VERSITA

Bull Vet Inst Pulawy 57, 301-304, 2013
DOI: 10.2478/bvip-2013-0052

New herpesvirus isolated from geese in Poland

Wojciech Kozdruń, Grzegorz Woźniakowski, Hanna Czekaj, Wojciech Kozaczyński[1], Elżbieta Samorek–Salamonowicz

Department of Poultry Viral Diseases,
[1]Department of Pathology,
National Veterinary Research Institute, 24-100 Pulawy, Poland
wkozdrun@piwet.pulawy.pl

Received: April 30, 2013 *Accepted: August 12, 2013*

Abstract

Neoplastic changes characteristic of Marek's disease (MD) in the geese flock were described. The investigations were performed on White Italian reproductive geese kept on a farm where MD was previously diagnosed in broilers. Neither antibodies against MD virus (MDV) were detected by AGID method, nor MDV antigen was found by RID. The histopathological examination revealed the presence of lymphoid infiltrations characteristic of MD in all examined tissues. No lesions typical for avian leukosis or reticuloendotheliosis were observed. PCR products characteristic of meq, and ICP4 and pp38 genes were not observed, but real-time PCR for gB gene of MDV were positive in DNA samples from visceral organs. The real-time PCR results may indicate the presence of a new MDV variant or a new herpesviral infection among geese.

Key words: geese, herpesvirus, glycoprotein B, real – time PCR, Poland.

Introduction

Naturally occurring neoplasms of domestic birds are associated with infections of either Marek's disease virus (MDV) or avian C-type retroviruses belonging to the avian leukosis group (ALV), or to the reticuloenotheliosis group (REV) viruses.

Marek's disease (MD) is a lymphoproliferative and neuropathic disease of poultry caused by a highly contagious, cell – associated oncogenic virus classified into the family *Herpesviridae*. The infection with MDV is thought to be transmitted by the respiratory route, by the inhalation of infected dust in poultry houses. MDV is a lymphotropic virus targeting B lymphocytes, which are one of the principal cells of the immune system (4).

In general, there are different clinical forms of the disease, which have been recognised in flocks infected with MDV, including classical and neurological form, acute form, transient paralysis, and acute mortality syndrome.

Up till now, MD was described in chickens, turkeys, and Japanese quails (1, 2). The disease has been occasionally recognised in other species of poultry, especially in waterfowl.

The paper describes the case of neoplastic disease in geese flock. The presented data suggests that the etiological agent of the disease may be related to MDV.

Material and Methods

Geese. The study was performed on White Italian reproductive geese suspected of Marek's disease. The geese were kept in the farm, in which MD was previously diagnosed in broilers. In the flock, about 1200 birds in the third laying season were kept. The symptoms of paralysis, especially legs and wings were observed, as well as an increase in the mortality up to 20 birds per week. The serum, feathers of follicles, and the spleen and liver were collected from the affected geese.

AGID and RID. The agar gel immunodiffusion test (AGID) for the detection of MDV antibodies and radial immunodiffusion test (RID) for the detection of viral antigen in feather follicles were used (6, 9).

Isolation of DNA. The extraction of a total cellular DNA from the visceral organs and feather follicles was performed with a QIamp DNA Extraction Kit (Qiagen, Germany), according to the instruction of the producer.

Standard PCR for *meq* gene. For the amplification reaction, the following primers complementary to oncoprotein sequence *meq* of MD genome were used: meq: 5' GCA CTC TAG AGTGT AAA GAG ATGTCT CAG 3', meq2: 5' TAA CTC GAG GAG AAG AAA CAT GGG GCA TAG 3' (1,2). The DNA of Rispens CVI 988 vaccine strain and DNA

of virulent $HPRS_{16}$ strain of MDV were used as positive controls. Negative control was DNA isolated from non-infected chicken embryo fibroblasts (CEF).

PCR was done in BIOMETRA SYSTEM in a final volume of 50 µL of reaction mixture containing: 5 µL of PCR buffer (500 mM KCl, 100 µM Tris – HCl, 15 µM Triton X – 100); 2 µL of total DNA (1 µg/mL); 2 µL of each primer; 2 µL of dNTP (0.2 µM); 2 µL of $MgCl_2$ (5 mM); 1 µL of thermostable polymerase DNA, and 34 µL of sterile water. The following conditions were applied: 35 cycles, initial denaturation at 94°C - 2 min, denaturation at 94°C – 1 min, annealing at 60.2°C – 1 min, elongation at 72°C – 1 min, final elongation at 72°C – 10 min. The amplification products were detected by agarose gel electrophoresis in 2% agarose gel. The gel was stained with ethidium bromide, and photographed under ultraviolet light.

Standard PCR for ICP4 and pp38 genes. For amplification of the ICP4 and pp38 fragments, the following primers complementary to the destination sequences were used: ICP4 F: 5:' GCA GCA AGG AGG AAT GAT AA 3'; ICP4 R: 5' CAT CTG AGG CAT TTC ACA CA 3'; pp38 F: 5' CCC CAT CTG CTT CAT ACC CAT 3'; pp38 R: 5' GTG ATG GGA AGG CGA TAG AA 3'. The primers were designed using 'Primer 3' web-available software on the basis of genome sequence of MD5 strain, accession number: AF243438 and CVI988, accession number: DQ530348. The specificity of the used primers was tested using the BLAST search in NCBI Gene Bank database. The DNA of Rispens CVI 988 vaccine strain and DNA of virulent $HPRS_{16}$ strain of MDV were used as positive controls. Negative control was DNA isolated from non-infected chicken embryo fibroblasts (CEF). The thermal conditions were identical to the thermal protocol for the amplification reaction of *meq* gene.

Electrophoresis. PCR products were separated in 2 % agarose gel in TBE buffer (10.8 g of TRIS Base; 5.5 g of boric acid; 4 mL of 0.5 M EDTA set at pH 8.0) for 1 h under 120 V. Ten microlitres of reaction mixture and 2 µL of loading buffer 6 x DNA Loading Dye (Fermentas) were loaded into each well. The lenght of the obtained products was determined on the basis of DNA length fragment marker: GeneRuler™ 100 bp DNA Ladder (Fermentas). After electrophoretic separation, the gel was stained in ethidium bromide (1 µg/mL) for 15 min and then analysed under UV light transiluminator (Vilber-Lourmat).

Real – time PCR. Oligonucleotide primers specific for gB encoding region were designed using 'Primer 3' web-available software on the basis of gB encoding region of MDV complete genome of MD5 strain, accession number: AF243438 from NCBI GeneBank. The primer sequences were: gB F: AGT TGT TTC GAG CGT CCA GT, gB R: TAT ATG TCG TCC CCG TCC AT. The fluorescence probe was labelled on the 5' end with FAM (6-carbo-xyfluorescein) reporter dye and TAMRA (5-carbo-xytetra methylrhodamine) as a quencher dye on the 3' end. The gB probe sequence was: 5'-CTA GAA CCG CCG CGA AAA ATG-3' and was also designed on the basis on MD5 complete genome sequence. The melting temperature of the fluorescence probe was calculated on 5°C below the primers melting temperature. The used positive controls were DNA extracted from CVI988 Rispens vaccine strain (Merial) and DNA of MDV-1 reference strain $HPRS_{16}$ obtained from Dr A.M. Fadly (ADOL, USA). Negative control was represented by total DNA extracted from homogenates of 11-day-old SPF chicken embryos. Real-time PCR was performed on Applied Biosystems 7500 machine with installed ABI collection and analysis software. The real-time PCR mixture volume was 25 µL and contained: 12.5 µL of 2x concentrated QuantiTect Probe PCR Master Mix; 0.5 µM of gB F primer; 0.5 µM of gB R primer; 0.1 µM of gB specific probe; 2 µL of DNA sample, and 8 µL of RNase free water. The following thermal conditions were used: 50°C for 2 min (elimination of dUMPs by uracil-N-glycosylase), 95°C for 15 min (initial denaturation), then 40 cycles at 94°C for 1 min (exact denaturation) and at 60°C for 1 min (primers annealing and prolongation of the chain). The data was collected during the last thermal step.

Histological examinations. The sections of the liver, spleen, kidneys, proventriculus, and sciatic nerves were fixed in buffered 10% formalin and paraffin sections stained with haematoxylin and eosin were prepared.

Results

During the necropsy, white and gray stained tumours were found in the liver, pancreas, kidneys, and proventriculus, which protruded out from the visceral organs. The sciatic nerves were enlarged and yellow. The clinical symptoms, as well as pathological changes suggested the MD.

Neither MDV antibodies in sera nor MDV antigen in feather follicles were found. The PCR products characteristic for *meq* MDV, and for ICP4 gene and pp38 gene were not observed in the DNA samples isolated from the visceral organs and feather follicles used in the amplification reaction.

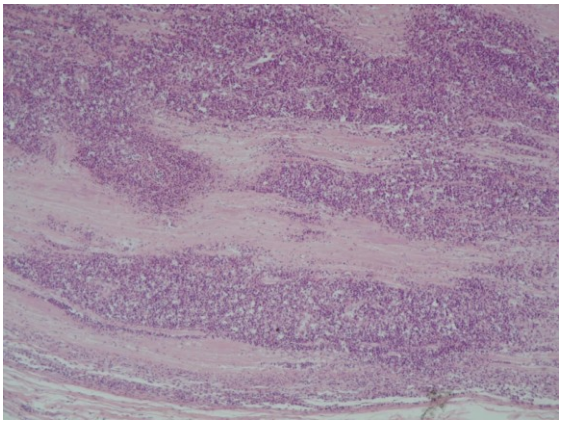

Fig. 1. Lymphoid cell infiltration in peripheral nerves (H&E, Magnification 100x)

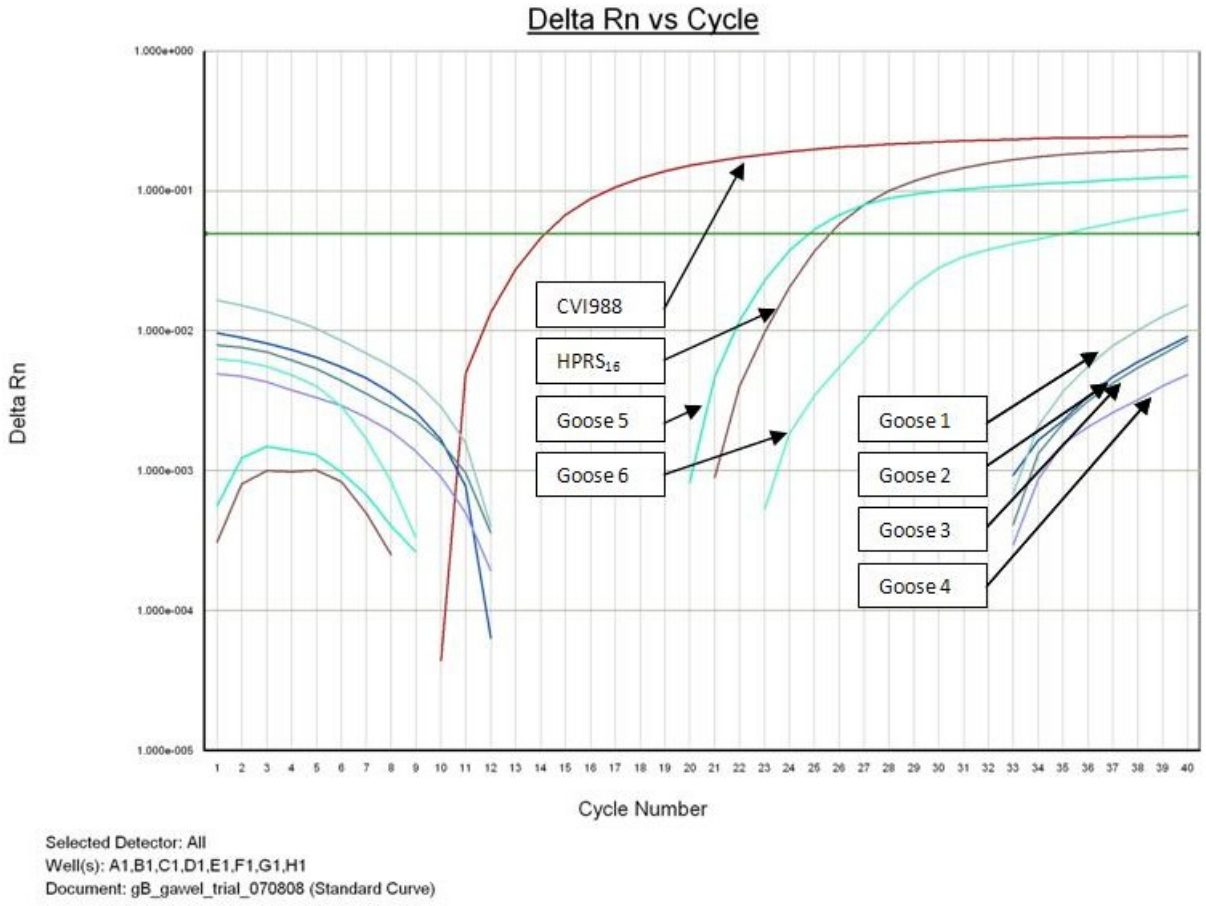

Fig. 2. Fluorescence curves for gB encoding region for DNA samples extracted from spleen of infected geese

However, the histopathological examinations revealed the presence of lymphoid infiltrations characteristic for MD in all of the examined tissues. No lesions typical for avian leukosis or reticuloendotheliosis were observed (Fig. 1).

Because the standard PCR used for detection of *meq* gene, ICP4 gene, and pp38 gene of MDV provided negative results, the real-time PCR for gB gene was used. During the reaction, the positive signal specific for gB encoding region was detected in case of CVI 988 and $HPRS_{16}$. For negative control (NTC), no significant signal was detected. For the two samples collected from infected geese, the C_t values ranged from 21.7 to 37.9, whereas four other geese DNA samples were negative. After gel electrophoresis, the presence of one band about 242 bp long, which was specific for MDV was confirmed for positive controls, as well as the DNA sample with the highest C_t value for one goose. The negative curves for four others samples were slightly below the threshold line (Fig. 2).

Discussion

The first case of MD among wild geese in Japan and Eastern Russia was described by Murrata *et al*. (7). Using the nested-PCR method, the authors have confirmed MDV in feather follicles in 30% of the examined birds. The further studies confirmed the high homology between virus strain isolated from geese and MDV strains isolated from chickens. The authors suggest that wild waterfowl can be a reservoir of MDV for the domestic poultry (8).

In 1988, Dren *et al*. (3) isolated a reticuloendotheliosis-like virus from naturally occurring lymphoreticular tumours of domestic geese. Diffuse and/or nodular lymphoma-like tumours occurred in the spleen, liver, pancreas, intestines, and rarely in other internal organs. In these geese proliferative lesions consisting of large, slightly different lymphoblast-type cells were seen in the visceral organs.

In 2005, Kozaczyński *et al*. (5) describes the case of well-differentiated cholangiocarcinoma in a 2-year old goose. Gross pathological examinations revealed the presence of large yellow-grey nodules scattered throught all the liver lobes. Histopathology examinations exposed a neoplasma composed of structures resembling bile ducts and irregularry arranged cells in the form of nests.

Despite the negative results in AGID and RID, it seems that MD occurred in the examined geese. Additionally, detection of gB gene by real-time PCR

indicates the presence of a new MDV variant, or a new herpesviral infection among geese (Gene Bank No. HQ645961).

References

1. Chang K.S., Lee S.I., Ohashi K., Ibrahim A., Onuma M.: The detection of the *meq* gene in chickens infected with Marek's disease virus serotype 1. J Vet Med Sci 2002, **64**, 413–417.
2. Davidson J., Smith E.J., Perl S., Malkinson M.: Application of the polymerase chain reaction for the diagnosis of natural infection of chickens and turkeys with avian oncogenic viruses. Isr J Vet Med 1995, **50**, 97–103.
3. Dren C.N., Nemeth I., Rath F., Glavits R., Somogyl P.: Isolation of a reticuloendotheliosis – like virus from naturally occurring lymphoreticular tumours of domestic goose. Avian Pathol 1988, **17**, 259–277.
4. Jarosiński K.W., Tischer B.K, Trapp S., Osterrieder K.: Marek's disease virus: lytic replication, oncogenesis and control. Expert Rev Vaccines 2006, **5**, 761–772.
5. Kozaczyński W., Kozdruń W., Reichert M., Samorek-Salamonowicz E., Jasik A., Koźluk A.: A case of cholangiocarcinoma in goose. Med Weter 2005, **61**, 715-717.
6. Marquardt W.M.: Avian complement-fixation. Avian Dis 1973, **17**, 236-250.
7. Murata S., Chang K.S., Yamamoto Y., Okada T., Lee S.I., Konnai S.: Detection of the virulent Marek's disease virus genome from feather tips of wild geese in Japan and the Far East region of Russia. Arch Virol 2007, **152**, 1523–1526.
8. Murata S., Hayashi Y., Kato A., Isezaki M., Takasaki S., Onuca M., Osa Y., Asakawa M., Konnai S., Ohashi K.: Surveillance of Marek's disease virus in migratory and sedentary birds in Hokkaido, Japan. J Vet 2012, **192**, 538–540.
9. Yates V.J., Rhee Y.O., Fry D.E., El-Mishad A.M., Milornick K.J.: The presence of avian adenoviruses and adeno-associated viruses in health chickens. Avian Dis 1976, **20**, 146-152.