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Case Report—

Persistent Goose Hemorrhagic Polyomavirus Infection on a Belgian Goose Farm

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SUMMARY. Goose hemorrhagic polyomavirus (GHPV) is the causative agent of hemorrhagic nephritis enteritis of geese (HNEG), one of the major diseases of domestic geese in Europe. This case report describes a persistent outbreak of a GHPV infection on a Belgian goose farm. Clinical symptoms, necropsy lesions, and histopathologic lesions observed were compatible with previous reports of HNEG outbreaks. PCR analysis confirmed the diagnosis of GHPV. To our knowledge, this is the first report of an outbreak of a GHPV infection on a Belgian goose farm. This is evidence that GHPV is not only present in countries known for extensive waterfowl production, but disease outbreaks also occur in countries with less extensive goose production.

RESUMEN. *Reporte de caso*- Infección persistente por poliomavirus hemorrágico del ganso en una granja de gansos en Bélgica. El poliomavirus hemorrágico de ganso (con las siglas en inglés GHPV) es el agente causal de la nefritis y enteritis hemorrágicas de los gansos (HNEG), una de las principales enfermedades de los gansos domésticos en Europa. Este reporte de caso describe un brote persistente de una infección por el poliomavirus hemorrágico de ganso en una granja de gansos en Bélgica. Los signos clínicos, las lesiones a la necropsia y las lesiones histopatológicas observadas fueron compatibles con reportes previos de brotes de la nefritis y enteritis hemorrágicas. El análisis de PCR confirmó el diagnóstico del poliomavirus hemorrágico de ganso. Hasta donde sabemos, este es el primer informe de un brote de una infección por poliomavirus hemorrágico de ganso en una granja de gansos belga. Esto es evidencia de que el poliomavirus hemorrágico de ganso no solo está presente en países conocidos por su producción extensiva de aves acuáticas, sino que también se producen brotes de enfermedades en países con una producción de gansos menos extensa.

Key words: goose hemorrhagic polyomavirus, PCR, avian viral pathogens, Belgium

Abbreviations: GHPV = Goose Hemorrhagic Polyomavirus; HNEG = Hemorrhagic Nephritis Enteritis of Geese

Goose hemorrhagic polyomavirus (GHPV) is the causative agent of hemorrhagic nephritis enteritis of geese (HNEG), one of the major diseases of domestic geese in Europe. This systemic disease affects young goslings from 4 to 10 wk old, frequently with a lethal outcome (6). Until now, cases of HNEG have only been described in Hungary, Germany, France, and Poland (1,2,6,10), countries in which waterfowl are extensively bred for meat and foie gras production. In Belgium, industrial waterfowl production is not common, but this case report evidences that also in this country GHPV is present. This is the first report of a persistent outbreak of HNEG in a Belgian goose farm.

MATERIALS AND METHODS

Case history. In the summer of 2014, high mortality was observed in young goslings on a Belgian goose and duck farm. Both waterfowl species are bred and reared for meat consumption, but a small amount of birds are also sold as backyard waterfowl. In total, approximately 2000 birds are present on the farm. Only young 8-wk-old goslings were affected, showing signs of leg weakness for a short period of time (24 hr) after which death occurred with a total morbidity and mortality rate of 40%. Clinical signs in adult geese or in ducks were not observed.

In the summer of 2015, a similar disease outbreak occurred on the same farm, showing a short period of leg weakness and leg paralysis in young gosling of a few weeks old, often resulting in death shortly after onset of the clinical signs.

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Necropsy. In August 2014, four dead 8-wk-old goslings were delivered to the Department of Pathology, Bacteriology and Avian Diseases, Faculty of Veterinary Medicine, Ghent University for postmortem examination. In July 2015, one dead 8-wk-old gosling was presented. Necropsy procedures were as follows: birds were weighed and after external inspection, carcasses were plucked, skinned, and the sternum removed. A macroscopic investigation of all tissues and internal organs was performed. For cytology, organs (lung, liver, kidney, and spleen) were blotted on microscope slides, stained using Hemacolor® (Merck; Darmstadt, Germany) and examined microscopically. In addition, microscopic examination of smears from the intestinal content and fresh unfixed kidney tissue was performed for parasitologic control. During necropsy, organs were collected for microbiologic and histopathologic examination, and PCR analysis as described below.

Microbiology. Samples of kidneys (both necropsies) and liver (second necropsy) were cultured using commercial growth media for bacteriologic and mycologic analysis.

Histopathology. Samples of kidneys and intestines (first necropsy), and of the kidneys and liver (second necropsy) were fixed for 48 hr in 10% buffered formalin and embedded in paraffin. Sections of the tissue were stained with hematoxylin and eosin and microscopically examined.

PCR analysis and sequencing. From both cases, viral DNA was extracted from kidney tissue by using a DNeasy Blood & Tissue Kit (Qiagen, Hilden, Germany). From the first case, only formalin fixed, paraffin-embedded kidney tissue was available. This tissue was pretreated with xylene according to the manufacturer's instructions for preliminary extraction of paraffin. PCR analysis was performed as previously described (5) for amplification of a 144-bp fragment of the VP1 region, a structural GHPV protein. PCR reaction products were analyzed with gel electrophoresis and subsequently sequenced (https://www.gatc-biotech.com). The obtained nucleotide sequence was then

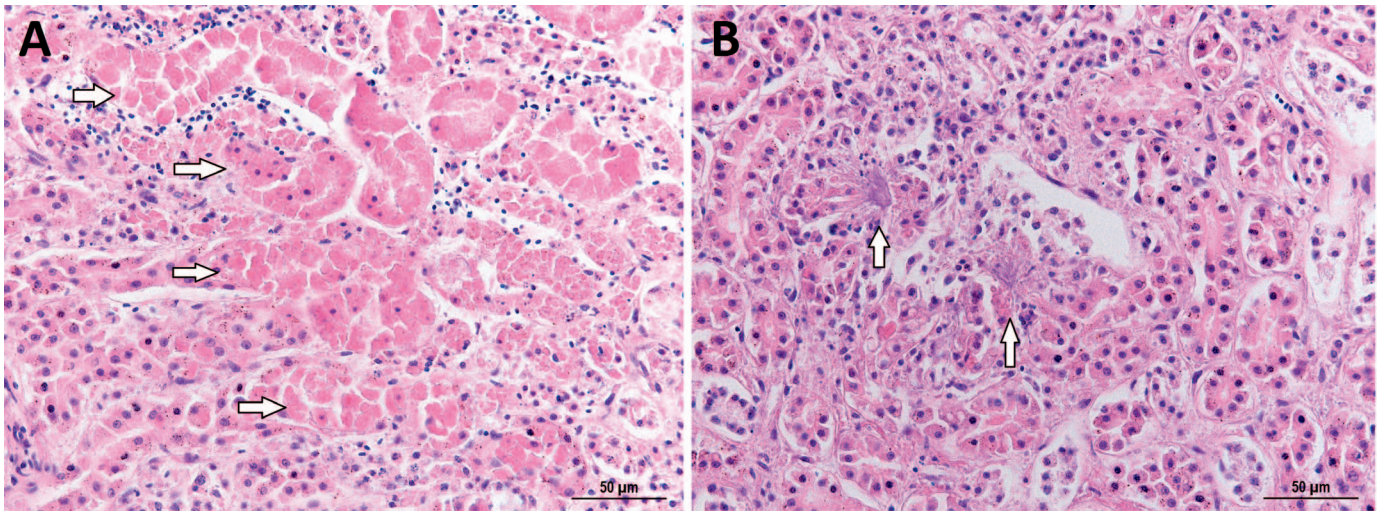

Fig. 1. Histology kidney (hematoxylin and eosin, 400×). (A) Multifocal necrosis of the kidney tubular epithelia (arrow) and (B) multiple intratubular accumulations of necrotic debris containing basophilic, radiating crystalline material (urate tophi) (arrow) were observed.

compared to other GenBank sequences by using the BLAST database (http://www.ncbi.nlm.nih.gov/BLAST/).

RESULTS

Necropsy. In August 2014, all four goslings showed poor body condition and severe swollen, urate infiltrated kidneys. In addition, three birds showed urate deposits on visceral organs (pericardium, liver, airsacs) and in the articulations (tibiotarsal, tibiofemoral, and shoulder). These lesions are compatible with visceral gout. Further, in three birds, the lining of the ventriculus was detached, with small ulcerations in the koilin layer and blood loss, lesions suggestive for an *Amidostomum anseris* infection. Nematodes in the proventriculus or ventriculus were not observed. Cytology of organs revealed the image of regenerative anemia in two birds. Infiltration of lymphocytes in the kidneys was observed in two birds. Fresh, unfixed smears from kidney tissue were examined for evidence of renal cocciodiosis, but proved negative. Smears from gut contents were positive for *Capillaria* sp. and *Ascaridia* sp. in two birds.

In July 2015, distinct macroscopic lesions could not be observed in the dead gosling. Cytology of internal organs was compromised because of postmortal decay. Fresh, unfixed smears from kidney tissue and gut contents proved negative for coccidiosis and verminosis.

Microbiology. Bacterial or fungal growth was not observed after microbiologic culture of the kidneys and liver, excluding bacterial or fungal origin of the kidney lesions (first necropsy) or origin of death (second necropsy).

Histopathology. In 2014, histopathologic examination of the kidneys revealed tubular necrosis, as well as multiple intratubular accumulations of necrotic debris containing basophilic, radiating crystalline material (urate tophi). Occasional calcification of the urate tophi was observed. In the gut, mild infiltration of lymphocytes and heterophils were observed in the lamina propria. In 2015, histopathologic examination of the kidneys revealed multifocal tubular necrosis (Fig. 1). In the liver, increased numbers of leukocytes within the hepatic sinusoids were observed.

PCR analysis and sequencing. PCR analysis and gel electrophoresis revealed positive product bands of the corresponding size (5) in both cases. Results of the nucleotide BLAST revealed high similarity with the GHPV genomic sequences of the major capsid protein VP1 present in BLAST Database (97%–98% similarity).

DISCUSSION

To our knowledge, this is the first report of an outbreak of a GHPV infection on a Belgian goose farm. During the first outbreak on the farm, in August 2014, clinical signs (a short period of lameness in 8-wk-old goslings, soon followed by death), macroscopic lesions (swollen kidneys, visceral, and joint gout) and histologic kidney lesions (epithelial tubular necrosis) were all typical for a chronic feature of GHPV infection (5,8,9). One year later, during the second outbreak, clinical signs were again suggestive for a GHPV infection, but macroscopic lesions were less distinctive. Nevertheless, histologic kidney lesions were consistent with and results of the PCR analysis were proof of the recurrence of the GHPV infection.

GHPV has a tropism for endothelial cells, resulting in vascular dysfunctions (9) and thereby inducing characteristic lesions such as renal tubular necrosis and necrosis of the intestinal epithelium. In this case, the latter lesion could not be observed, but, although mentioned in the disease's name, enteritis is less frequently present than the kidney lesions (4). Besides endothelial cell tropism, the polyomavirus targets lymphoid cells (7), which leads to immunosuppression. This might explain the severe gastrointestinal parasitosis in the goslings during the first outbreak in 2014.

In geese that survive the GHPV infection, the virus can become persistent (10). Because the GHPV infection recurred on the farm, there's a high probability that carrier animals are present on the farm. The breeding couples might spread the virus to their offspring, but also the ducks present on the farm might act as reservoirs of the virus, because ducks are regarded as refractory for GHPV infection (4). Because of this, first introduction of the virus on the farm might come from these ducks. Another possibility is that the virus might have been introduced on the farm by droppings of wild waterfowl, because the goose are kept outrange.

GHPV spreads from carriers and clinically affected birds by fecal route (5,8). Therefore, cleaning and disinfection procedures might

prevent or help to interrupt the disease outbreak. In addition, reduction of stress can prevent non-clinically infected birds from developing HNEG. However, management procedures are unlikely to be sufficient in controlling GHPV infections. Vaccination of breeding couples before the laying period and growing goslings could provide a preventive solution (3). Unfortunately, a commercial vaccine is not yet available.

CONCLUSION

To our knowledge, this is the first report of an outbreak of a GHPV infection on a Belgian goose farm. This evidences that GHPV is not only present in countries known for extensive waterfowl production but disease outbreaks also occur in countries with less extensive goose production. Because there is a lack of vaccines and polyomavirus infection can persist in carrier animals, outbreaks might become a recurrent problem on affected farms.

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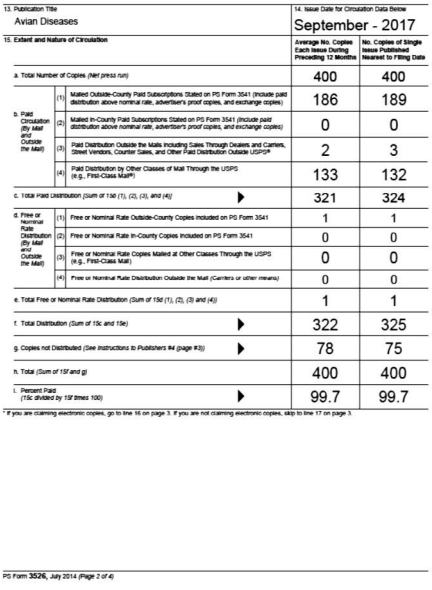

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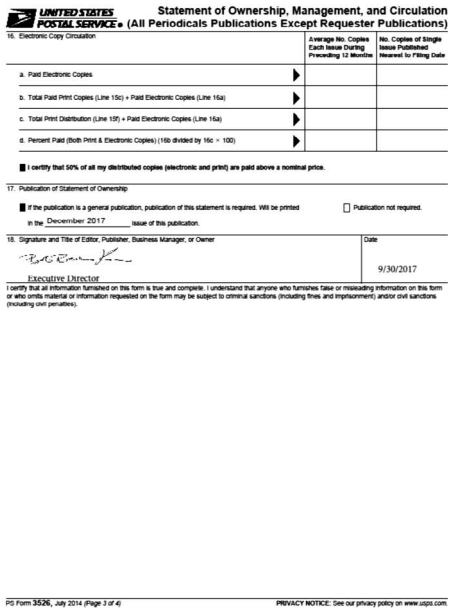

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