

Occurrence of Marek's disease in Poland on the basis of diagnostic examination in 2015–2018

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Abstract

Introduction: Marek's disease (MD) is a tumourous disease caused by Marek's disease virus (MDV) and most commonly described in poultry. The aim of the study was to determine the occurrence of Marek's disease virus infections in Poland and analyse clinical cases in the years 2015–2018. **Material and Methods:** The birds for diagnostic examination originated from 71 poultry flocks of various types of production. Birds were subjected to anatomopathological examination post mortem, during which liver and spleen sections and other pathologically changed internal organs were taken. These sections were homogenised with generally accepted methods, then total DNA was isolated and amplified with a real-time PCR. A pair of primers complementary to the MDV genome region encoding the *meq* gene were used. **Results:** MDV infection was found predominantly in broiler chicken flocks (69.01%), and also in layer breeder (9.85%) and commercial layer flocks (7.04% each). **Conclusion:** The results of research conducted in the years 2015–2018 clearly indicate that the problem of MDV infections is still current.

Keywords: poultry, Marek's disease, real-time PCR.

Introduction

Marek's disease is a disease of poultry whose aetiological agent is a herpesvirus called Marek's disease virus (MDV) belonging to the *Herpesviridae* family. This disease is most commonly described in poultry (1, 10).

There are three serotypes of Marek's disease virus. Serotype 1 includes pathogenic strains and the Rispens CVI988 vaccine strain, serotype 2 includes apathogenic strains isolated from chickens, and serotype 3 includes the herpesvirus HVT FC 126 isolated from turkeys. In serotype 1 there are 4 pathotypes with mild (MDV), virulent (vMDV), very virulent (vvMDV), and very virulent plus (vv+MDV) pathogenicities (10, 13, 14).

Preventive vaccinations are carried out in day-old chicks. The *in ovo* vaccination method can also be used, however, due to prohibitively high costs, this method has not found wide application. This vaccination does not protect birds against infection with the virulent field strain of MDV, but only against the occurrence of

clinical symptoms and anatomopathological changes in the internal organs (5, 15, 16).

Marek's disease clinically presents several forms, from hyperacute, through visceral, and classical to nervous. Very often there are none of the clinical symptoms typical for this disease. Only weight loss and an increase in the number of deaths among birds are observed. In the internal organs of infected birds, mainly enlargement of the liver and spleen is noted with the presence of necrotic foci in these organs (2, 3). Virological methods (isolation in cell cultures or chicken embryos) and methods based on molecular biology principally using amplification reactions (PCR) together with their variants, e.g. real-time PCR, are used for routine diagnosis of MDV infection (7, 11, 17, 18).

To avoid the possibility of infecting birds with the virulent field strain of MDV, it is recommended to follow the biosecurity rules strictly in the first three weeks of life after their introduction onto the farm (16, 17).

Based on our own research, in recent years an increase in the pathogenicity of isolated MDV strains has been

observed (unpublished data). Therefore, the aim of the study was to determine the occurrence of MDV infections in Poland in the years 2015–2018 using molecular methods and the analysis of clinical cases.

Material and Methods

Birds. The research material consisted of birds from 71 flocks of poultry suspected of being infected with MDV, sent to the Polish National Veterinary Research Institute for diagnosis: 53 flocks of broiler chickens, 10 flocks of layer breeder chickens, 6 flocks of commercial layer chickens, 1 flock of broiler turkeys and 1 flock of broiler geese. In these flocks, antibiotic administration did not impart any therapeutic effects. Therefore, MD was suspected on the basis of clinical symptoms and pathological changes. These birds were decapitated according to the official procedure, and during the necropsy the occurrence of anatomopathological changes was assessed, and liver and spleen sections and sections of the other infected internal organs were taken. The birds in 43 flocks of broiler chickens, and in all layer breeder and layer commercial flocks were vaccinated against Marek's disease on the first day of life. The flocks of turkey and geese were not vaccinated against MD. Only a molecular biological method was included in the laboratory diagnosis, and no histopathological examination was performed.

Total DNA isolation. The sections of visceral organs were homogenised under a generally accepted procedure. Isolation was performed using the QIAmp Viral DNA Mini Kit commercial kit (Qiagen, Germany). The isolated DNA was stored for further examination at a temperature below -20°C .

Primers for real-time PCR. A pair of primers specific to the MDV genome region encoding the *meq* gene were used with the sequences of 5'-GGA AAC CAC CAG ACC GTA GA-3' for the forward MEQ F primer and 5'-ACG CTC AGC TTT GTC CTG TT-3' for the reverse MEQ R primer. The starters were synthesised at Genomed in Warsaw, Poland.

Control DNA. The DNA from the G48 reference strain of MDV was used as the positive control, and total DNA isolated from uninfected SPF chicken embryo fibroblast (Specific Pathogen Free, Lohmman, Germany) cell culture was used as the negative control.

Real-time PCR. The reaction was carried out in a thermal cycler (Biometra, Germany), the volume of the reaction mixture was 25 μL , and the composition was: 13.5 μL of QuantiTect SYBR Green (Qiagen, Germany); 0.5 μL of MEQ F primer; 0.5 μL of MEQ R primer; 8.5 μL of RNase-Free Water (Qiagen, Germany), and 2.0 μL of total DNA from visceral organs. The reaction was performed in 40 cycles and the temperature and duration parameters were as follows: the holding stage was at 50°C for 2 min and 95°C for 10 min, the cycling stage was at 95°C for 15 s and 60°C

for 1 min, and the melting curve stage was at 95°C for 15 s, 60°C for 1 min, 95°C for 30 s, and 60°C for 15 s.

Results

In the clinical examinations of birds during the years 2015–2018, growth inhibition (5.49%) and paralysis (4.44%) were observed. In necropsies, anatomopathological changes were most often found in the form of hepatomegaly (76.25%) with necrotic foci (60.1%), and splenomegaly (57.54%) with necrotic foci (34.7%). Pathological changes in other internal organs were rarely reported.

Based on the results of real-time PCR in 2015, infection with the virulent field strain of MDV was detected in only three flocks. The affected birds were broiler chickens of the Ross 308 line at the age of 5–6 weeks located in the Łódź (two flocks) and Mazovian (one flock) regions. No anatomopathological changes in internal organs were observed in these flocks, apart from subnormal body weight.

In 2016, no MDV genetic material was found in flocks of broiler chickens, and in only two flocks of layer breeders. HyLine line infected chickens at 29 and 34 weeks of age were located in the Lower Silesian and the Kuyavian-Pomeranian regions. Necropsy in one flock at 29 weeks of age revealed significant liver and spleen enlargement and necrotic foci.

The presence of genetic material of the virulent field strain of MDV was detected in the next year in a total of 36 flocks, including 28 flocks of broiler chickens of the Ross 308 (15 flocks) and Cobb500 (13 flocks) lines aged 2–6 weeks, 3 flocks of layer breeders of the Ross 308 (2 flocks) and HyLine (1 flock) lines aged 32–40 weeks, and 3 flocks of commercial layer chickens of the Lohmann line at 29–30 weeks of age. In addition, the presence of MDV was confirmed in 1 flock of Big 6 broiler turkeys at 18 weeks of age and in 1 flock of broiler geese at 7 weeks of age. These flocks with positive results in real-time PCR were located in the Mazovian (18 flocks), Lublin (12 flocks), Łódź (1 flock), Świętokrzyskie (2 flocks), Podlasie (4 flocks) and Podkarpackie (1 flock) regions. The flock of broiler turkeys was located in the Kuyavian-Pomeranian region, and the flock of broiler geese in the Greater Poland region. In both broiler chicken flock types, layer breeders, and commercial layers, a very small percentage of birds evidenced growth retardation, paralysis, and anatomopathological changes in the form of liver and spleen enlargement with multiple necrotic foci. In the flock of broiler turkeys, only growth inhibition was noticed, but in the broiler geese flock the presence of tumorous lesions in the liver, spleen and intestines were observed. The results of real-time PCR confirmed the infection in broiler geese, which were kept in a building where the Marek's disease infection was noted in the past in layer breeder chickens.

In 2018, MDV infection was confirmed in 22 flocks: 18 broiler chicken flocks of the Ross 308 (9 flocks) and Cobb500 (9 flocks) lines aged 5–6 weeks, 2 flocks of layer breeders of the Ross 308 line aged 29 and 39 weeks and in 2 flocks of commercial layers of the Lohmann line aged 36 and 42 weeks. These flocks came from the Mazovian (11 flocks), Lublin (3 flocks), Świętokrzyskie (2 flocks), Podlasie (3 flocks), Podkarpackie (2 flocks) and Greater Poland regions (4 flocks). In broiler chickens from these flocks, subnormal growth was noted, while in flocks of layer breeders anatomopathological changes in the liver and spleen were observed.

In summary, out of all the flocks examined, MDV genetic material was most often found in broiler chickens (49 flocks; 69.01%), and then in descending order in layer breeders (7 flocks; 9.85%), in commercial layers (5 flocks; 7.04%) and broilers turkeys (1 flock; 1.40%) and broiler geese (1 flock; 1.40%). The results are shown in Table 1 and Fig. 1.

The *meq* gene is very often used to determine the pathogenicity of MDV of serotype 1 strains (8, 9), and was therefore used in this study. All the results obtained with the use of primers for the *meq* gene proved that the MDV strains detected in Poland belong to serotype 1 and pathotype 4, i.e. the so-called vv+MDV strains.

Table 1. Positive results of real-time PCR during the years 2015–2018

| Year | Type of production | | | | | Total |
|-------|--------------------|----------------|-------------------|-----------------|---------------|-------|
| | Broiler chickens | Layer breeders | Commercial layers | Broiler turkeys | Broiler geese | |
| 2015 | 3 | - | - | - | - | 3 |
| 2016 | - | 2 | - | - | - | 2 |
| 2017 | 28 | 3 | 3 | 1 | 1 | 36 |
| 2018 | 18 | 2 | 2 | - | - | 22 |
| Total | 49 | 7 | 5 | 1 | 1 | 63 |

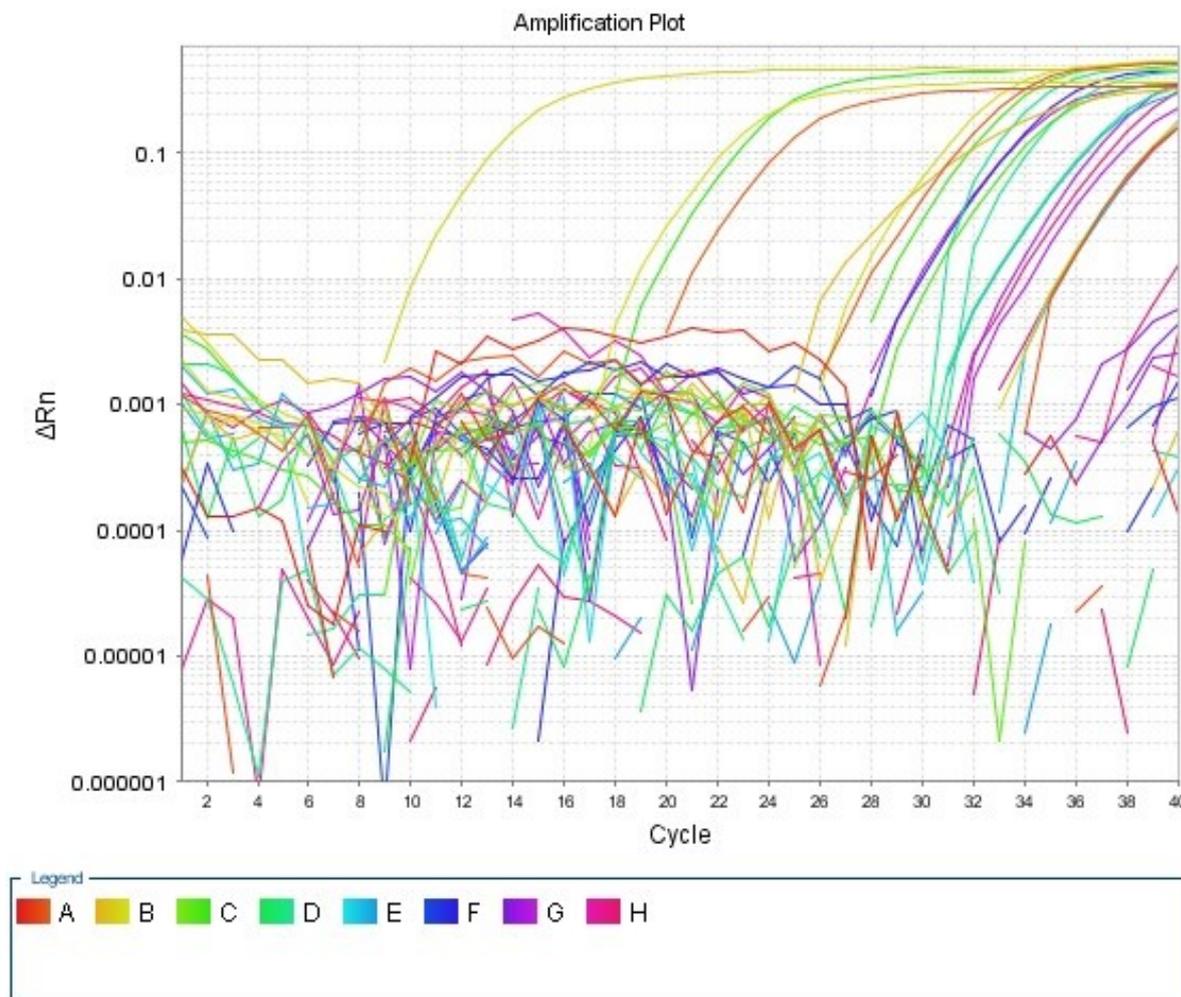


Fig. 1. The positive results of real-time PCR for detection of MDV in field samples

Discussion

Marek's disease is one of the most serious epizootic threats to large scale poultry production. The reasons for the losses it can inflict are the inhibition of birds' growth, the induction of anatomopathological changes in the internal organs and consequential meat condemnation in slaughterhouses, and an increased number of dead birds (6, 14, 15).

For many years, an increase in the pathogenicity of MDV strains has been observed despite immunoprophylaxis being used in day-old chicks. Such an increase was observed as early as 1997 in the vaccinated flocks of birds (3, 4).

As per the results of the presented research, an upward trend in the number of flocks of MDV-infected poultry has been visible since 2015. According to preliminary analyses, an upward trend has been continuing in the in the years since the end of the investigated period (unpublished data).

Similar tests were carried out in Turkey in layer breeder flocks. The study birds came from three regions of the country and were necropsied in 2017 and 2018. The study used the same research method and primers for the *meq* gene as the present research. In the birds studied, tumorous lesions were also most frequently seen in the liver and spleen in the form of significant enlargement of these organs and the presence of necrotic foci. MDV genetic material was detected in 120 out of 602 samples tested, which constituted 19.93%. The analysis confirmed that the Turkish strains belong to the pathotype of vv+MDV strains and have a high degree of similarity to strains isolated in other countries, e.g. in Italy and Hungary (6, 19).

In the USA, material for analogous research came from 104 poultry farms of various types of production located in the state of Pennsylvania. MDV genetic material was detected in birds from 36 farms (34.61%). The percentage of the infected birds on farms ranged from 12% to 63.4% (1). Also these birds' clinical symptoms and anatomopathological changes were similar to those observed in the birds in the present study.

Investigations in Brazil also confirmed that MDV infection is a significant problem. In addition, double infection with MDV and reticuloendotheliosis virus was diagnosed in samples from a flock from the vicinity of San Paulo (4). Birds from this flock had not been vaccinated against Marek's disease. Apathy and a decrease in appetite as well as a significant fall in body weight were observed in the sick birds. Sections gave a clear indication of significant enlargement of the internal organs and the presence of tumours, i.e. as observed in the authors' research.

Similar cases came to light in studies performed in India. In the period from January to March 2015, examinations were carried out on two farms in the province of Ri Bhoi. On the first farm, bird mortality was recorded at 5.5% over a three-day period, while on the

second, mortality was much higher and amounted to approximately 34%. It was presumed that the reason for such a high percentage of dead birds was secondary bacterial infections. Laboratory diagnosis was pursued with primers specific to the MDV genome region encoding for the *ICP4* gene. To confirm the correct diagnosis, *meq* gene primer sequences were also used. The presence of reticuloendotheliosis virus genetic material was not detected in either flock (12, 13).

Based on our study and research carried out in other countries, it can be concluded that MDV infection is still a problem for global poultry production. It is of particular concern that the serotype and pathotype apparently highly prevalent in Poland is very virulent, and the isolated strains need to be investigated further to elucidate their characteristics in detail.

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Animal Rights Statement: Samples for research were collected from birds sent for routine diagnostic examination. Therefore, no animal experiments were designed or performed. In addition, the owners of the birds consented to the use of the samples from the examined birds for scientific purposes.

References

1. Bell A.S., Kennedy D.A., Jones M.J., Cairns C.L., Pandey U., Dunn P.A., Szpara M.L., Read A.F.: Molecular epidemiology of Marek's disease virus in central Pennsylvania, USA. *Virus Evolution* 2019, 5, 1–13, doi: 10.1093/ve/vey042.
2. Bertzbach L.D., Conradie A.M., You Y., Kaufer B.B.: Latest insights into Marek's disease virus pathogenesis and tumorigenesis. *Cancers* 2020, 12, 647–658, doi: 10.3390/cancers12030647.
3. Boodhoo N., Kamble N., Sherif S., Behboundi S.: Glutaminolysis and glycolysis are essentials for optimal replication of Marek's disease virus. *J Virol* 2020, 94, e01680-19, doi: 10.1128/JVI.01680-19.
4. Chacon R.D., Astolfi-Ferreira C.S., Guimares M.B., Torres L., Torre D.D., de Sa L.R.M., Ferreira P.J.: Detection and molecular characterization of a natural coinfection of Marek's disease virus and reticuloendotheliosis virus in Brazilian backyard chicken flock. *Vet Sci* 2019, 6, 92–106, doi: 10.3390/vetsci6040092.
5. Haq K., Schat K.A., Sharif S.: Immunity to Marek's disease: Where are we now? *Dev Comp Immunol* 2013, 41, 439–446, doi: 10.1016/j.dci.2013.04.001.
6. Kennedy D.A., Cairns C.L., Jones M.J., Bell A.S., Salathé R.M., Baigent S.J., Nair V., Dunn P.A., Read A.F.: Industry-wide surveillance of Marek's disease virus on commercial poultry farms. *Avian Dis* 2017, 61, 153–164, doi: 10.1637/11525-110216-Reg.1.
7. Kheimer A., Previdelli R.L., Wight D.J., Kaufer B.B.: Telomeres and telomerase: role in Marek's disease virus pathogenesis, integration and tumorigenesis. *Virus* 2017, 9, 173–178, doi: 10.3390/v9070173.

8. McPherson M.C., Delany M.E.: Virus and host genomic, molecular, and cellular interactions during Marek's disease pathogenesis and oncogenesis. *Poultry Science* 2016, 95, 412–429, doi: 10.3382/ps/pev369.
9. Mescolini G., Lupini C., Davidson I., Massi P., Tosi G., Catelli E.: Marek's disease viruses circulating in commercial poultry in Italy in the years 2015–2018 are closely related by their *meq* gene phylogeny. *Transbound Emerg Dis* 2020, 67, 98–107, doi: 10.1111/tbed.13327.
10. Nair V.: Latency and tumorigenesis in Marek's disease. *Avian Dis* 2013, 57, 360–365, doi: 10.1637/10470-121712-Reg.1.
11. Nair V.: Spotlight on avian pathology: Marek's disease. *Avian Pathol* 2018, 47, 440–442, doi: 10.1080/03079457.2018.1484073.
12. Prathibha Y., Sreedevi B., Kumar N.V., Srilatha C.H.: Molecular characterization and phylogenetic analysis of oncogenes from virulent serotype 1 Marek's disease virus in India. *Acta Virol* 2018, 62, 277–286, doi: 10.4149/av_2018_221.
13. Puro K., Bhattacharjee U., Baruah S., Sen A., Das S., Ghatak S., Doley S., Sanjukta R., Shakuntala I.: Characterization of Marek's disease virus and phylogenetic analyses of *meq* gene from an outbreak in poultry in Meghalaya of Northeast India. *Vir Dis* 2018, 29, 167–172, doi: 10.1007/s13337-018-0448-2.
14. Reddy M.S., Izumiya Y., Lupiani B.: Marek's disease vaccines: Current status, and strategies for improvement and development of vector vaccines. *Vet Microbiol* 2017, 206, 113–120, doi: 10.1016/j.vetmic.2016.11.024.
15. Samorek-Salamonowicz E., Kozdrun W.: Isolation of Marek's disease virus strains from chickens from cases of post-vaccination immunity. VII Symp. Poultry, 1997, Polanica, 188.
16. Schat K.A.: History of the first-generation Marek's disease vaccines: the science and little known facts. *Avian Dis* 2016, 60, 715–724, doi: 10.1637/11429-050216-Hist.
17. Tai S.S., Hearn C., Umthong S., Agafitei O., Cheng H.H., Dunn J.R., Niikura M.: Expression of Marek's disease virus oncoprotein *meq* during infection in the natural host. *Virology* 2017, 503, 103–113, doi: 10.1016/j.virol.2017.01.011.
18. Trimpert J., Groenke N., Jenckel M., Kunec D., Szpara M.L., Spatz S.J., Osterrieder N., McMahon D.P.: A phylogenetic analysis of Marek's disease virus reveals independent path to virulence in Eurasia and North America. *Erd Appl* 2017, 10, 1091–1101, doi: 10.1111/eva.12515.
19. Yilmaz A., Turan N., Bayraktar E., Tali H.E., Aydin O., Umar S., Cakan B., Sadeyn J.R., Baigent S., Iqbal M., Nair V., Yilmaz H.: Molecular characterization and phylogenetic analysis of Marek's disease virus in Turkish layer chickens. *Br Poult Sci* 2020, doi: 10.1080/00071668.1758301.