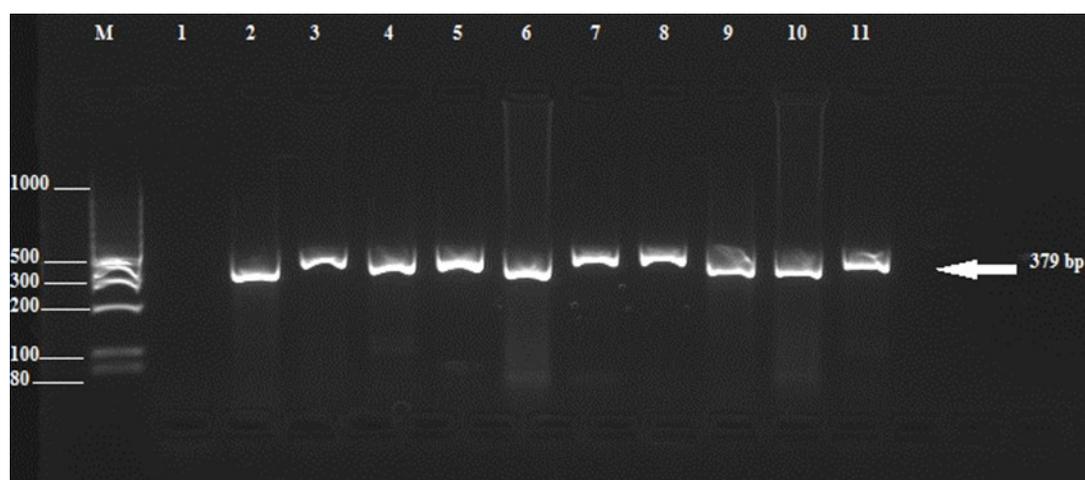


Fig. 1. Electrophoresis of PCR products in 2% agarose gel stained with GelRed™ solution for selected samples. Descriptions: M - MassRuler Low Range DNA Ladder, 1 - negative control (DNA extracted from non-infected GEFs), 2 - positive control (DNA of 20/13 GHPV strain), 3 - sample of the intestine from white stork-67/14j1, 4 - sample of the heart from white stork-67/14s1, 5 - sample of the intestine from seagull-57/15j4, 6 - sample of intestine from rock pigeon-57/15j7, 7 - sample of intestine from grey heron-57/15j9, 8 - sample of the liver from western jackdaw-61/15w7, 8 - sample of the intestine from common kestrel-61/15j2, 9 - sample of the liver from western jackdaw-61/15w4, 10 - sample of the gizzard from western jackdaw-61/15z1, 11 - sample of the gizzard from white stork-61/15z4

Table 2. Origin of GHPV positive samples

Species of GHPV positive birds	Number of GHPV positive internal organ samples				
	Intestine	Gizzard	Heart	Liver	Total
<i>Anas platyrhynchos</i>	1	-	-	-	1
<i>Ardea cinerea</i>	1	-	-	-	1
<i>Ciconia ciconia</i>	3	1	3	-	7
<i>Columba livia</i>	2	-	-	-	2
<i>Corvus monedula</i>	-	1	-	3	4
<i>Cygnus olor</i>	1	-	-	-	1
<i>Falco tinnunculus</i>	2	-	-	-	2
<i>Larus argentatus</i>	1	-	2	-	3
<i>Buteo buteo</i>	1	-	-	-	1
Total	12	2	5	3	22

The presence of GHPV genetic material was confirmed in three samples of the intestine, three samples of the heart, and one of the gizzard from seven white storks (*Ciconia ciconia*), three samples of the liver and one of the gizzard from four western jackdaws (*Corvus monedula*), two samples of the heart and one of the intestine from three seagulls (*Larus argentatus*), two samples of the intestine from common kestrels (*Falco tinnunculus*), two samples of the intestine from rock pigeons (*Columba livia*), and four samples of the intestines from wild ducks (*Anas platyrhynchos*), grey heron (*Ardea cinerea*), mute swan (*Cygnus olor*), and common buzzard (*Buteo buteo*) (Table 2). Birds with positive results belonged to different orders: *Anseriformes*, *Ciconiiformes*, *Pelecaniformes*, *Charadriiformes*, *Columbiformes*, *Falconiformes*, and *Passeriformes* (Table 1).

Discussion

There is strong evidence that APV occurs in wild birds on various continents (16). APV infection was demonstrated in several species of wild birds: buzzards, falcons, and red-faced lovebirds (9). Furthermore, high prevalence of APV antibody was found in free-ranging greater sulphur-crested cockatoos in Australia (16). It is likely that many other species of birds are susceptible to infection.

Despite the fact that previous cases of GHPV infections indicate a narrow host range, even among waterfowl species, Guerin *et al.* (7) reported the onset of investigations on GHPV infections in other species of birds.

Corrand *et al.* (4) confirmed that ducks may be asymptomatic carriers of GHPV, in which the virus is not able to induce lesions at the cellular level. These birds are considered as a potential source of viral infection for other poultry species (4). Molecular analyses of duck-GHPV strains isolated in 2008 showed their high similarity with the genome of goose

strains (17). It is suggested that GHPV strains circulating in duck and goose flocks belong to a common genotype. Furthermore, duck-GHPV isolates proved to be virulent for domestic geese as confirmed by experimental GHPV infection of 1-day-old goslings in which typical clinical signs of HNEG were observed. These symptoms did not occur in ducklings infected by a reference GHPV strain. The detection of antibodies in the sera of these birds confirmed GHPV infection, which in ducklings, in contrast to goslings, does not lead to the development of the disease. The results of the experimental study in ducklings revealed that it is unlikely that GHPV was a significant immunosuppressive agent in ducks (4).

The demonstrated presence of GHPV genome fragment in the tissues of white storks, western jackdaws, seagulls, common kestrels, rock pigeons, wild ducks, grey herons, mute swans, and common buzzards extends the range of birds susceptible to infection with this virus. Infections of wild birds with GHPV are of particular interest because of their role as reservoirs and vectors of the infectious agents in the epidemiology of diseases.

On the basis of these findings, we can assume that contact between wild birds and domestic geese may be a potential way of spreading the virus, so the risk of transmission of GHPV to farmed geese in Poland remains very serious. The presence of the genetic material of GHPV was demonstrated in the liver, intestine, and gizzard, which confirms tropism of the virus to endothelial cells that are present in all organs (5). The restricted cell type specificity of GHPV is reflected by the fact that attempts to propagate the GHPV in goose embryo fibroblasts (GEF) were unsuccessful in contrast to APV, which reached higher titres in cultures of chicken embryo fibroblasts (CEF) (10, 15).

The complete characterisation of GHPV strains isolated from wild birds, which consists of sequential analysis of VP1 GHPV gene and their comparison to those described so far in classical GHPV strains

circulating in duck and goose flocks will be explored in further studies. This will be crucial to the assessment of the role of wild birds as an epidemiological reservoir of GHPV for goslings.

Conflict of Interests Statement: The authors declare that there is no conflict of interests regarding the publication of this article.

Financial Disclosure Statement: The study was supported by Project KNOW No. K/02/0.1 “New molecular methods for diagnostic of viral diseases of poultry”.

Animal Rights Statement: None required.

References

- Bernáth S., Farsang A., Kovács A., Nagy E., Dobos-Kovács M.: Pathology of goose haemorrhagic polyomavirus infection in goose embryos. *Avian Pathol* 2006, 35, 49–52.
- Bernáth S., Szalai F.: Investigations for clearing the etiology of disease appeared among goslings in 1969. *Magyar Állatgyógyászati Lap* 1970, 25, 531–536.
- Bozeman L.H., Davis R.B., Gaudry D., Lukert P.D., Fletcher O.J., Dykstra M.J.: Characterization of a papovavirus isolated from fledgling budgerigars. *Avian Dis* 1981, 25, 1083–1092.
- Corrand L., Gelfi J., Albaric O., Etievant M., Pingret J.L., Guerin J.L.: Pathological and epidemiological significance of goose haemorrhagic polyomavirus infection in ducks. *Avian Pathol* 2011, 40, 355–360.
- Dobos-Kovács M., Horváth E., Farsang A., Nagy E., Kovács A., Szalai F., Bernáth S.: Haemorrhagic nephritis and enteritis of geese: pathomorphological investigations and proposed pathogenesis. *Acta Vet Hung* 2005, 53, 213–223.
- Gaweł A., Woźniakowski G., Samorek-Salamonowicz E., Kozdrun W., Bobrek K., Bobusia K., Nowak M.: Hemorrhagic nephritis and enteritis in a goose flock in Poland-disease course analysis and characterization of etiologic agent. *Avian Dis* 2014, 58, 8–22.
- Guérin J.-L.: Hemorrhagic nephritis enteritis of geese. In: *Disease of poultry*, edited by Swayne D.E., Blackwell Publishing Ltd., Ames, 2013, pp. 440–443.
- Guérin J.L., Gelfi J., Dubois L., Vuillaume A., Boucraut-Baralon C., Pingret J.L.: A novel polyomavirus (goose hemorrhagic polyomavirus) is the agent of hemorrhagic nephritis enteritis of geese. *J Virol* 2000, 74, 4523–4529.
- Johne R., Müller H.: Avian polyomavirus in wild birds: genome analysis of isolates from Falconiformes and Psittaciformes. *Arch Virol* 1998, 143, 1501–1512.
- Johne R., Müller H.: Polyomaviruses of birds: etiologic agents of inflammatory diseases in a tumor virus family *J Virol* 2007, 81, 11554–11559.
- Johne R., Müller H.: The genome of goose hemorrhagic polyomavirus, a new member of the proposed subgenus Avipolyomavirus. *Virology* 2003, 308, 291–302.
- Johne R., Wittig W., Fernández-de-Luaco D., Höfle U., Müller H.: Characterization of two novel polyomaviruses of birds by using multiply primed rolling-circle amplification of their genomes. *J Virol* 2007, 80, 3523–3531.
- Lacroux C., Andreoletti O., Payre B., Pingret J.L., Dissais A., Guerin J.L.: Pathology of spontaneous and experimental infections by goose haemorrhagic polyomavirus. *Avian Pathol* 2004, 33, 351–358.
- Leon O., Corrand L., Bich T.N., Le Minor O., Lemaire M., Guérin J.L.: Goose hemorrhagic polyomavirus detection in geese using real-time PCR assay. *Avian Dis* 2013, 57, 797–799.
- Palya V., Ivanics E., Glávits R., Dán A., Mató T., Zarka P.: Epizootic occurrence of haemorrhagic nephritis enteritis virus infection of geese. *Avian Pathol* 2004, 33, 244–250.
- Phalen D.N., Papillomaviruses and Polyomaviruses. In: *Infectious Diseases of Wild Birds*, edited by Nancy J.T., Hunter D.B., Atkinson C.T., Blackwell Publishing, Oxford, 2007, pp. 206–215.
- Pingret J.L., Boucraut-Baralon C., Guérin J.L.: Goose haemorrhagic polyomavirus infection in ducks. *Vet Rec* 2008, 162, 164.
- Schettler C.H.: Clinical picture and pathology of haemorrhagic and enteritis in geese. *Tierärztliche Prax* 1980, 8, 313–320.
- Schettler C.H.: De'tection en France de la néphrite hé'morragique et ente'rite de l'oie (NHEO). *Rec Med Vet* 1977, 153, 353–355.