

Research Note: An overview on distribution of fowl adenoviruses

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ABSTRACT Fowl adenoviruses (FAdV), detected during routine diagnostic investigations from 38 countries (5 continents) over a decade, were partially sequenced and grouped by phylogenetic analysis. The partial polymerase gene nucleotide sequences of the 365 fowl adenovirus isolates resulted in the following species distribution: 11% FAdV-A; 3% FAdV-B; 2% FAdV-C; 34% FAdV-D; and 50% FAdV-E. Noticeably, only 79 of the detected strains could be associated with adenovirus-specific pathologic conditions: 62 (79%) with inclusion body hepatitis; 9 (11%) with gizzard erosion; and 8 (10%) with hepatitis hydropericardium syndrome. The remainder of the FAdV strains was detected as

concomitant infection from other disease conditions almost exclusively in boilers of 27 to 42 d of age: the majority of them was FAdV-E followed by FAdV-D, and to a lesser extent of FAdV-A, B, and C, the latter ones have not been associated with any of the established adenovirus-caused syndromes in our collection. The highest ratio of coinfections was observed for FAdV-B (62%), while it was about 30% for the rest of the FAdV species. The most frequent coinfection, in connection with all FAdV species, was with the avian infectious bronchitis virus. The presented database will serve as the basis for comparative whole genome and cross-neutralization analysis of selected FAdV isolates.

Key words: fowl adenovirus, distribution, epidemiology, prevalence, chicken

2021 Poultry Science 100:101052
<https://doi.org/10.1016/j.psj.2021.101052>

INTRODUCTION

Fowl adenoviruses (FAdV) have been getting into focus more frequently owing to the increasing awareness of their spread and associated disease conditions. For a recent review, refer to the study by Schachner et al. (2018).

Fowl adenoviruses are medium-sized, nonenveloped icosahedral DNA viruses that were recognized in the middle of the last century and have since been reported to affect poultry globally (Harrach et al., 2019), though FAdV can be demonstrated from birds regardless of disease conditions (McFerran and Smyth, 2000). The initial skepticism regarding FAdV as primary pathogens for poultry was gradually replaced by the awareness of their role in several, by now well-characterized syndromes, such as inclusion body hepatitis (IBH), hepatitis hydropericardium syndrome (HHS), and gizzard erosion (GE) and ulceration, affecting primarily young broiler chicks (Hess, 2017). The 12 described serotypes (1 to 8a and

8b to 11) are classified into 5 species from FAdV A through E (Hess, 2017; Schachner et al., 2018). Inclusion body hepatitis was mainly associated with species D (FAdV serotype 2 and 11), but in recent years, FAdV E (FAdV serotypes 8a and 8b) strains have also been reported as etiologic agents for the syndrome (Kajan et al., 2013). Species C (FAdV serotype 4) and species A (FAdV serotype 1) strains were identified as causative agents for HHS and GE, respectively (Harrach et al., 2019), though FAdV-D was already identified from HHS cases and the disease was reproduced experimentally with the isolate (Zhao et al., 2015). The main economic impacts are seen in relation with IBH and HHS (Ojkic et al., 2008).

Fowl adenovirus infection of poultry may occur at any age, the severity of lesions is directly related to the age of the birds and the level of maternally derived antibodies. The most serious clinical and economic impact is seen in young birds. Beyond the aforementioned ones, the outcome of the infection depends on several other factors such as pathogenicity of the virus strain and immunosuppressive conditions. Vertical transmission may result in significant mortality (up to 80–85%). Vaccination is practiced to reduce the losses, including either live or inactivated vaccines, virus-like particles, subunit vaccines, commercial, and autogenous products (Hess, 2017; Schachner et al., 2018).

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Received December 15, 2020.

Accepted February 4, 2021.

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Taking into account that there is a limited range of commercially available registered vaccines to control FAdV infections, autogenous vaccines are expected to provide a solution for the poultry industry. For that purpose, knowledge on the prevalent FAdV types is crucial, and furthermore, the obtained data provide useful information on the role of FAdV in the different disease conditions.

In alignment with that, the aim of this investigation was to assess the diversity of FAdV based on the records of our laboratory, originating from poultry diagnostic sample submissions over more than a decade (i.e., 2008–2019) from broad geographical sources.

Fowl adenovirus infections associated with well-defined disease conditions can be diagnosed on the basis of flock disease history, necropsy and histopathology findings, and confirmed by virus isolation, and PCR (Ojkic et al., 2008).

MATERIALS AND METHODS

Samples

During the routine virus detection and characterization processes by using standard diagnostic techniques, for example virus isolation in embryonated eggs and cell cultures (LMH, chicken hepatocellular carcinoma cell line; CEF, chicken embryo fibroblast), and detection of virus-specific nucleic acid by molecular diagnostic assays (traditional and real-time PCR, restriction fragment length polymorphism, nucleotide sequencing), several viral pathogens of chicken have been detected at the Scientific Support and Investigation Unit, Ceva-Phylaxia Co. Ltd., Budapest, Hungary, over more than a decade (2008–2019).

Various organ samples (liver, spleen, gizzard, respiratory and gastrointestinal organs, bursa, caecal tonsil, kidney) were submitted from the chicken (*Gallus gallus domesticus*) either with or without adenovirus-specific diseases (e.g., IBH; HHS; GE), in the latter case with other disease suspicion or with monitoring purposes from geographically diverse regions (Figure 1). Either organ samples or their prints on FTA Cards (Flinders Technology Associates, Whatman) were submitted for testing.

Those samples that proved to be positive by virus isolation for FAdV were selected for further analysis. From 193 submissions, representing 38 countries through 5 continents, 570 samples were found as FAdV positive. Three hundred sixty-five selected FAdV sequences – representing all the 570 positive samples on the flock level – were characterized by partial nucleotide sequencing and phylogenetic analysis of the polymerase gene.

Data Analysis

A database was constructed with BioNumerics database management software (Biomerieux, Applied Maths, NV) containing relevant/available metadata

(e.g., sampling yr, geographic region of origin, coinfecting agents, see Supplemental Table 1.).

Nucleic Acid Extraction

DNA was extracted from both organ samples (homogenized in PBS in a ratio 1:10) and FTA prints using a QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) as per the manufacturer's instructions. If the sample was tested for RNA viruses, RNA was extracted from organ samples with an RNeasy Mini Kit (Qiagen, Hilden, Germany), while a MagMAX Viral RNA Isolation kit (Thermo Fisher Scientific) was used for RNA extraction from FTA prints.

Adenovirus Detection

Universal adenovirus DNA polymerase gene-specific nested PCR was performed as per a published protocol (Kajan et al., 2011). The primers were designed to amplify the genomic region nt 8912 to 9183 of a FAdV 2 strain, 685 (accession number, KT862805) and were shown to be able to detect any adenovirus species with appropriate sensitivity.

Virus Isolation

The adenovirus PCR-positive organ sample homogenates were inoculated on chicken hepatocellular carcinoma cell line LMH. When cytopathogenic effect indicated the replication of adenovirus, confirmation by the PCR test described previously was performed. Adenovirus isolates have been stored in a deep freezer at -70°C.

Histopathology

The tissue samples were fixed in 10% neutral buffered formalin and processed by routine paraffin-embedding technique. Briefly, 4- to 5-µm-thick sections were deparaffinized and stained by hematoxylin and eosin staining method for detailed microscopic examination.

Nucleotide Sequencing

Sanger sequencing of the nested PCR product was performed by the Biomi Ltd. (Gödöllő, Hungary) using a BigDye Terminator v3.1 Cycle Sequencing Kit. The resulting 272-bp-long partial pol gene sequences were aligned, and isolates were identified on species level (e.g., FAdV A-E; goose adenovirus; duck adenovirus; and turkey adenovirus).

Phylogenetic Analysis

Maximum parsimony tree of the sequences was constructed using the “Advanced Cluster Analysis” tool of BioNumerics software. For compact representation, the

unrooted spanning tree branch lengths were transformed to logarithmic scale.

RESULTS AND DISCUSSION

Clinical samples were received from almost all over the world (Figure 1). The sample history was either suggestive of FAdV-related syndromes or unrelated to that. In the former case, targeted investigations were pursued, such as detection and isolation of the virus, and histopathologic examinations. In the latter case, the additional diagnostic tests revealed the presence of FAdV in many cases.

The IBH, HHS, and GE cases were diagnosed on the basis of case history, including postmortem gross pathologic investigations, where available, the characteristic histopathologic findings (e.g., degeneration of hepatocytes, necrosis, mononuclear cell infiltration, and the presence of basophilic intranuclear inclusion bodies for IBH; hydropericardium and inflammation in the heart muscles for HHS; gizzard lesions, ulceration, erosion for GE), accompanied by isolation of FAdV from the relevant organs.

The samples originated mainly from South-East Asia, while the lowest number of samples was submitted from Central Asia. The geographic distribution of the origin and approximate ratio of the samples are shown in Figure 1.

Most FAdV-positive cases were recorded in South East Asia (proportional to the submitted samples ratio), but there were positive cases from all regions included in the study.

The partial polymerase gene phylogeny allowed the species level identification (Kajan et al., 2011), and as per that, half (50%, 181 samples) of the detected strains belonged to species E, while species C was present only in 2% of the samples (6 samples; Figure 2).

All 5 FAdV species were detected in South-East Asia and in Europe; however, the sample numbers were unevenly distributed among the geographic regions.

Regarding the phylogenetic grouping of the polymerase gene fragment, there were larger homogenous groups with a global distribution, but evidently, individual or distinct smaller groups could also be observed (Figure 2). The relevance of this separation could further be confirmed or rather refined by analyzing larger genomic fragments.

The majority of detections could not be linked to established FAdV-related syndromes, that is 78, 100, 100, 82, and 72% for FAdV-A, B, C, D, and E, respectively.

Concerning the FAdV-associated syndromes, confirmed by histopathologic examination (data not shown), FAdV-D and FAdV-E strains were identified in 24 and 76% of the total number of IBH cases, respectively (Figure 2). The majority of the positive samples originated from broilers at around 30 d of age but in the range of 10 to 44 d of age. Furthermore, some breeders were found to be affected as early as 7 and 18 d of age.

Gizzard erosion condition was already described in the 1930s, while its first association with FAdV-1 was established in 2001 only (Ono et al., 2001) but sporadically with FAdV-8 also (Mase and Nakamura, 2014). The disease was mainly found in Europe and Asia. We also found it in association with FAdV-A and in samples originating from South East Asia and Southern and Central Europe. Regarding the age distribution, where it was known, it ranged from 23 d of age (broilers) to 189 (layers) d of age.

Hepatitis hydropericardium syndrome or Angara disease was first described in Pakistan in the late 1980s (Jaffery, 1988; Schachner et al., 2018), which subsequently spread over Asia, parts of Middle-East countries, and Latin America. We diagnosed the disease in low representation (8 cases) and in association with species D and E viruses at negligible proportion (5.6 and 1.6%, respectively). All cases originated from South East Asia, the Middle East, and South America. It was demonstrated in broilers and breeders of 7 to 23 d of age. Finding FAdV-E in a reportedly HHS field case is unusual, since it has yet been reported from experimental infections in SPF chickens (Nakamura et al., 2000). Nevertheless, it could also be the consequence of the simultaneous presence of different FAdV species in flocks/individuals and the shift of virus species composition on isolation. Such phenomenon, which otherwise would provide opportunities for recombination between the FAdV strains (Schachner et al., 2019), could best be revealed by deep sequencing, which was beyond the scope of this study.

FAdV-B and C were not found to be associated with any of the established syndromes in our collection, which could either be the consequence of the older age of birds the samples originated from and the virulence characteristics of the identified strains (Liu et al., 2016).

The relatively low recognition of FAdV-related syndromes should be evaluated taking into consideration that FAdV may cause subclinical infections, which calls for the evaluation of the zootechnical performance of the birds to demonstrate the detrimental effect of viral infection (Matos et al., 2018). To prove the effect of subclinical adenovirus infection, however, careful investigation to exclude the effects caused by concomitant infection with other agents and by nutritional or husbandry problems is needed.

The age of the FAdV-positive birds with syndromes ranged between 7 and 280 d of age, 50% of that between 25 and 35 d of age, while in the detection with no established syndromes, it was 9 to 245 days. In this latter case, one third of the submissions did not provide the age-related data, while 40% of the FAdV detections were carried out from birds between 30 and 42 d of age. Furthermore, from the nonsyndrome-related detections, FAdV-E was isolated in most of the cases (46%), followed by FAdV-D (36%), FAdV-A (11%), FAdV-B (5%), and FAdV-C (2%).

Because the resolution or cross-reactivity of the available serologic tests is not informative enough and we did not have such data anyway, it is assumed that the

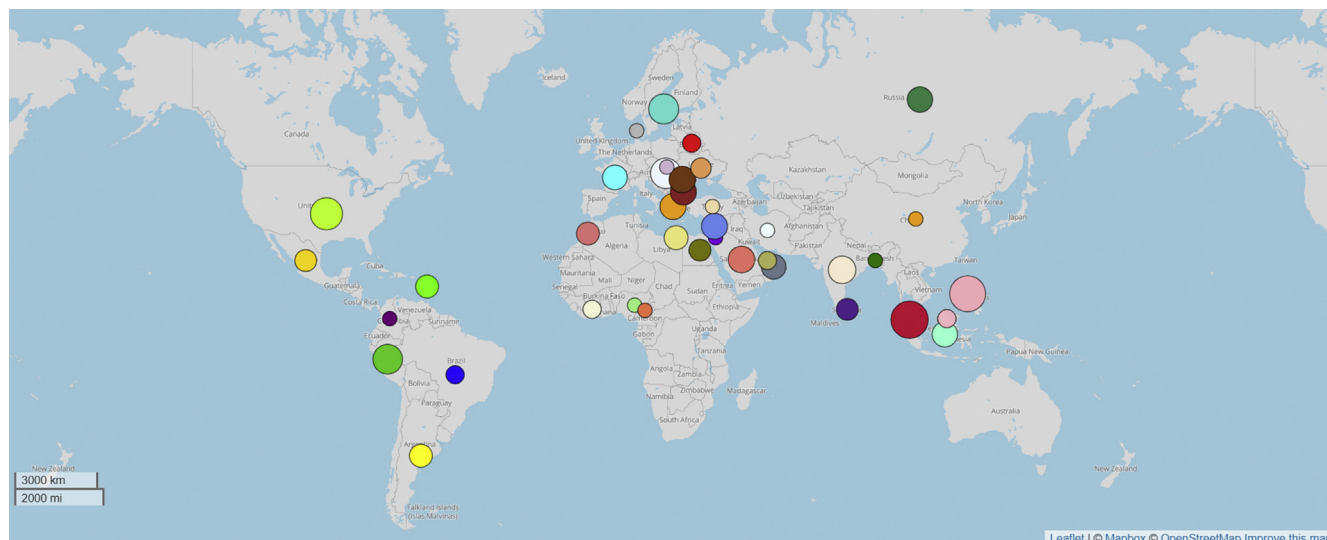


Figure 1. Geographical origin of the FAdV-positive submitted samples. The sizes of circles are indicative of sample numbers but not in a linear correlation. The map was prepared by MicroReact visualization software (Argimon et al., 2016). Abbreviation: FAdV, fowl adenovirus.

depletion of maternally derived antibodies against FAdV preceded this period of time.

Regarding the coinfecting agents adjoining the established syndromes, IBH was found in connection with reovirus (FAdV D), very virulent Gumboro virus (FAdV E), and IBV (FAdV D), while GE was found in connection with MDV (FAdV-A).

The highest ratio of the coinfections was detected in connection with species B (62%), while it was rather homogenous among the other species, for example 35, 33, 36, and 29% for species A, C, D, and E, respectively, mainly including IBV.

The steady improvements of molecular techniques used in routine diagnostic procedures led to more

frequent detection of numerous pathogens, which were previously considered and presented as secondary agents. The coevolution of such pathogens and their hosts certainly also has contributed to their increasing role and recognition in the poultry industry.

The purpose of this study was to investigate the ratio of FAdV in diagnostic sample submissions with or without the history of suspected FAdV-associated disease conditions. We used a conservative approach for FAdV detection: a PCR targeting a portion of the viral polymerase gene, which enables broad-range detection of the target viruses with relatively high sensitivity (Kajan et al., 2011). The species based classification of field isolates was performed by phylogenetic analysis of the

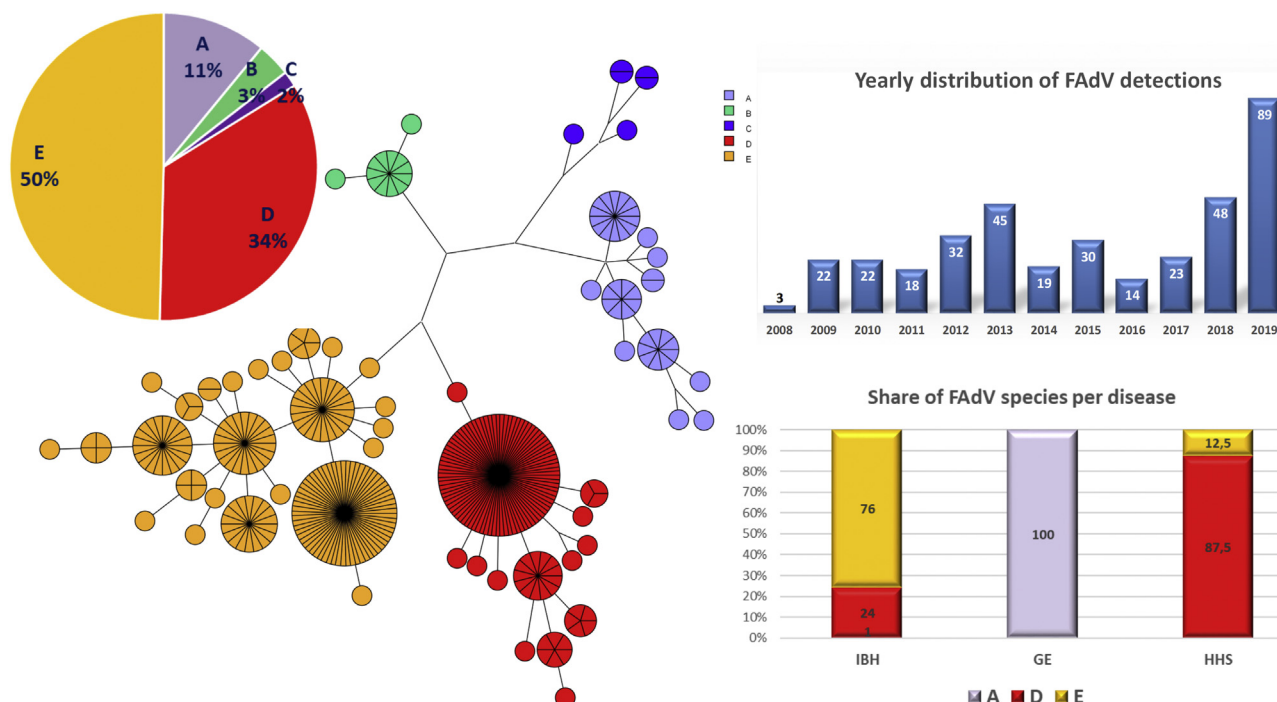


Figure 2. FAdV species distribution, phylogeny, yearly detection rate, and species ratio in the associated diseases. Abbreviation: FAdV, fowl adenovirus.

partial DNA polymerase gene. This approach was indicative of the serotype also, but for the correct nucleotide sequence-based serotype assignment, the characterization of other genes, especially the hexon (gene), is necessary.

Our broad-scale collection provided an overview of the occurrence of FAdV and further contributed to the preexisting findings on FAdV species and disease syndrome association. Notably, FAdV-E was found to be a predominant species in our collection of FAdV isolates regardless of its association to established syndromes or not. We confirmed the primary association between FAdV infection and GE, HHS, and IBH; however, FAdV were also isolated from other diseases, mainly as coinfecting agents. The synergistic etiologic role of FAdV in these diseases, if any, needs to be better understood.

It is also noteworthy that one third and two third (regarding the different FAdV species) of detections was part of coinfections, highlighting the epidemiologic complexity of poultry health management.

Further analysis of the viral genome and associated disease forms is warranted as it was demonstrated, for example, that nucleotide sequences of the short fiber genes of FAdV-4 isolates differed between HPS and non-HPS case-related viruses (Mase et al., 2010).

In alignment with that and based on this analysis, representative strains will be selected from the established groups of the strains (based on phylogeny, geographical origin, syndrome relatedness) and submitted to whole genome sequencing. In addition, strains will be selected for immunization of chickens to perform cross neutralization tests for deeper molecular and antigenic characterization.

ACKNOWLEDGMENTS

KB was supported by the Hungarian Scientific Research Fund (NKFI-OTKA K120201). Edit Fodor is acknowledged for the excellent technical assistance.

DISCLOSURES

The authors declare no conflicts of interest.

SUPPLEMENTARY DATA

Supplementary data associated with this article can be found in the online version at <https://doi.org/10.1016/j.psj.2021.101052>.

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