



## CASE REPORT

### Adenoviral Gizzard Erosions in Commercial Layer Chickens

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#### ABSTRACT

This case report describes a series of gizzard erosion outbreaks in two farms of commercial layers flocks at the age of 19, 25, 30 and 73 weeks observed in the period between 2012 and 2015. The clinical signs, course of disease and mortality in all cases were similar. The post-mortem examinations revealed dark-brownish contents in crops, esophagus, proventriculus, gizzards, and duodenums, as well as haemorrhagic changes in proventriculus and ablations of the overlaying keratinoid layers in the gizzards. Histopathology showed the presence of intranuclear inclusion bodies in gizzard epithelial cells and molecular findings confirmed the presence of fowl adenoviruses A. It seems that the virus persisted on the farm during this four-year period and temperature fluctuations were to be the probable factors predisposing the clinical course of disease.

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#### INTRODUCTION

The aetiological factor of gizzard erosions belonged to the family *Adenoviridae*, the genus *Aviadenovirus*, the species *fowl adenovirus A* or *E* (FAdV-A, FAdV-E) (Lim *et al.*, 2012; Mase and Nakamura, 2014). In recent years, outbreaks of gizzard erosions in chickens were reported in different parts of the world. Most commonly, broilers were affected and vertical transmission of the disease from breeders to progeny was indicated (Manarolla *et al.*, 2009; Marek *et al.*, 2010; Schade *et al.*, 2013). Signs of gizzard erosion in older chickens were rarely reported and concerned 10-, 21- and 39-40-week-old commercial layers (Lim *et al.*, 2012; Matczuk *et al.*, 2017).

The data on the effect of FAdV infections in adult chickens are scarce and sometimes contradictory. This paper describes the outbreaks of adenoviral gizzard erosions in commercial layer flocks at the age of 19, 25, 30 and 73 weeks recorded between 2012 and 2015 in Poland.

**Cases presentation:** All disease cases occurred between 2012 and 2015 on two farms (A and B), which belonged to the same owner, but were located at about 12 km distance from each other. Farms A and B consisted of three and two buildings, respectively and the birds were reared in cages. The buildings (flocks) were of various sizes, from 20,000 to 40,000 birds in each. Usually, birds

of one age were kept in individual buildings, but the farm could have chickens of different ages. The first case of disease occurred in February 2012 in one flock of 25-week-old commercial layers of farm A and manifested by increased mortality of up to 18 birds per day. Elevated mortality lasted about 3 weeks (0.25% per week). A few days before disease occurrence, sudden temperature drop below  $-30^{\circ}\text{C}$  was recorded. The second case was one year later, in February 2013. Disease conditions were observed in one of two flocks of farm B consisting of 30-week-old layers. As previously, clinical signs of disease lasted about 3 weeks. In this case mortality was higher and reached 21 birds per day (0.4% per week). The temperature a few days before the beginning of clinical symptoms of disease fell below  $-15^{\circ}\text{C}$ . In 2014 there was no such case, while in February 2015, a similar disease condition was again diagnosed in 25-week-old layers in one flock of farm A. Signs of clinical disease lasted about 3.5 weeks with mortality of 0.3% per week and as previously, sudden temperature drop below  $-10^{\circ}\text{C}$  just before disease outbreak was observed. The next two outbreaks were noticed in June 2015: first in the flock of 19-week-old layers in farm A, and a few days later in the flock of 73-week-old layers of farm B. The mortality rate in the flock of layers at the age of 19 weeks was about 0.2% per week while in the flock of 73 week-old even lower, about 0.05%. Again, temperature drop by 18 degrees occurred a few days before disease cases.

Chickens maintained on both farms originated from different breeders. All birds were immunized with the same vaccination program. No decrease in either egg production or egg quality, regarding both external (size, shape and shell) and internal features was observed. There was no reduction in feed and water intake. Dead chickens were found randomly between batteries and floors. Zoohygienic conditions in the affected farms were good with the exception of the 2012 case when the occurrence of mites was observed. The housing was equipped with non-automatic heating and did not have air conditioning.

**Postmortem examination and histopathology:** Dead chickens exhibited paleness of combs and wattles. The post-mortem examination revealed similar gross lesions in birds from all mentioned outbreaks. The crop, esophagus, proventriculus, gizzard, and duodenum were filled with dark-brownish fluid. Hemorrhagic changes, such as congestion, petechiae and erosions in proventriculus, especially in the proximity of the interventriculus, were observed (Fig. 1). Numerous erosions, including even an ablation of the overlying keratinoid layer in the gizzard, were found. Furthermore, due to the perforation of the gizzard walls, the efflux of its haemorrhagic contents to the body cavity was observed in some birds. In most of the dead, necropsied layers, fully developed yolks as well as the presence of properly formed eggs in the uterus were found. Additionally, the presence of fat in abdominal cavity and around internal organs was observed (Fig. 2). There were no lesions in the livers or spleens.

Histopathological examination showed the presence of focal degeneration of koilin layer and necrosis of the gizzard epithelium. They were surrounded by massive inflammatory cells infiltrations which reached the submucosa and muscle layer of the gizzard. The presence of intranuclear inclusion bodies in gizzard glandular epithelial cells were observed (Fig. 3).

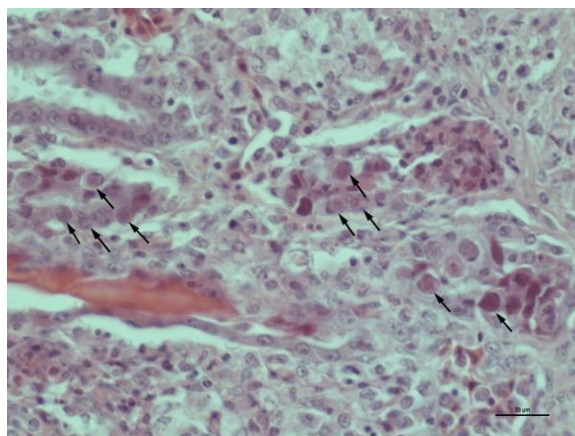
**Diagnostic PCR and sequence analysis.** Samples of gizzards, duodenum and livers from five birds from the mentioned cases were homogenized and checked for the genome presence of FAdV which was suspected to be responsible for observed disease symptoms. In nucleic acids suspensions extracted from supernatants of tissues/organs of field samples delivered to our laboratory in years 2012-2015 the presence of 52K gene fragment of FAdV was detected (Gunes *et al.*, 2012). Viruses from disease outbreaks in 2012 and 2013 were isolated in chicken embryo fibroblasts cells and fragments of their hexon (deposited in GenBank under Accession # KY027456 for PL/G026/12 and KY027457 for PL/G018/13), short and long fibers were sequenced (Accession # KY027458, KY027459 and KY027460, KY027461 for PL/G026/12 and PL/G018/13, respectively) (Domanska-Blicharz *et al.*, 2011). The nucleotides homology between them in analyzed fragments were 99.5-99.9% and some of these changes were nonsynonymous. Similarities of studied genome fragments to the Polish FAdV PL/60/08 isolated from gizzard erosions case in broilers in 2008, European reference chicken embryo lethal orphan (CELO) and Japanese JM1/1 strains from diseased broilers identified in 2000 were also high in the range of 98.7-99.9%, 98.8-99.7% and 99.4-100%, respectively.



**Fig. 1:** Gizzards from 25-week-old commercial layers infected with FAdV A (PL/G026/12). The detachment of the overlying keratinoid layer and even its partial decay in the gizzard.



**Fig. 2:** The presence of fat in the abdominal cavity and around organs as well as fully developed yolks. Visible dark brown content flows from the damaged intestines.



**Fig. 3:** Gizzard tissue from 25 week-old layers of the farm A infected with the FAdV A (PL/G026/12). Arrows show intranuclear inclusion bodies in epithelial cells (Hematoxylin stain, 600x).

## DISCUSSION

The condition described as adenoviral gizzard erosion mostly affected broilers and only occasionally older chickens. In Poland at the beginning of 2008, the outbreak of adenoviral gizzard erosions was recorded in a few farms of broiler chickens in central Poland and epidemiological investigations suggested vertical transmission of FAdV (Domanska-Blicharz *et al.*, 2011). In the present study, we detected FAdV-A strains in two

farms of commercial layer chickens at the age between 19 and 73 weeks. Both affected farms belonged to the same owner, therefore the probable introduction of FAdVs into farms was horizontal, i.e. through the same means of transports of chickens, feeding stuff or sewage. Erosions in keratinoid layer of gizzard could be also caused by nutrition factors, but in described cases such possibility should be excluded as all animals in affected farms received the same feed premixes, whereas afflicted birds were only observed in a single building of each farm. It seems that the virus circulated among the birds in the farms, passing from older to younger replacements during the period between February 2012 and June 2015 and the maintenance of multiple-age groups of chickens in the farms and the lack of "all-in-all-out" replacement system especially fostered the continued presence of the virus. It is probable that during the period when the virus propagated in birds it acquired new genetic features seen as the altered sequences in the hexon as well as in short and long fiber proteins. However, it should be mentioned, that FAdV strains isolated in 2012 and 2013 differ from each other in studied genome fragments in a similar range as from virus isolated from gizzard erosions case in broilers identified in the same territory (Poland), but in different time (4-5 years earlier) and even from virus detected in country very distant spatially (Japan) and temporarily (12-13 years earlier) (Domanska-Blicharz *et al.*, 2011; Thanasut *et al.*, 2017). It would be interesting to determine the whole genome sequence of these strains as well as to check them *in vivo* whether the identified changes are related to the pathogenicity of these strains.

In presented cases, clinical symptoms of the disease were observed only in a minute percentage of flocks. The presence of fat in the abdominal cavity and around organs as well as properly functioning reproductive system with fully developed yolks in ovary and shelled eggs in uterus or vagina of dead layer chickens suggested a very fast course of the disease. Furthermore, there was no reduction in overall flock performances. Recent observations of the effects of FAdV-A infection in adult chickens are contradictory; in some cases older chickens were not susceptible to FAdV-A while in others they were vulnerable and developed clinical signs of disease (Grafl *et al.*, 2012; Matczuk *et al.*, 2017; Naseem *et al.*, 2018). Similar to herein described lack of disturbances in flock performance was outlined by Grafl *et al.* (2012) during the outbreak of gizzard erosions in Germany. Broiler breeders which vertically transmitted FAdV-A to broilers did not manifest any clinical signs and egg production remained normal (Grafl *et al.*, 2012). In turn, clinical signs as elevated mortality rates, reduction in laying performance and decreased total egg weight were observed in FAdV-affected flock of laying hens at 38 weeks of age (Matczuk *et al.*, 2017). This may suggest the participation of other factors in the induction of clinical symptoms of FAdV-A infection of chickens, however, not identified in either paper cited.

We have traced the weather conditions that prevailed in time of presented outbreaks and they appeared to be of particular interest. In the years 2012 and 2013, the disease occurred in February, and in the year 2015 in February and June. The winter in 2012, a few days before disease occurrence, was very frosty, with the temperature

dropping even below  $-30^{\circ}\text{C}$ . The weather one year later was warmer, but again the temperature a few days before start of clinical symptoms of disease fell below  $-15^{\circ}\text{C}$ . Similarly, in February 2015 the temperatures again dropped below  $-10^{\circ}\text{C}$  during a few nights just before gizzard erosions outbreaks. In turn, in June 2015 the temperature anomaly was observed which involved a sudden drop from over  $30^{\circ}\text{C}$  to less than  $12^{\circ}\text{C}$ . The winter of 2014 was the 9<sup>th</sup> warmest winter since the mid-twentieth century and this fact seems to correlate with the lack of clinical signs of disease. Recently, it was shown that heat stress induced negative effects on immune function as well as changes in the intestinal mucosa of young chickens (Quinteiro *et al.*, 2012). Furthermore, an increase in temperature of 8 degrees for several hours over a few days immunomodulated the immune response of chickens to the vaccine against Newcastle disease (Honda *et al.*, 2015). It couldn't be excluded that sudden temperature fluctuations accompanying observed disease cases altered the immune system ability to function normally, making some birds more sensitive to infection with FAdV but the influence of other contributory factors not assessed in this study was also possible.

**Conclusions:** In this study, we have described an outbreak of gizzard erosions in commercial layer flocks at the age of 19, 25, 30 and 73 weeks caused by FAdV-A. Our observations suggest that sudden temperature fluctuations which could have disturbed the immunity of adult chickens were the factor that influenced the emergence of clinical signs of gizzard erosions caused by adenovirus infections in described cases.

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**Authors contribution:** KDB participated in conceiving, designing and coordination of the study, general supervision of the research group, and drafting of the manuscript. RB participated in material and data collection in the field, described the gross lesions. All authors read and approved the final manuscript.

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