Outbreak of duck viral hepatitis in duckling flocks in Poland

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Abstract

The aim of the study was to determine the aetiologic agent causing deaths in two flocks of Pekin ducklings at the age of 12 d in Poland. on the basis of clinical symptoms and pathological changes, viral hepatitis infection was suspected in the birds. During the necropsy, liver sections were collected, from which total cellular RNA was isolated. Then, real-time polymerase chain reaction (RT-PCR) was performed using primers complementary to the pre-S region of the duck hepatitis virus genome. In all liver samples, the presence of a 530 bp PCR product was detected. The RT-PCR demonstrated the presence of genetic material of duck hepatitis virus type 1 (DH type 1) in the examined ducklings.

Keywords: ducklings, duck viral hepatitis, Poland.

Introduction

Duck viral hepatitis (DH) is an infectious and highly contagious disease of ducklings. They are most susceptible to infection in the first weeks of age but this susceptibility and the occurrence of the disease decrease with bird age. Adult birds become asymptomatic carriers and shed the virus for lifetime. The incidence of viral hepatitis in ducklings is recorded under three subclassifications, which differentiate the three different viruses responsible for DH. Recently, it has been found that DH type 2 is caused by an astrovirus and types 1 and 3 by picornaviruses. The virus of DH type 3 is less virulent than the virus of DH type 1. There are no antigenic similarities between these viruses (8, 10).

Duck viral hepatitis (DHV) is on the list of diseases notified to the World Organisation for Animal Health (OIE) (5). The disease has been described for the first time in ducks in the United States in the 1950s. Currently, it is noted in many countries around the world (2). In Poland, duck viral hepatitis was described for the first time in the 1960s and 1970s, but the disease was not reported in subsequent years (9).

The aetiological agent of duck hepatitis type 1 is a virus belonging to the family Picornaviridae. It is a RNA virus of 20-40 nm diameter and its genome consists of 7691 nucleotides (4). The virus is characterised by a high resistance to disinfectants, acidic environments, and trypsin (7).

The infection occurs mainly by ingestion. Less important is infection through the respiratory system. Vertical transmission of the virus has not been reported. The spread of the virus in the flock is usually achieved by direct contact with sick birds, contaminated equipment, vehicles, or employees. Wild birds may play an important role in the spread of the virus, because they can transmit the virus mechanically. Specific antibodies were found in wild ducks living in the immediate vicinity of ducks for breeding (2).

In the flock the disease spreads very quickly, and within 4-5 d all the birds are sick and mass mortality follows. The viral infection has a pantropic character, but the virus has a specific tropism to the liver cells as evidenced by the localisation of the lesions mainly in this organ (2, 8).

The aim of the study was to determine the aetiologic agent causing deaths in two flocks of ducklings in Poland.

Material and Methods

Birds. The material consisted of 12-day-old dead Pekin ducklings from two flocks which contained 500
and 800 birds respectively. The flocks were located in the province of Pomerania. All ducklings were from the same hatchery located in one of these farms, where geese were also incubated. During this time, ducklings imported from France were also housed. The first deaths were observed in five to six-day-old ducklings and the number of cases increased on subsequent days. Microbiological testing of the ducklings’ visceral organs revealed an increase of infection with Staphylococcus sp. and Streptococcus sp. Based on susceptibility testing, antibiotic treatment turned out to be ineffective. Data obtained during the interview with the flocks’ owners indicated that the birds displayed difficulty in moving, walked on ankle joints, threw their heads back, and suddenly died despite being in a good condition. During the course of the disease, total deaths in these flocks were approximately 80% of the birds.

Post-mortem examination. Ten dead ducklings from each flock were necropsied and sections of the liver were collected.

RNA isolation. Samples from the liver were homogenised and total cellular RNA was isolated using the commercial QIAamp Viral RNA Mini Kit (Qiagen, USA) according to the manufacturer’s instructions.

Primers. For the detection of genetic material, DHV primers based on the sequence of the pre-S region of the DHV genome were synthesised at the Institute of Biochemistry and Biophysics of the Polish Academy of Sciences in Warsaw. The primer sequences were as follows: DHVF1 5’ ACA ATG ACC AGC CTT AG 3’; DHVR2 5’ CCA CTG TAT CTT CCC TTC 3’ (1).

Amplification reaction. RT-PCR was performed using the commercial One Step RT-PCR kit (Qiagen). Amplification was conducted in 25 µL of reaction mixture for 30 cycles. Denaturation was performed at 94°C during 1 min, annealing at 52°C for 30 s, elongation at 72°C for 2 min, and the final elongation at 72°C for 10 min. RNA from a vaccine strain against DH type 1 obtained from the Institute of Experimental and Clinical Veterinary Medicine in Charkov, Ukraine served as a positive control in the amplification reaction. The negative control was liver RNA isolated from uninfected SPF chicken embryos.

Analysis of PCR products. Products of the amplification reaction (cDNA) were separated electrophoretically on a 2% agarose gel for 1 h at 120 V. Afterwards, the gel was stained with ethidium bromide solution (1 µg/mL) and assayed by UV comparing the sizes of the products obtained from the sample and the positive control template DNA mass (DNA MassRuler, Fermentas, part of Thermo Fisher Scientific, Lithuania). The result was considered positive if the PCR product for the stain used was 530 bp.

Results

According to a veterinarian’s declaration, a characteristic body position with the head tilted upwards and towards the rear of the body was observed in birds.

At necropsy, the main pathological changes in the liver were noted. In all ducklings examined, the liver was significantly enlarged and congested. Numerous petechiae and extravasations were also observed. In addition, enlarged kidneys with congestion were found in two birds.

In all liver samples examined, the amplification reaction product revealed the expected 530 bp size characteristic of the primers used, based on the sequence of the pre-S region of the DHV genome. The same product was obtained in the positive control. In contrast, no amplicon was observed in negative control sample.

![Fig. 1. Electrophoresis of RT-PCR products](image)

Lane 1-DNA MassRuler; Lanes 2-15-Field samples; Lane 16-Positive control; Lane 17-Negative control
Discussion

Duck viral hepatitis type 1 is one of the most dangerous infectious diseases and causes great losses, especially in Pekin ducks, in many countries. The disease is often acute or subacute and spreads very quickly through the flock. Within a few days the birds are sick and die in a great number. Exhibiting limb spasms and tremors, sick birds have difficulty in movement and fall down suddenly with their heads tilted upwards and towards the rear of the body (opisthotonus) (2).

As regards the tested flocks, the first sudden deaths occurred in five to six-day-old birds and it was related to ducklings being in good condition. Difficulty in moving, walking on ankle joints, and throwing the head back indicated DH type 1 (2, 8). Similar symptoms have been described by Marek (9) and by Jin et al. (6). The clinical symptoms described by Jin et al. (6) were observed in five-day-old ducklings, i.e. much earlier than in the birds examined in the study, the majority of which presented symptoms closer to their twelfth day of life.

In the course of the disease, pathological changes are localised mainly in the liver, which is enlarged and has olive colour with numerous petechiae and haemorrhages. Pathological changes may also affect the spleen and kidneys, which are enlarged and congested. In older birds, inflammation of the pericardium and air sacs is also observed. In experimentally infected ducklings, pathological changes were demonstrated in the liver, and also in the kidneys of some birds (2, 8, 11), and in the examined birds likewise some of these changes were manifested.

To detect the presence of picornavirus genetic material, RT-PCR was used. The technique has been developed during previous studies performed in the Department of Poultry Viral Diseases (data not published). Primers complementary to the sequence of the pre-S region, or to the RNA polymerase gene of DHV were used. Positive results in the amplification reaction in all liver samples examined indicate the usefulness of this method for diagnosis of DH. Chen et al. (3) demonstrated the advantages of multiplex RT - PCR, because all three viruses causing DH were detected by the method in one reaction.

On the basis of clinical signs, pathological changes, and the results of molecular tests, the occurrence of DH type 1 in two flocks of Pekin ducklings in Poland was confirmed. It should be noted that although no diagnoses of the disease in the population of ducks have been recorded over the last 40 years, it constitutes a serious epidemiological threat and could be responsible for large economic losses.

References