Occurrence, characteristics and control of pigeon paramyxovirus type 1 in pigeons

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Abstract

Newcastle disease (ND) is a highly contagious and devastating viral disease of poultry and other birds that has a worldwide distribution. ND in pigeons is called paramyxovirosis and is caused by antigenic “pigeon variant” of the virus (pigeon paramyxovirus type 1, PPMV-1).

During PPMV-1 infections, central nervous system symptoms and sometimes high mortality are observed. In the case of infection with viscerotropic strains which exhibit specific affinity for the kidneys, the first observed sign is polyuria, and neural symptoms appear only in individual birds in the flock.

Due to the similarity of symptoms of paramyxovirosis to the pigeon herpes virus infection (PHV), sodium chloride poisoning, overdose of ronidazole or vitamin B1 deficiency, it is necessary to perform laboratory tests to make a correct diagnosis. After virus isolation PPMV-1 can be detected initially by haemagglutination assay (HA). PPMV-1 can be confirmed by conventional serological tests such a haemagglutination inhibition test (HI) or molecular-based techniques.

In the prophylaxis of paramyxovirosis in pigeons, inactivated vaccines are used, administered by subcutaneous injection in various prevention programs. However, vaccination should be only one component of a strategy of PPMV-1 control, on a par with effective biosecurity and proper, effective methods of prevention and diagnostics of paramyxovirosis.

Key words: pigeons, pigeon paramyxovirus type 1, PPMV-1

Brief history of paramyxovirus infections in pigeons

Newcastle disease (ND) is on the A List of the World Organisation for Animal Health (OIE) as a highly infectious and contagious viral disease of birds, sometimes causing even 100% mortality. The occurrence of Newcastle disease was first diagnosed in chickens in Newcastle-on-Tyne in England and on the island of Java in 1926. The disease spread very rapidly in Asia and over the next 40 years became a panzootic (Alexander et al. 2012). In the late ’60s of the twentieth century, the second pandemic broke out, and within only four years, the ND virus escaped from the Far East through the Middle East to Europe (Śmietanka and Minta 2011a). Newcastle disease in pigeons is called paramyxovirosis and is caused by antigenic “pigeon variant” of the virus (pigeon para-
myxovirus type 1, PPMV-1). PPMV-1 was first isolated in 1978 from meat pigeons in Iraq. A detailed characterization of the virus was carried out by Kaleta et al. (1985). In the same year, the virus from the Middle East regions reached the eastern part of Europe and North Africa, causing respiratory and neural symptoms in pigeons, and in the years 1981-1983 it spread across Europe and the world (Alexander et al. 1985). It is believed that such a rapid spread of PPMV-1 was closely related to trade, competition flights, and exhibitions of carrier and ornamental pigeons (Aldous et al. 2012). In Poland, the first cases of clinical symptoms of PPMV-1 infection in pigeons were observed in 1983, but they have not been confirmed by virological or serological tests (Śmietanka et al. 2006). First isolation and preliminary identification of the virus in Poland took place in the late ’80s of the last century (Wawrzkiewicz et al. 1989).

**Etiology**

The pigeon variant of the Newcastle disease virus is an avian paramyxovirus serotype 1 (APMV-1), and with eleven other bird paramyxovirus serotypes (APMV-2 to APMV-12) it belongs to the genus *Avulavirus*, subfamily *Paramyxovirinae* and family *Paramyxoviridae* (Alexander and Senne 2008, Miller et al. 2010, Briand et al. 2012, Terregino et al. 2013). The paramyxovirus genome is a single-stranded RNA chain of negative polarity, consisting of approximately 15000 nucleotides, encoding at least seven proteins: RNA polymerase (L), haemagglutinin-neuraminidase (HN), fusion protein (F), matrix protein (M), phosphoprotein (P) and nucleocapsid protein (NP). During transcription of the gene encoding protein P, additional nonstructural protein V is produced by means of mRNA processing (Dortmans et al. 2010b). The complex of proteins NP, P and L is responsible for the transcription and replication of the viral genome. NP protein forms a nucleocapsid enclosing the viral RNA, protein P enables the synthesis of RNA and protein L functions as an RNA-dependent RNA polymerase and is responsible for the post-transcriptional modifications of mRNA (Dortmans et al. 2011). HN and F glycoproteins, forming two types of viral surface projection, play a special role in the course of infection and are the major antigens, which cause an immune response. HN glycoprotein has haemagglutinin activity, which allows the attachment of the virus-infected cells and the activity of neuraminidase, which enables a virus to leave the infected cell. Breakdown of the F protein into subunits F1 and F2 determines the virulence, and is necessary for the spread of the virus in the host organism (Seal et al. 2000).

ND viruses show a high antigenic variation. Fourteen antigenic groups were distinguished based on the reactivity of the APMV-1 strains with 9 types of monoclonal antibodies (Alexander et al. 1997a). Comparative analysis of the nucleotide sequences of the segment of the gene encoding protein F in 174 isolates of ND virus with 164 nucleotide sequences of ND virus isolates available in GenBank, allowed six major genetic groups marked with numbers from 1 to 6 to be distinguished. Of these, groups 3 and 4 were secondarily divided into 4 subgroups (a – d) and group 5 into 5 subgroups (a – e) (Aldous et al. 2003). The pigeon variant of the ND virus has been classified into antigenic group P (pigeon variants) and genetic group 4b (Alexander et al. 1997a, Aldous et al. 2003). Further genetic studies conducted by Aldous et al. (2004) found a high genetic variability within the 4b group. On this basis, two distinct groups, 4bi and 4bii, were formed and these were divided into subgroups 4bia-c and 4biid-f. Phylogenetic analysis of PPMV-1 isolated from ornamental pigeons in Poland (PPMV-1/Poland/H2/10) showed that although the virus belongs to antigen group P, genetically it should be classified to the group 4a, which is characteristic for the APMV-1 isolated from poultry (Śmietanka and Minta 2011b).

**Pathogenicity of the virus**

Over 250 species of birds are susceptible to ND virus infection (Aldous and Alexander 2001). However, PPMV-1 attacks mostly pigeons and less frequently chickens, but there are cases of this virus being isolated from birds kept in captivity as well as from wild birds, including partridges, pheasants, swans, falcons, blackbirds, cockatoos and budgerigars (Alexander et al. 1997b, Aldous et al. 2004, Irvine et al. 2009, Krapež et al. 2010).

The course of the infection can vary widely depending on the virulence of the virus. Studies on the pathogenicity of the ND virus based on observing clinical symptoms in infected chickens were carried out under laboratory conditions by Beard and Hanson (1984). In this way, the following five pathotypes of avian paramyxoviruses serotype 1 have been distinguished: velogenic, causing high mortality and having an affinity for the gastrointestinal tract (viscerotropic) or central nervous system (neurotropic); mesogenic of moderate virulence, causing clinical symptoms affecting the nervous and respiratory systems; low virulence lentogenic strains causing mild respiratory system infections; asymptomatic causing subclinical infections of the gastrointestinal tract (Alexander 2000).
The pathogenicity of this virus is determined by the nucleotide sequence of the cleavage site of the fusion protein. During the replication of PPMV-1, precursors of fusion proteins are formed ($F_0$), which are cut by the host cell proteases into $F_1$ and $F_2$ proteins in the post-translational modification (Alexander, 2000). Velogenic and mesogenic viruses have at least three basic amino acid residues at the cleavage site, arginine or lysine between positions 112-116 at the C-terminus of the $F_2$ protein, and phenylalanine at position 117 at the N-terminus of the $F_1$ protein. This amino acid composition ($C^{112}{R/K-R-O/K/R-}$-$K/R-R-F^{117}$-$N$) allows the protein to be cut by intracellular furin-like proteases present in all tissues of the host, which results in pantropic properties of the virus. However, lentogenic viruses have only two basic amino acids, and leucine at position 117 at the cleavage site ($C^{112}{G/E-K/R-O/G/E-R-L^{117}}$-$N$), which can be cleaved only by trypsin-like extracellular proteases present in certain tissues, mainly in the intestine and respiratory tract, so that the virus is replicable only in these organs (Dortmans et al. 2009, Nidzworski et al. 2011). Most PPMV-1 strains contain polybasic cleavage sites of the $F_0$ protein, characteristic for highly pathogenic viruses. However, despite this fact, some PPMV-1 strains have low virulence. Determination of the virulence can be done using biological assays such as ICPI (intracerebral pathogenicity index) for one-day-old specific pathogens free (SPF) chickens (Dortmans et al. 2011). As defined by the OIE (2008), Newcastle disease, among other factors, occurs when the avian paramyxovirus serotype 1 has ICPI equal to 0.7 or higher. It has been shown that some pigeon paramyxoviruses have ICPI below 0.7, even though they have a cleavage site of the $F$ protein specific for virulent strains. Meulemans et al. (2002) compared 240-bp sequences at the cleavage site $F_2/F_1$ of PPMV-1 strains isolated in the years 1983-1984 and 1998-1999. The results showed that, during this period, three amino acid substitutions occurred at the cleavage site (from $112{GRQKF^{117}}$ to $112{RR(O/K/R)KRF^{117}}$). The motif of $112{RRQKF^{117}}$ occurs in the majority of strains from 1998 and 1999; however, the ICPI of these viruses is much lower (average 0.69) than in PPMV-1 isolates in 1983 and 1984 (average 1.44). Similar results were obtained by Dortmans et al. (2010a). They demonstrated that two closely related genetic PPMV-1 variants, having a specific $F$ protein cleavage site for velogenic viruses, at the same time have different ICPI values (0.025 and 1.3, respectively). Results of these studies show that for the proper description of PPMV-1, both $F_2/F_1$ sequence analysis and ICPI test must be performed. Scientists are increasingly casting doubt on the validity of the OIE (2008) definition, which allows the characterization of the ND virus just by using one of the above two methods. They have started to believe that only the results of both tests allow the pathogenicity of the virus to be determined.

Some PPMV-1, with a cleavage site specific for velogenic viruses, show low virulence for chickens (Meulemans et al. 2002). However, this may increase with three or four passages from bird to bird, during the spread of the virus in the infected flock of chickens (Alexander and Parsons 1986). Sequencing of the whole genome revealed that the increase in virulence of the virus passaged in chickens was not associated with changes in the $F$ protein, but with mutations which occurred in genes encoding $L$ and $P$ proteins. These proteins are responsible for replication of the genome, including the synthesis of RNA strands of positive polarity, which serves as a template for the RNA strand of negative polarity (Dortmans et al. 2011). Therefore, the pathogenicity of PPMV-1, and thus the ICPI ratio, is determined not only by the amino acid composition of the $F$ protein, but also by the viral replication complex (proteins NP, P and L) (Dortmans et al. 2011).

### Course of infection

The incubation period of paramyxoviriosis in pigeons varies from 4-6 days to 3-4 weeks. Clinical signs of PPMV-1 infection are generally similar to the symptoms caused by viruses from the neurotropic velogenic group of ND viruses. However, some currently occurring field cases are caused by viscerotropic strains that exhibit specific affinity for the kidneys, so that the first sign is polyuria, then neural symptoms which appear only in individual birds in the flock. The incidence varies from 30 to 70%, with mortality not exceeding 10%. In case of associated bacterial or parasitic infections, mortality may reach more than 30% (Marlier and Vindevogel 2006). Paramyxoviriosis much more often occurs as an associated infection with pigeon circovirus (PiCV) infection (Stenzel et al. 2012). The most common neural signs that occur during infections with PPMV-1 include head and neck 180° twists (torticolis), imbalance, paralysis of wings and legs or difficulties in food intake. Infected birds sometimes have watery (increased urinary volume fractions resulting from kidney infection) or bloody diarrhea. If infection occurs during moulting, problems with feather development may occur – feathers appear deformed, poorly developed and fragile (Marlier and Vindevogel 2006).
Diagnostics

Due to the similarity of symptoms of paramyxovirosis to the pigeon herpes virus infection (PHV), sodium chloride poisoning, overdose of ronidazole or vitamin B1 deficiency, it is necessary to perform laboratory tests to make a correct diagnosis.

The first step in proper diagnosis of PPMV-1 is virus isolation. According to OIE (2008) samples from live birds should consist of tracheal or oropharyngeal and cloacal swabs. From dead birds, besides swabs, samples should include part of organs such as the lungs, kidneys, spleen, brain, liver and intestine. Samples placed in phosphate buffered saline (PBS), containing antibiotics, are inoculated into the allantocic cavity of embryonated SPF chicken eggs of nine to eleven days incubation. The eggs are incubated until the embryos die or for 4-7 days. Harvested allantocic fluids are tested for haemagglutination (HA) activity using chicken erythrocytes. Fluids which do not exhibit HA activity should be passed into at least one batch of chicken eggs.

Suspension of homogenated organs, samples of faeces or swabs prepared in the same way as for virus isolation using SPF eggs, may also be used for isolation in cell cultures. NDV indicates capability to replicate in a variety of cell cultures, inter alia chicken embryo liver (CEL) cells, chicken embryo kidney (CEK) cells, and African green monkey kidney (Vero) cells. However, for NDV replication, primary cell cultures of avian origin are the most suitable. Presence of NDV in cell cultures induces cytopathic effects represented by disruption of the monolayer and formation of syncytia.

Performing only virus isolation and HA assay is insufficient to make a correct identification of PPMV-1, since all serotypes of avian paramyxoviruses and avian influenza viruses (AI) have haemagglutination activity. Therefore, it is recommended that a haemagglutination inhibition (HI) test with monospecific sera of chickens immunized with a specific strain of ND virus should be conducted (Śmietanka and Minta 2011a). Serological tests used for antibody detection are of limited diagnostic value, which is related to the immune status of the flock. Using the HI method, recommended by the OIE (2008), it is possible to detect antibodies specific for the ND virus, which does not necessarily indicate an ongoing infection, and can only be a post-vaccinal immune response. In addition, these tests do not allow the virulence of the virus to be determined (Alexander 2000). Due to the fact that most of the birds are immunized against paramyxovirosis, detection of specific antibodies to the ND virus cannot be the basis for making a diagnosis. Therefore, molecular biology techniques are most frequently applied. The technique of reverse transcription polymerase chain reaction (RT-PCR) is a fundamental method for the in vitro differentiation and detection of ND viruses (Aldous and Alexander 2001). RNA isolation can be performed with the internal organs, including the brain, intestines, liver, lungs, kidney or spleen. However, due to the ease of sampling and a small amount of non-viral genetic material, tracheal, cloaca and direct feces swabs are also recommended (Creelan et al. 2002). Amplification of a specific region of the genome can be done using universal and specific primers for a given pathotype or antigenic variant of the ND virus (Aldous and Alexander 2001). It is also possible to use two pairs of primers in the nested RT-PCR. This method has a higher sensitivity than conventional RT-PCR and can detect single copies of the PPMV-1 genome in the organs of birds which are in the late stage of infection, when viral RNA isolation is very difficult (Barbezange and Jestin 2002). The RT-PCR technique, expanded by restriction fragment length polymorphism analysis (RFLP) and sequencing of the gene fragment encoding the protein F cleavage site, not only allows the virulence of the virus to be partially determined (Śmietanka and Minta 2011a), but also has a high educational value in the studies of epidemiological and phylogenetic analyses of the ND virus, including PPMV-1 (Kou et al. 1999, Aldous et al. 2003). Conventional RT-PCR is increasingly being replaced by real time RT-PCR (RRT-PCR), which has a high sensitivity and allows for quantitative measurement of viral RNA in the samples. RRT-PCR can be performed in a single tube, which significantly reduces the time required for the reaction and reduces the possibility of cross-contamination of samples (Farkas et al. 2009). According to Nidzworski et al. (2011), using SYBR Green and a pair of degenerate primers, it is possible to distinguish non-virulent from virulent strains of ND. The results are based on the analysis of the melting curve of RRT-PCR product. Melting temperature (Tm) of non-virulent strains ranges from 80.00 to 83.80°C, while for the mesogenic and velogenic strains, Tm is over 83.80°C. This method is an alternative to the TaqMan RRT-PCR, where the use of two or three pairs of primers and TaqMan probes of various types in one reaction is necessary to distinguish pathotypes of ND virus, which significantly increases the cost of the test.

Disease control

In prophylaxis of paramyxovirosis in pigeons inactivated vaccines are used. The vaccines are administered by subcutaneous injection in various prevention
programs. The vaccines are based on the different strains of paramyxoviruses and the LaSota strain is one of the most commonly used. It has been shown that, despite the antigenic differences between LaSota and PPMV-1 strains, this vaccine effectively protects against disease caused by PPMV-1 (Marlier and Vin-devogel 2006). However, an increasing number of breaking post-vaccination immunity cases may be associated with immunosuppression caused by very prevalent PiCV infection (Stenzel et al. 2012, Stenzel and Pestka 2014). The immunoprophylaxis of paramyxoviruses in pigeons does not involve live vaccines, because they are less immunogenic, and in the case of carrier pigeons the spread of vaccine virus may occur in areas where vaccinations against the ND virus are not applied (Smietanka and Minta 2011a). However, it is worth noting that immunization does not prevent infection or replication of PPMV-1, and only prevents the occurrence of clinical symptoms (Alexander 2011). Therefore, vaccination should be only one component of a strategy of PPMV-1 virus control on a par with effective biosecurity (Smietanka and Minta 2011a). However, the specificity of pigeon breeding (competition flights, exhibitions), significantly impedes the principles of biosecurity.

Poland follows the Council Directive 92/66/EEC of 14 July 1992 introducing Community measures for the control of Newcastle disease, the Act of 11 March 2004 on the protection of animal health and control of infectious animal diseases as well as the regulation of 29 July 2005 on the control of Newcastle disease in poultry, pigeons and other birds kept in captivity. A regulation of the Ministry of Agriculture and Rural Development describes in detail the procedure to be followed in case of suspecting, diagnosing and controlling of ND also in domestic pigeons. In accordance with this Regulation, feral/urban pigeons are not included within the definition of poultry. In connection with the above, the occurrence of Newcastle disease in these birds does not affect the loss of the country’s ND free status. Paramyxoviruses originating from wild or feral pigeons (‘city doves’) may first cause minor infections in poultry, which, during the spread of the virus in a flock, may lead to a more virulent virus and the outbreak of ND. This happened during an epizootic infection in poultry in the United Kingdom, where at least 20 outbreaks of the disease occurred due to contamination of poultry feed with pigeon feces containing PPMV-1 (Dortmans et al. 2010a). Economic losses resulting from infection of poultry can be very high, as evidenced in the United Kingdom in 1984 where PPMV-1 caused losses in the poultry industry of almost 3.4 million dollars (Seal et al. 2000). For these reasons, proper and effective methods of prevention, control and diagnostics of NDV is highly important, especially for PPMV-1, as increasing outbreaks of pigeon paramyxoviriosis, including Europe, show that for the last 30 years the control of PPMV-1 has been conducted inefficiently (Alexander 2011).

**References**


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