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RESEARCH ARTICLE

Avian astrovirus caused mortality in pheasant (*Phasianus colchicus*, Linnaeus, 1758) farm in Hungary

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ABSTRACT

We present the clinical symptoms, pathologic lesions and diagnostic possibilities of the avian astrovirus-related mortality in a pheasant colony. In addition to enteritis in chicks, we also confirmed acute nephrosis. The genome section of the astrovirus was detected and verified by polymerase chain reaction (PCR) testing. After sequencing the isolated genome section based on BLAST driver analysis (601-base pair-long) avian astrovirus has the same ORF-1b gene as turkey astrovirus 1 (TAstV1).

KEYWORDS

pheasant, astrovirus, enteritis, nephrosis, mortality, phylogenetic tree

INTRODUCTION

The pheasant (*Phasianus colchicus*) plays a significant role in domestic small game farming. The pheasant is bred in the largest number of avian wild game species to be reintroduced to habitats for hunting purposes. During rearing, many non-infectious and infectious causes of death can be encountered, which occur more and more often with large numbers of pheasants being kept and bred together. In day-old chicks, poor hatching causes losses in the first 4–7 days of life, which can basically result from hatching technology errors in this species as well (Bicsé et al., 2010; Dobos-Ková, 2014). In chickens with a weight smaller than normal, incomplete absorption of the yolk, sometimes its inflammation and degeneration can also be observed during dissection (Dobos-Ková, 2014; Morishita and Porter, 2023).

Inadequate housing temperature, errors in feeding and watering can also cause significant losses. Due to improperly designed drinking technology, thirst and gout can occur, during which, in addition to signs of dehydration, the deposition of uric acid salts in the kidneys can be observed (Dobos-Ková, 2014). If the position of the feeders, or the lighting intensity of the room in the first days of life are not suitable, the chicks will not learn to eat. In such cases,

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the losses will be significant due to starvation, and in this case the stomach of the carcasses is empty, erosions and consequential bleeding in the glandular stomach may also develop (Dobos-Ková, 2014; Morishita and Porter, 2023).

If the quality of the litter material is not adequate for the chicks, it is often observed that the young chicks pick up pieces of the litter, which fill the stomach's cavity as indigestible material (Morishita and Porter, 2023). Fungi can multiply in the mouldy substrate and their spores can enter the airways and cause pneumonia with exudation, granuloma formation and alveolar inflammation (Bicsé et al., 2010; Morishita and Porter, 2023).

An infection caused by *Escherichia coli* (*E. coli*) can also cause inflammation in the small intestine with a significant amount of fluid accumulation in infancy (Morishita and Porter, 2023). In this case, contaminated drinking water can be a frequent source of pathogens, especially if young individuals drink it from open drinking fountains. In chicks and young pheasants, subacute enteritis caused by *Salmonella* bacteria can be a common problem, which often causes changes in the large intestine and mainly in the caecum. In addition to the necrosis of the intestinal wall, caseous debris can accumulate in the intestinal lumen (Morishita and Porter, 2023). Coccidiosis can cause significant losses in young pheasants if the coccidiostat in the rearing food is not chosen properly. In such cases, in the small intestine, rarely in the caecum and the large intestine, inflammation is characterized by the accumulation of caseous, fragile, clastic content (Dobos-Ková, 2014; Morishita and Porter, 2023).

Acute enteritis caused by pathogens belonging to the group of enteroviruses (rota-, reo-, parvoviruses) can also occur in young chicks (Gál et al., 2019; Mándoki et al., 2019; Varga et al., 2018). In such cases, the intestinal villi become desquamated due to diarrhoea and acute, catarrhal enteritis can be observed by pathological examination (Dobos-Ková, 2014; Mándoki et al., 2019).

Viral infections of the intestinal tract are common in young poultry, with a wide range of clinical manifestations, from asymptomatic infection to serious disease, causing severe economic losses (Guy, 1998). In pheasants, viral gastrointestinal diseases have been linked to astro-, calici-, corona-, picorna-, parvo- and rotaviruses (Toffan et al., 2016; Baxendale and Mebatsion, 2004; Farkas et al., 2012; Hauck et al., 2016; Kisary et al., 1984; Otto et al., 2006; Kaszab et al., 2024). Avian astroviruses (AAstVs) belong to the genus *Avastrovirus*, are non-enveloped, single-stranded positive-sense RNA viruses. The genome of AAstV is 6.1–7.9 kb long and contains three open reading frames (ORFs: ORF1a, ORF1b and ORF2), a 5′ untranslated region (UTR), a 3′ UTR, and a poly (A) tail (Wan et al., 2018).

MATERIALS AND METHODS

In the spring of 2023, five, approximately 2.5-week-old pheasant chicks were sent for autopsy to the Department of Exotic Animal and Wildlife Medicine, University of Veterinary Medicine. The pheasant chicks were dissected according to the rules of the profession (Vetési et al., 1998) and small pieces of the organs showing changes (mainly the small intestine and the kidney) were fixed in 10% buffered formaldehyde solution. After a few days of fixation, the organ parts were embedded in paraffin, then 3–4 µm thin sections were cut, mounted on a glass slide and, after hematoxylin-eosin staining and coverslipping, they were examined under a light microscope.

Samples from the proximal part of the small intestine of the pheasant carcasses, from the opened lumen duodenum, were inoculated onto Columbia blood agar and Drigalski agar (Biolab Zrt.), and then these were incubated at 37 °C for 24 h under aerobic conditions. Bacteria were identified based on colony morphology, growth characteristics and biochemical properties.

During the dissection of the pheasants, as a first step, small pieces of the small intestine and kidney were homogenized using 500 µL of phosphate buffered saline (PBS) buffer for virological testing. At the end of the process, centrifugation followed and a 200 µL sample was taken from the supernatant for further nucleic acid isolation. This step was performed using the IndiSpin Pathogen Kit based on the product description and the QIAGEN QIAcube nucleic acid detection and isolation automata (Qiagen, Hilden, Germany). For the RT-PCR test, we used the product of Avian Nephritis Virus (ANV) with a length of 607 base pairs (Fw: (5′-AGATACGCTTGCTCGTCTTG-3′), Rev: (5′-CCTCTAACCGGCGATATTCT-3′)) (Mándoki et al., 2006). The Genesy 96T gradient PCR machine (Tianlong Science and Technology Co., Ltd., China) and the QIAGEN OneStep RT-PCR kit (Qiagen, Hilden, Germany) were used in the study. After agarose gel electrophoresis, the amplicon was excised and re-isolated from the gel using the QIAquick GEL Extraction Kit (Qiagen, Hilden, Germany) according to the manufacturer's recommendations. Sequencing and sequence reading were the final steps of the process, which we carried out in cooperation with the staff of Eurofins BIOMI Kft. (Gödöllő).

The MAFFT algorithm of Geneious Prime 2024.0.2 was used for making alignments of nt sequences (https://www.geneious.com) (Biomatters, Auckland, New Zealand). Maximum likelihood phylogenetic tree of the RdRp nt sequences was generated and visualized using MEGA-X with the best fitted model (GTR+G+I model) and with 500 bootstrap replicates (Kumar et al., 2018). Sequence identity values were calculated using the Geneious Prime software.

RESULTS

The pheasants that arrived for the examination were well developed, but in an average state of nutrition. Their plumage was contaminated with diluted excrement around the cloaca. The ends of the toes were also covered with small balls from faecal contamination. In the opened body cavity, the air sacs were intact and transparent. The liver in all

individuals was normal in shape and size, brownish red. In the cavity of the glandular and muscular stomachs, a small amount of rough content was found. The lumen of the small intestine was significantly extended and stretchy, mucous contents accumulated in it (Fig. 1). The pancreas showed no abnormality. The kidneys were slightly enlarged, had a paler brownish-red colour and showed granularity on their surface.

Bacteriological examination of the proximal part of the small intestine resulted in the isolation of few bacterial colonies belonging to the *Enterobacteriaceae* family.

During the histopathological examination, the desquamation of the villi of the small intestine, the necrosis and detachment of the epithelial cells were evident. The affected intestinal villi were significantly shortened and widened and merged in several areas (Fig. 2). More or less intact epithelial cells were also observed in the crypts of the intestine. Multiple, solitary epithelial cell degeneration was seen in the renal tubules. The nucleus of the affected cells often showed signs of pyknosis, but rhexis was also observed in some places, accompanied by vacuolization of the cytoplasm (Fig. 3).

The genetic material of astrovirus could be detected in the kidney and intestine samples using the PCR test. After sequencing the isolated genome, the ORF-1b gene segment of avian astrovirus (601 base pairs long) was identical to turkey astrovirus 1 (TAstV1) based on BLAST driver analysis.

The partial (601bp)(601bp) ORF-1b sequence of avian astrovirus was deposited in GenBank database. Avian astrovirus isolate Avast_HUN_Ph_1 (OR700006.1) shared 94.12% nt identity with the most closely related GenBank reference sequence turkey astrovirus (NC_002470). In the phylogenetic analysis, the avian astrovirus isolate Avast_HUN_Ph_1 clustered together with turkey astrovirus strains (Fig. 4.).

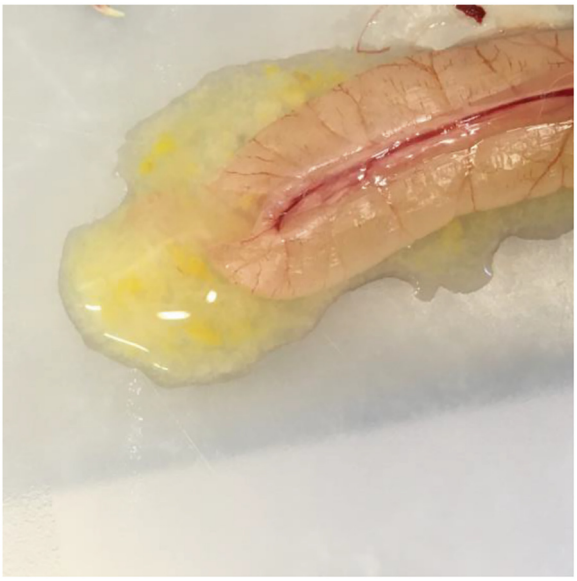

Fig. 1. Acute catarrhal enteritis in the duodenum

DISCUSSION

In pheasants, acute enteritis caused by several viruses has recently been described in the literature, such as diseases caused by rota-, reo-, corona-, calicivirus (Morishita and Porter, 2023), and in a previous study, diseases caused by astrovirus (Mándoki et al., 2019) were also reported. The disease caused by the astrovirus was described in pheasants at the age of 19–22 days in domestic studies (Mándoki et al., 2019). In our case, we confirmed the disease in 17-day-old pheasants.

The viruses multiply in the intestinal epithelium and cause the destruction of the epithelial cells (Dobos-Ková, 2014; Gál et al., 2019; Morishita and Porter, 2023), as we also have observed, which causes the villi to become

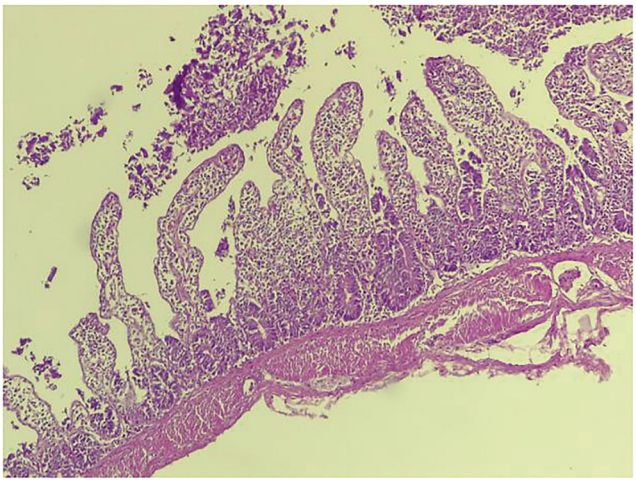

Fig. 2. The fusion and shortening of the denudated intestinal villi (staining: H. E., 100x)

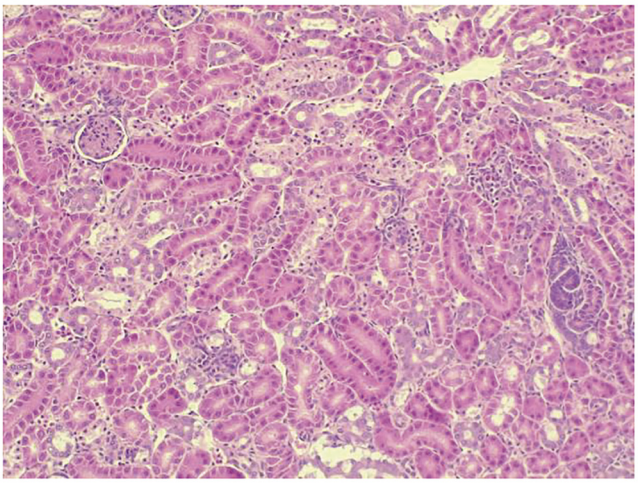

Fig. 3. Degeneration of the tubular epithelium in the kidney (staining: H. E., 100x)

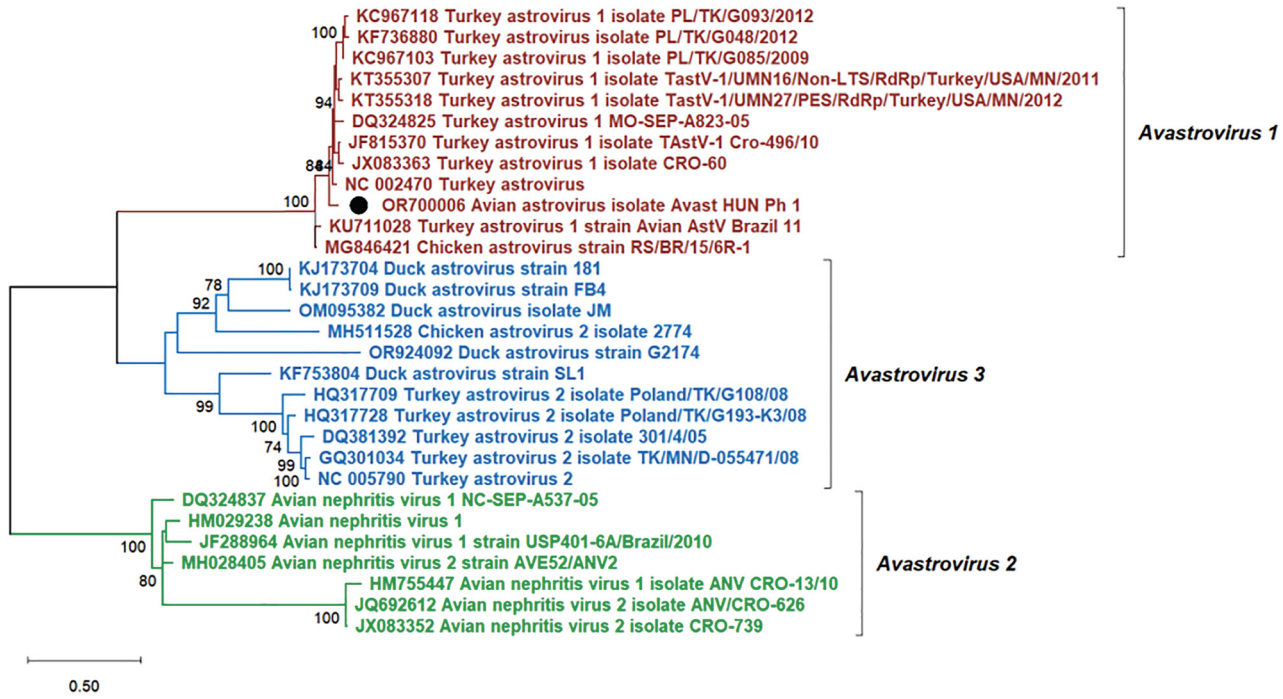

Fig. 4. Maximum likelihood phylogenetic tree of *Avastrovirus* partial RdRp gene sequences. Sequence identified in this study was highlighted by a dot. The tree was generated by the MEGA-X software with the best fitted GTR + G + I model and with 500 bootstrap replicates. Branch support values lower than 70 were hidden

desquamated. A large amount of fluid enters the intestinal lumen from the exposed vessels of the denuded villi. Damage to the intestinal epithelium also causes digestive and absorption disorders (maldigestion, malabsorption) in chicks, which results in clinical symptoms of diarrhoea. Pathological abnormalities of this nature were also observed in ostriches (Gál et al., 2024), where damage to the intestinal villi was also reported.

The so-called nephrotropic strain of astroviruses (Morishita and Porter, 2023) is known in ostriches (Mándoki et al., 2019), which reaches the epithelium of the renal tubules during viraemia, as we observed in the pheasant chicks examined. As result of the multiplication of the virus, due to the damage to the tubular epithelial cells, an excretion disorder can also be observed, though it cannot be verified if it was caused by primary virus replication, or it was a secondary consequence by dehydration or endotoxication.

In connection with the case we observed, we confirmed the susceptibility of pheasants (*P. colchicus*) to turkey astrovirus 1 (TAstV1).

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