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MICROBIOLOGY

Fowl Aviadenovirus E associated with hepatitis-hydropericardium syndrome in broiler breeders

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ABSTRACT: *Fowl aviadenovirus (FAdV) is an important pathogen in the global poultry industry and the etiology of inclusion body hepatitis-hydropericardium syndrome (HHS) in chickens. Since the 1990s, several outbreaks of HHS have occurred in poultry producing areas, including South America. The coinfection of FAdV and chicken anemia virus (CAV) may markedly impact the incidence of HHS. This study describes an outbreak of HHS in coinfection with CAV in industrial broiler breeders and characterizes the FAdV isolate. The three-week-old male broiler breeders had pale bone marrow, enlarged and yellowish liver, splenomegaly, and atrophied thymus; one chicken was also found with hydropericardium. Virus isolation was performed in SPF chicken embryos of liver and thymus. Tissues of the naturally infected chickens and the inoculated embryos were evaluated by PCR and histopathology. All affected chickens and inoculated embryos were positive for FAdV and CAV. The inoculated embryos had enlarged, greenish and hemorrhagic livers, and 30% died within 7 days of inoculation. Phylogenetic analysis of the FAdV isolate hexon gene partial sequence enabled grouping with E species. The E species has recently become a relevant species in several countries. The association of FAdV with CAV in breeders is of further concern due to both being capable of vertical transmission. Within the last decade, a worldwide upsurge of HHS in broiler breeders owing to failed biosecurity has occurred. In this episode, the failure on biosecurity may have enabled challenge with both FAdV and CAV, with pathological synergism. The CAV-impaired adaptive immunity may have benefited the FAdV infection.*
Key words: *Aviadenovirus E, CAV, chicken anemia, FAdV, Gallus gallus domesticus, hepatitis, inclusion body hepatitis, liver disease.*

Síndrome da hepatite-hidropericárdio por Aviadenovirus e em reprodutores de corte

RESUMO: *Adenovírus aviário (FAdV) é um importante patógeno na indústria avícola global e a etiologia da síndrome da hepatite por corpúsculo de inclusão-hidropericárdio (SHH) em galinhas. Desde a década de 1990, vários surtos de SHH foram descritos em todas as áreas de produção de aves, incluindo na América do Sul, e a coinfecção entre FAdV e vírus da anemia das galinhas (CAV) pode ser agravante para todos os aspectos da SHH. Objetiva-se descrever um surto de SHH em matrizes de frangos corte, caracterizar a estirpe de FAdV envolvida e destacar a coinfecção com CAV. Foram avaliados machos reprodutores de corte com três semanas de idade, com medula óssea pálida, fígado aumentado e amarelado e esplenomegalia, timo atrofiado e um com hidropericárdio. Fígado e timo foram coletados para isolamento do vírus em embriões de galinhas SPF, PCR e histopatologia. Todas as aves acometidas e embriões inoculados foram positivos para FAdV e CAV. Os embriões inoculados tiveram óbito de 30% em até sete dias após a inoculação e alterações hepáticas por fígados esverdeados e aumentados. A análise filogenética de FAdV com base em parte da sequência do gene que codifica a proteína hexon revelou identidade com a espécie E, que se tornou disseminada em vários países. A coinfecção de FAdV e CAV resulta em maior intensidade de lesões, maior morbidade e mortalidade e em reprodutores tem alta relevância epidemiológica, em razão da transmissão vertical de ambos e da ampla distribuição geográfica das progênies infectadas. Na última década, ocorreu um aumento mundial na ocorrência de SHH em frangos de corte relacionado a falhas em biosseguridade, especialmente em reprodutores, condição que pode ter ocorrido neste episódio. A presença de FAdV e CAV em reprodutores é motivo para preocupação por reflexos negativos à imunidade e viabilidade das progênies.*
Palavras-chave: *Aviadenovirus E, CAV, anemia das galinhas, FAdV, Gallus gallus domesticus, hepatite, hepatite por corpúsculo de inclusão, doença hepática.*

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INTRODUCTION

Adenoviridae contains five genera, with three found in chickens, namely *Atadenovirus*, *Aviadenovirus*, and *Siadenovirus*. In *Aviadenovirus*, Fowl aviadenovirus is subdivided into five species, A to E, and 12 serotypes (1-8a-8b-11), based on molecular and serum neutralization analyses, respectively. *Aviadenovirus* has been identified in several avian species, such as chicken (fowl aviadenovirus), duck, falcon, goose, pigeon, psittacines (ICTV, 2011; HESS, 2013; MAREK et al., 2014), and curassow (MARQUES et al., 2019).

All species and serotypes of *Aviadenovirus* occur globally (HESS, 2013). Fowl Aviadenovirus (FAdV) species (A to E) may be the cause of inclusion body hepatitis (IBH), and inclusion body hepatitis-hydropericardium syndrome (HHS), which are liver-cardiac-circulatory diseases, potentially also involving the digestive and respiratory systems (HESS, 2013; ONO et al., 2007). FAdV has also been detected in clinically normal chickens, suggesting the relevance of aggravating factors on pathology (KAJÁN et al., 2013; NICZYPORUK et al., 2016; MCFERRAN & SMYTH, 2000). A strain of FAdV-A, but not FAdV-E, was associated to ventricular erosion in replacement layers (GRAFL et al., 2018).

The pathogenesis of FAdV, despite being considered primary (HESS, 2017), may be associated with pathogens that impair the immune system, such as chicken anemia virus (CAV, *Gyrovirus*, Anelloviridae) and infectious bursal disease virus (IBDV, *Avibirnavirus*, Birnaviridae) (TORO et al., 2000); however, a study of coinfections in Canada did not find a statistical association between these viruses (EREGAE et al., 2014). The vertical transmission of FAdV is considered to be the most important variable associated with early age disease, which usually occurs at one to three weeks post-hatching (TORO et al., 2001).

Increasing cases of HHS have been reported worldwide. For instance, during 2011–2013, strains involved in HHS were mainly found to be of the FAdV E species (serotype 8b) and D species (serotype 11) in Spain (OLIVER-FERRANDO et al., 2017). Further, studies between 2015 and 2019 (CHEN et al., 2019) revealed the occurrence of C (FAdV-4) and E (FAdV-8a and 8b) species in the broiler industry in China. Based on studies during 2012–2019, FAdV-8b (E species) is the most prevalent strain in South Korea, thereby substituting FAdV-4 (C species). According to the authors, FAdV-8b emergence may be a consequence of FAdV-4 vaccines; thus, they recommended that such vaccines be updated (LAI et al., 2021). However, serotype 4 remains as the most prevalent in Mexico (VERA-HERNANDES et al., 2016). The impact of HHS on the performance of broilers has been associated with reduced activity and apathy, resulting in poor growth (SCHACHNER et al., 2018).

The contamination of live poultry vaccines with FAdV in addition to CAV and avian *Orthoreovirus* was previously described (BARRIOS et al., 2012), and may have modified the epidemiology of these viruses in the field.

The aim of this study was to describe the viral isolation and hexon gene-based molecular characterization of a FAdV strain coinfected with CAV in an outbreak of HHS in broiler breeders.

MATERIALS AND METHODS

Broiler breeders

Three-week-old broiler breeder chickens of flocks I (n = 3) and II (n = 5) were examined in this study and original from the same breeding company and hatch. Males only of mixed male and female flocks, of the same hatch and origin, were selected in reason of virus presence semen (HESS, 2013) and the excess of males due to the regular use of 10% male to female ratio during mating (AVIAGEN, 2022). Both flocks were vaccinated against Marek's disease via subcutaneous injection at hatching (HVT). An intermediate infectious bursal disease vaccine strain was administered to the 15-day-old chickens. The grandparent stocks were administered regular infectious bronchitis, infectious bursal disease, and Newcastle disease active virus vaccines at an early age, and an inactivated multivalent oil-emulsion vaccine containing the above vaccines and reovirus at puberty. No CAV vaccines were administered to grandparents or their progeny stocks. Chickens of flocks I and II had mortality rates of 4.5% and 1.2%, respectively. Flocks were obtained from a biosecurity managed farm. At necropsy, tissue samples (liver, spleen, and thymus) were collected and frozen (-20 ºC) for FAdV isolation or PCR detection, or fixed in neutral-buffered 10% formalin for histopathology.

FAdV isolation

Livers were homogenized with a mortar and pestle in equal parts with sterile 1X PBS and centrifuged (2000 *g* for 10 min at room temperature). The supernatant was collected and treated with antibiotics and antimycotic (potassium penicillin G 1,000 units, streptomycin 500 µg, and amphotericin

B 1 µg/mL) for 30 min at 25 °C. After 10 days of incubation, 10 specific pathogen-free chicken eggs (Valo Biomedia, Uberlandia, Brazil) were inoculated with the liver extract (0.1 mL) via the chorioallantoic membranes (CAMs) and candled daily. Seven days post-infection, embryos and CAMs were examined, and CAMs were collected for histopathology and PCR analysis.

Histopathology

Tissue samples of the necropsied chickens, including the liver, spleen, and thymus, were fixed in 10% buffered formalin, routinely processed, embedded in paraffin, and sectioned at 4 µm thickness for hematoxylin-eosin (HE) staining (LUNA, 1968). The chorioallantoic membranes of the inoculated embryos were also collected for histopathological analysis.

PCR

The total DNA of tissues (liver, spleen, and thymus) or embryo (CAM) was extracted using the chaotropic agent sodium iodide (6 M; VOGELSTEIN & GILLESPIE, 1979; BOOM et al., 1990). DNA purification was performed using silicon dioxide microspheres (BOOM et al., 1990), and the concentration and quality of DNA were determined (NanoVue®, GE Healthcare, Chalfont, UK).

All amplifications were performed in a thermocycler (MaxyGene®, Axygen, Union City, California, USA; or PTC 100®, MJ Research, Watertown, MA, USA; or Mastercycler® Nexus, Eppendorf, Hamburg, Germany) according to previously described protocols (MEULEMANS et al., 2001; CARDONA et al., 2000). The primer oligonucleotides were obtained from Thermo Fisher Scientific (São Paulo, Brazil). To detect Aviadenovirus FAdV DNA, primers A 5'- CAARTTCAGRCAGACGGT-3' and B 5'-TAGTGATGMCGSGACATCAT-3' were used to amplify a conserved sequence adjacent to the L1 loop of the hexon protein gene (MEULEMANS et al., 2001). The FAdV Phelps strain, which served as the positive control, was kindly provided by Dr. Jane K.A. Cook (Houghton Trust, Huntingdon, UK). To detect CAV DNA, a nested PCR protocol was employed with external primers O3F: 5'-(CAAGTAATTTCAAATGAACG)-3' and O3R: 3'-(TTGCCATCT TACAGTCTTAT)-5', and internal primers N3: 5'-(CCACCCGGACCATCAAC)-3' and N4: 3'-(GGTCCTCAAGTCCGGCACATTC)-5', to amplify a segment of the VP1 gene (CARDONA et al., 2000). The CAV positive control DNA was obtained from a commercial vaccine (AviProThymovac®, *Lohman Animal Health*, Elanco, Santo Amaro, Brazil). A FAdV broiler strain (01/2019) (MW 960053), detected in a previously observed HHS case (unpublished), was included in the evaluation to enable a comparative analysis.

The amplified products were evaluated by electrophoresis [agarose 1.5%, 100V in TBE (100 mM Tris-base pH 8.3, 25 mM EDTA and 50 mM boric acid)] and the DNA was assessed using *GelRed*® (Biotium, Fremont, CA, USA) at UV light (280 nm).

Sequencing and phylogenetic analysis

The PCR amplified FAdV products were sequenced using dideoxinucleotide (SANGER et al., 1997) in a capillary automated sequencer (ABI 310®, Applied Biosystems, Thermo Fisher Scientific, Waltham, Massachusetts, USA), with Big Dye Terminator Mix® (Thermo Fisher Scientific, Waltham, MA, USA) according to the manufacturer's instructions. The obtained sequences were deposited under accession numbers MN462585 and MN462586, and were evaluated using Sequencing Analyses Version 5.2 (Applied Biosystems). The algorithms BLAST 2.0 (Basic Local Alignment Search Tool) and BLASTn (https://blast.ncbi.nlm.nih.gov/Blast.cgi?-PAGE_TYPE=BlastSearch) were employed for sequence identity search (ALTSCHUL et al., 1997) and alignment to published sequences in GenBank (National Centre for Biotechnology Information, NCBI, http://www.ncbi.nlm.nih.gov/). Alignments and dendrogram trees were constructed using neighbor-joining clustering and substitution methods in MEGA 10 (KUMAR et al., 2018). The evolutionary history was inferred using the Maximum Parsimony method. The percentage of replicate trees, where Aviadenovirus hexon sequences clustered together in the bootstrap (TAMURA et al., 2004) test (1000 replicates), was displayed next to the branches (FELSENSTEIN, 1985). The MP tree was obtained using the Subtree-Pruning-Regrafting (SPR) algorithm (NEI & KUMAR, 2000). The analysis involved 40 nucleotide sequences. All positions with less than 70% site coverage were eliminated (i.e., fewer than 30% alignment gaps, missing data, and ambiguous bases were allowed at any position [partial deletion option]). A total of 559 positions were included for each sequence in the final dataset. All evolutionary analyses were conducted in MEGA X (KUMAR et al., 2018).

RESULTS

Necropsy

The major macroscopic changes for chickens of flock I included enlarged and pale-red livers (3/3) (Figure 1a), hydropericardium (1/3), pale-

red kidneys (3/3, atrophy of the thymus and cloacal bursa (3/3), pale-red to yellowish femoral bone marrow (3/3), and pale conjunctivae (3/3). No macroscopic lesions were found in chickens of flock II.

Histopathology and virus isolation

Histologically, the livers of three and two chickens of flock I and flock II, respectively, had multifocal areas with moderate lymphocytic, histiocytic, and plasma cell infiltrations, and areas of vacuolar degeneration and lytic necrosis of hepatocytes (Figure 1b). There were also numerous hepatocytes with basophilic intranuclear inclusions that filled the entire nucleus and displaced the chromatin to the periphery. Few hepatocytes with eosinophilic intranuclear inclusion bodies comprising a halo were also found. In two chickens of flock I, intranuclear inclusions in the macrophages of spleen, similar to those observed in the liver, reticular cell hyperplasia, and rarefaction of lymphoid follicles were present. The thymus was reduced in size due to the rarefaction of lymphocytes. In a few epithelial cells of thymus, it was possible to observe eosinophilic intranuclear inclusion bodies.

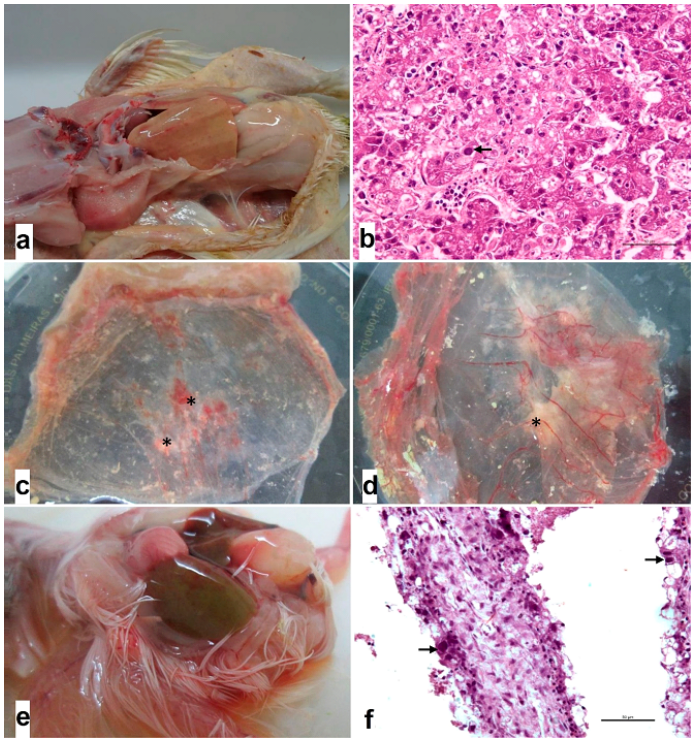

Figure 1 - a. Three-week-old broiler breeder. Note the enlarged, diffusely yellow liver, with multifocal millimetric red areas. b. Microscopy of liver showed in a. Necrotic hepatocytes are seen in the central area associated with mild mononuclear cells infiltration. At the periphery there are hepatocytes with basophilic nuclear inclusion bodies filling the entire nucleus and dislocating the chromatin to the periphery (arrow). There is also lipidic degeneration. HE. Bar = 50 µm. c, d. Chorioallantoic membranes. At seven days post-infection of SPF chicken embryos, some thick and whitish foci were observed (*). e. Enlarged, greenish and hemorrhagic hepatic changes in the inoculated embryo. This embryo survived the seven days of infection, up to the 17 days of incubation, although 3/10 of the inoculated embryos died. f. Microscopy of chorioallantoic membrane (Fig. 1c or 1d) of FAdV infected embryo 7 dpi. Basophilic nuclear bodies (arrows) are present in the mesoderm layer, with degeneration and necrosis of the chorionic epithelium. HE. Bar: 50µm.

At 7 days post-infection, chicken embryos had CAMs with multifocal whitish foci, edema, and thickening (Figure 1c–1d). The embryonic livers were enlarged, greenish, and had hemorrhage (Figure 1e). Of the 10 inoculated embryos, 30% died at 2 to 5 days post-inoculation. The histologic examination of CAMs inoculated with a liver extract from flock I revealed degeneration and necrosis in the chorionic epithelium, with basophilic nuclear bodies characterized by enlarged nuclei, mainly in the mesoderm layer (Figure 1f).

PCR and sequencing

The expected FAdV (897 bp) and CAV (183 bp) DNA products were detected in tissues (the liver, spleen, and thymus) of flock I, and CAMs of the inoculated embryos, but not of flock II. The obtained FAdV product sequences were aligned with sequences obtained in GenBank (Figure 2).

The evolutionary history, as inferred using the Maximum Parsimony method, enabled the clustering of *Aviadenovirus* hexon gene sequences with bootstrap (TAMURA et al., 2004) test (1000 replicates). The percentages of replicate trees are displayed next to the branches (FELSENSTEIN, 1985), indicating grouping the isolate with E species strains.

DISCUSSION

The breeders in this study and their progenitor stock (grandparent) were administered regular live and inactivated vaccines (infectious bursal disease, infectious bronchitis, Newcastle disease and reovirus) at puberty. However, no CAV vaccines were given, reducing the risk of confusion by the detection of a CAV vaccine strain. Chickens of flocks I and II had mortality rates of 4.5% and 1.2%, respectively, despite originating from the same biosecurity managed farm.

The macroscopic and microscopic lesions observed in the liver and heart were indicative of HHS, as described elsewhere (MAZAHERI et al., 1998). Unfortunately, because of the small sample size, the actual pathogenicity and respective lesions are unknown, and should thus be determined, possibly via experimental infections. Although, both flocks were derived from the same grandparent stock, differences in disease expression were reported, thereby highlighting the need for experimental infection studies to determine the necessary virus load for vertical transmission (MATOS et al., 2016; SCHACHNER et al., 2018). The grandparent stock was assumed to be protected and capable of transferring antibodies to the progeny (breeders of this study), such as to infectious bronchitis, infectious bursal disease, and Newcastle disease viruses. Despite CAV susceptibility, as no CAV vaccines were administered to the grandparent or their progeny stocks, the possibility of vaccine strain detection was considered negligible. However, the passive protection of flocks I and II, considering CAV and FAdV, might have been dissimilar, leading to differences in disease expression.

FAdV was successfully replicated in SPF chicken embryos and induced embryonic lesions similar to those described previously (ALEMNESH et al., 2012; COWEN et al., 1988), except for abundant basophilic intranuclear inclusion bodies, which were especially observed in the mesodermal layer of CAMs. Conversely, FAdV hepatocyte intranuclear inclusion bodies were previously reported to be mainly eosinophilic (ITAKURA et al., 1977).

The basophilic or eosinophilic properties of inclusion bodies may depend on the chronology of the infection. Further, the eosinophilic inclusion bodies are considered to be early empty capsid virion assembly sites that lack DNA (ITAKURA et al., 1977; SCHACHNER et al., 2018), and are superseded by the basophilic DNA-containing complete virions (MCFERRAN & ADAIR, 1977). In this study, the FAdV inclusions were mainly basophilic, suggesting that the evaluations occurred within sufficient infection time for complete DNA-containing particles formation. As the evaluation occurred on 21 day-old chicks, complete DNA-containing virus particle formation was enabled, aligning with previously described changes (GRIMES et al., 1978, MCFERRAN & ADAIR, 1977).

HHS is typically caused by FAdV strains of species D and E, and sporadic cases of the disease have been reported in broilers, breeders, and layers (CUI et al., 2020; SCHACHNER et al., 2018; MORSHED et al., 2017). The occurrence of strains of *Aviadenovirus* of D species was previously reported in Minas Gerais State, Brazil (PEREIRA et al., 2014). In our study, based on the comparisons of nucleotide composition to sequences of the L1 loop of the hexon protein gene (Figure 2), the strain detected in the breeders was considered to be belong to the E species. In São Paulo state, FAdV was associated with HHS in broilers (DE LA TORRE et al., 2018). The phylogenetic analysis has revealed that the HHS isolate 1904-2019 has been grouped with the E species has revealed that HHS strains have been grouped with the E species (FAdV-8a, FAdV-8b) or D species (FAdV-11). The detection of FAdV in 3-week-old breeders may indicate failed biosecurity at growing, but may also suggest a vertical

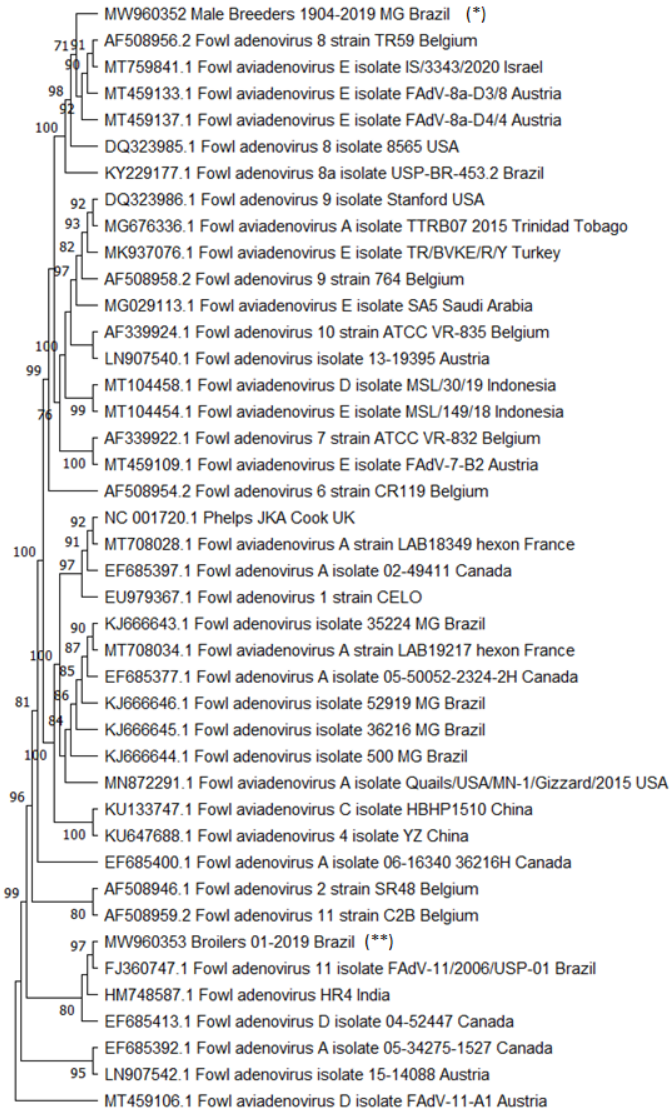

Figure 2 - Maximum parsimony phylogenetic analysis of *Aviadenovirus* hexon gene partial sequences.

The broiler breeder strain 1904-2019 (accession number MW960352) (*) involved in hepatitis-hydropericardium was grouped, with high confidence, with strains of E species. The identity to TR59 (AF508956.2) of Belgium (FAdV serotype 8) was of 99.6% in nucleotide composition. Closely related strains were IS/3343/2020 (MT759841.1) of E species of Israel, FAdV-8a-D3/8 (MT459133.1) and FAdV-8a-D4/4 (MT459137.1), described in broiler breeders in Austria, strain 8565 (DQ323985.1), described in case of inclusion body hepatitis the USA, and strain USP-BR-453.2 (KY229177.1), also typed FAdV 8a, described in normal broilers in São Paulo (Brazil). The broiler A species strain sequences obtained in 2014 in Minas Gerais, of isolates 500 (KJ666644.1), 35224 (KJ666643.1), 36216 (KJ6666645.1), 52919 (KJ666646.1), all detected in apparently normal broiler livers collected at processing plants, were closely related to the isolate LAB19217, described in pancreatitis in *Numida meleagris* in France (accession number MT708034.1). A recent broiler isolate 01/2019 (**) (MW960353) (unpublished), detected in broilers with hepatitis/hydropericardium was grouped (97% confidence) with a strain of D species (FAdV-11 /2006 of Brazil, FJ360747.1), described in the neighbouring state of São Paulo.

transmission; at vertical transmission, FAdV may be reactivated as maternal antibodies have waned (MCFERRAN & ADAIR, 1977). The infection of breeders will result in the generation of infected progenies (ADAIR & FITZGERALD, 2008), and infection may cycle through generations of breeders retained within imperfect biosecurity systems.

The coinfection of CAV and FAdV has been previously detected and described as a major cause of HHS, with the FAdV infection potentiated by the immune depression caused by CAV (TORO et al., 2000; 2001). In our study, atrophic thymuses were observed to be associated with the CAV detection (flock I), with a potential role in a reduced immune competence. The IBH-enhancing association of lymphocytic coinfections was previously found also for IBDV plus CAV in 51.5% of cases, and CAV was detected in all cases of clinical FAdV infection (CHOI et al., 2012). As a CAV vaccine was not administered to grandparents or their progeny stocks, such as the chickens evaluated herein, it is assumed that the accidental CAV challenge occurred via punctual biosecurity failure, which might explain also the FAdV infection.

The PCR protocol used in this study for FAdV was described as sensitive for the detection of any of the 12 FAdV serotypes (MEULEMANS et al., 2001). However, the results herein indicated that a single species was associated with the outbreak. Compared to published hexon gene sequences, the nucleotide composition of products and phylogenetic relationships, as determined by phylogenetic analysis, revealed the genetic identity to E species strain USP-BR-453.2 (KY229177.1), previously detected in São Paulo (DE LA TORRE et al., 2018), and may suggest an epidemiological association. The nested PCR protocol used for the detection of CAV DNA is highly specific and sensitive (CARDONA et al., 2000). CAV was previously detected in chickens (BRENTANO et al., 1991) and live vaccines (BARRIOS et al., 2012) in Brazil, suggesting that the synergistic association with FAdV might be underestimated in the country.

Despite being of a different species, FAdV A was more recently found to cause fatal hemorrhagic tracheitis in curassow in Brazil (MARQUES et al., 2019). Unpublished evidence of *Aviadenovirus* infection in native wild avian species in triage (article in preparation) has been demonstrated, indicating a complex natural local epidemiology for *Aviadenovirus*, aligning with previous reports (HESS, 2013). Nonetheless, unpublished information from abroad has revealed the occurrence of an uncharacterized FAdV infection in the precursor flocks of the genetic lineage studied herein.

The broiler breeder strain involved in HHS was grouped, with high confidence, with strains of E species, and had high genetic identity (99.6%) to strains TR59 (accession number AF508956.2) of Belgium; an FAdV serotype 8, IS/3343/2020 (MT759841.1) of Israel; FAdV-8a-D3/8 (MT459133.1) and FAdV-8a-D4/4 (MT459137.1) in broiler breeders in Austria; strain 8565 (DQ323985.1) described in IBH in the US; and strain USP-BR-453.2 (KY229177.1), with an identity of 99.3% with FAdV serotype 8a, described in normal broilers in São Paulo (Brazil) (DE LA TORRE et al., 2018).

The complexity of the local *Aviadenovirus* epidemiology may be underestimated. Previously, as we detected a D species isolate in normal broiler chickens (PEREIRA et al., 2014) and an A species isolate in young curassows with hemorrhagic tracheitis (Marques et al, 2019). The isolate 01/2019 (MW960353), detected in broilers with HHS in another outbreak in Minas Gerais in 2019 (unpublished), was grouped with 99% confidence with a strains of D species (FAdV-11 /2006, Brazil, FJ360747.1), described in the neighboring state of São Paulo (unpublished), and the same D species previously detected in Minas Gerais, Brazil (PEREIRA et al., 2014). *Aviadenovirus* D and E species were identified, with sharp increases from 2014, involved with HHS and HPS in the intensified poultry region of northern China (CHANGJING et al., 2016). Fowl adenovirus 11 (D species) was sequenced in Canada and six amino acid substitutions were detected in the L5 region, encoding three overlapping proteins; these changes were suggested to represent molecular markers for virulence (SLAINE et al., 2016). Such studies may validate the hypothesis of increasing virulence; however, it was not evaluated in our study. Previous isolates, obtained in broilers in 2014 in Minas Gerais (Brazil) (unpublished), including 500 (KJ666644.1), 35224 (KJ666643.1), 36216 (KJ6666645.1), 52919 (KJ666646.1), which were detected in normal broiler livers collected at processing plants, were phylogenetically grouped with A species. These strains had the closest identity (100%) to strain LAB19217 (MT708034.1), which is described to be involved with pancreatitis in *Numida meleagris* in France.

Increased numbers of HHS cases have been described globally. In Spain (2011–2013), such cases involved mainly the E species (serotype 8b) and D species (serotype 11) (OLIVER-FERRANDO et al., 2017); in China (CHEN et al., 2019) (2015–2019), associated (in the broiler industry) with C (FAdV-4) and E (FAdV-8a and 8b) species. In South Korea (2012–2019), the most prevalent strain, of the

E species (FAdV-8b), was thereby substituting FAdV-4 (C species), possibly due to selective pressure by the use of FAdV-4 vaccines (LAI et al., 2021). However, serotype 4 remains as the most prevalent in the geographically distant Mexico (VERA-HERNANDES et al., 2016).

HHS cases have been increasingly described worldwide, including in Spain (2011–2013), involving mainly the E species and D species strains (OLIVER-FERRANDO et al., 2017), in China (CHEN et al., 2019) (2015–2019), with C and E species strains , in South Korea (2012–2019), with E species substituted by C species (LAI et al., 2021) and C species strains in Mexico (VERA-HERNANDES et al., 2016)

In Brazil, FAdV contamination of live poultry vaccines was found with CAV and avian *Orthoreovirus* (BARRIOS et al., 2012), which might have modified the epidemiology of these viruses in the field. Additional characterization of the contaminating CAV has suggested that its epidemiology may have been modified in Brazil through the use of CAV-contaminated live vaccines (MARIN et al., 2013). Ensuring adequate innocuity, purity, and safety of vaccines is a permanent concern in vaccinology. Continuous efforts are necessary for the biosecurity of breeders to achieve the elimination of vertically transmissible infections, as well as permanently monitoring using sensitive and specific diagnostic methods.

CONCLUSION

The detection and characterization of a FAdV strain of E species and its association with HHS were achieved. However, only experimental infections might be able to elucidate the pathogenic potential of the strain, leading to HHS alone or with CAV. The histopathological and molecular diagnosis of IBH caused by *Aviadenovirus* in association with CAV in broiler breeder chickens are of concern and may lead to infected broiler progenies. Distinct FAdV strains were identified in the region, thereby indicating the epidemiological complexity. As biosecurity plays a key role in the epidemiology, stricter biosecurity of breeder flocks is paramount, especially to prevent the potential negative impact of both infections in the progeny, which will induce productivity losses.

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DECLARATION OF CONFLICT OF INTEREST

The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

AUTHORS' CONTRIBUTIONS

All authors contributed equally for the conception and writing of the manuscript. All authors critically revised the manuscript and approved of the final version.

BIOETHICS AND BIOSSECURITY COMMITTEE APPROVAL

All procedures herein were in accordance to the ethical use of research animals, and registered at the Ethical Committee with protocol 101/2018.

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