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ORIGINAL ARTICLE

Molecular characterization of fowl aviadenoviruses species D and E associated with inclusion body hepatitis in chickens and falcons indicates possible cross-species transmission

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ABSTRACT

During the period from 2015 to 2017, frequent outbreaks of inclusion body hepatitis (IBH) were observed in broiler chickens and falcons in Saudi Arabia. Fifty samples were collected from both species. The histopathological examination and polymerase chain reaction confirmed the IBH infection in eight samples (five samples from chickens and three samples from falcons). The genomic sequence and phylogenetic analysis based on nucleotide and amino acid sequences of Saudi strains, reference fowl aviadenoviruses (FAdVs) and field viruses available in Genbank revealed that all investigated FAdVs clustered into FAdV-2 (species D) and FAdV-6 (species E). The host-dependent characterization revealed that falcon origin strains showed low identity (~35%) with falcon adenoviruses isolated from USA, which clustered into a separate group. The identification of FAdV-D and FAdV-E in diseased falcons and chickens indicates cross-species transmission although falcons and chickens are phylogenetically different. The control of IBH infection in falcons and chickens should be based on the separation of carriers and susceptible chickens as well as falcons to prevent cross-species contact. Vaccination is an important method for prevention of IBH. The characterization of newly emerging FAdV strains provides valuable information for the development of an efficacious control strategy based on the molecular structure of current circulating FAdV strains in different species of birds.

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KEYWORDS
Chickens; falcons; fowl aviadenoviruses; hexon gene; inclusion body hepatitis; Saudi Arabia

Research Highlights

- For the first time IBH is described in Saudi Arabia in different bird species
- All strains from chickens and falcons clustered into FAdV-D and FAdV-E
- Saudi falcon strains showed high divergence to adenoviruses isolated from falcons worldwide
- Actual data indicate trans-species transmission and circulation of IBH in Saudi Arabia

Introduction

Adenoviruses are heterogeneous pathogens that generally have been identified in many species including fish, birds, reptiles, mammals and amphibians, and virus has been isolated from at least 40 vertebrate species (Benko & Harrach, 2003). Fowl aviadenoviruses (FAdVs) are non-enveloped dsDNA viruses belonging to the genus *Aviadenovirus* within the family *Adenoviridae*. They are classified into five diverse species (FAdV-A to FAdV-E), based on genomic differences, and numerous serotypes (FAdV-1 to 8a and 8b to 11) following cross-neutralization test (Harrach *et al.*, 2012). Many FAdVs have been isolated from cases of inclusion body hepatitis (IBH), which has been described in many countries all over the world, highlighting the wide distribution of FAdVs during the last 10 years (Schachner *et al.*, 2017). IBH is characterized by a sudden increase in chick mortality lasting for a short period (average 5 days) in broilers generally up to 5 weeks of age, although sporadic outbreaks are reported in layers and broiler breeders (McFerran & Adair 2003; Hess, 2013; Schachner *et al.*, 2016). Mortalities due to IBH outbreaks always ranged from 10–30% (Schachner *et al.*, 2016); however, mortalities up to 60–70% have also been stated (Dahiya *et al.*, 2002; Gomis *et al.*, 2006).

Affected chicks usually have light-coloured, soft, friable, enlarged livers with local to generalized necrosis and enormous basophilic intranuclear inclusion bodies in liver cells (Steer *et al.*, 2015). Histopathologically,

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basophilic inclusion bodies were distinguished in natural outbreaks of intranuclear IBH by haematoxylin and eosin (H&E) staining (Matos *et al.*, 2016). On the other hand, acidophilic inclusions contained little or no virus particles and corresponded to fibrillar, granular material (Itakura *et al.*, 1974). An adenovirus was detected by electron microscopy in tissues from falcons that died during an outbreak of IBH and enteritis that affected neonatal Northern palomino falcons (Schrenzel *et al.*, 2005).

On the other hand, adenovirus was detected by electron microscopy in tissues from falcons that died during an outbreak of IBH and enteritis. Molecular characterization identified the falcon virus (FaAdV) as a new member within the genus aviadenovirus (Schrenzel *et al.*, 2005). Molecular analysis supplied evidence that adenoviruses identified in falcons were distinct from the known fowl aviadenoviruses (Oaks *et al.*, 2005). In earlier reports on adenovirus infections of falcons (Schelling *et al.*, 1989; Forbes *et al.*, 1997), the source of infection was not determined. However, cross-species transmission through the food source was previously described (Forbes *et al.*, 1997) based on isolation of an adenovirus from chickens and turkeys used to feed falcons.

Conventional typing using serological methods such as virus neutralization and agar gel precipitation test were described for the typing of FAdVs using reference FAdV strains and antisera (Hess, 2000). With the increased number of serotypes the molecular pathotyping methods polymerase chain reaction (PCR) and genomic sequencing were successfully used as alternative methods for serological identification and circumventing the difficulty of preparing a full panel of antisera. Many PCR methods have been optimized for the characterization of FAdVs, targeting the hexon loop 1 (Hex L1) capsid protein gene (Raue & Hess, 1998; Meulemans *et al.*, 2004; Raue *et al.*, 2005; Mase *et al.*, 2009).

IBH was first reported in Saudi Arabia in 2015 causing great economic losses to the respective poultry industry, especially broilers, as well as falcons. In the present study, for the first time, a panel of FAdV isolates from actual field cases of IBH in falcons (gyrfalcons) and broiler chickens were investigated. The nucleotide sequence of partial hexon capsid protein gene was determined to explore the similarities, differences, genetic constitution and diversity of locally circulating FAdVs in chickens and falcons.

Materials and methods

Sample origin

Samples were collected from 42 broiler farms and eight falcon holdings presented to the Veterinary Teaching Hospital, King Faisal University, Saudi Arabia. Birds exhibited signs suspected to be related to IBH infection. Forty-two samples originated from broiler flocks (population size ranged from 20,000 to 40,000 birds) aged 3–5 weeks old, with mortalities ranging from 3% to 25%. Necropsy of the dead birds revealed congested, friable livers. Eight samples were obtained from falcons raised in separated geographical areas. Falcons were exhibiting weakness and in some cases mortality. Faecal samples from live birds and/or liver samples from freshly dead birds were collected and stored at −80°C for molecular investigation. Liver samples were homogenized in phosphate-buffered saline (pH 7.0–7.4) at a ratio of 1:10 w/v. After three freeze–thaw cycles, the homogenates were centrifuged at 3000 × g for 5 min and supernatants were collected for nucleic acid isolation and PCR. Specimens from liver tissues with necrotic focal lesions were collected in 10% neutral formalin solution for histopathological investigation, a portion (0.5–1 cm^3) from each tissue embedded in paraffin for 24 hours and stained with H&E for microscopic examination, as described by Kiernan (2008).

DNA extraction

The DNA of liver homogenates and faecal samples was extracted using DNeasy® Blood and Tissue Mini Kits and Stool® DNA Mini Kits (QIAGEN, USA) respectively according to the manufacturer's instructions. The DNA was stored at −80°C until used for PCR and sequencing.

Oligonucleotide primers

Primers used in PCR assays were selected according to sequence database analysed by Oligo Analyze 3.1 Integrated DNA Technologies, Germantown, MD, USA and synthesized by Macrogen, Korea (Supplementary Table 1).

PCR

The extracted DNA was screened for the presence of Herpesvirus (Woźniakowski *et al.*, 2013) and FAdVs using HotStartTaq® Plus Master Mix Kit (QIAGEN, USA). Two microlitres of purified genomic DNA was added to 20 µl of the final volume of a 2X HotStartTaq Plus Master Mix containing 1.5 mM $MgCl_2$, 200 µM of each dNTP, 1 unit HotStartTaq Plus DNA polymerase, and 10 µM of each forward and reverse primer set. Thermo-cycling conditions were enzyme activation and initial denaturation at 95°C for 5 min followed by 35 cycles at 94°C for 30 s, 60°C for 30 s and 72°C for 45 s and a final extension step at 72°C for 10 min. The amplified PCR products were electrophoresed in 1.2% agarose gel stained with ethidium bromide and documented using ultraviolet gel documentation system (BIORAD®, California, USA).

Sequencing and construction of phylogenetic tree

Hexon gene-specific bands were excised from the agarose gel, purified using Montàge DNA gel extraction kit (Millipore, Burlington, MA, USA) and sequenced in an automated ABI 3730 DNA sequencer (Macrogen®, Seoul, Korea). The obtained sequence was aligned by the Clustal W method. The obtained nucleotide sequences were compared with corresponding sequences available in Genbank by BLAST web tool of the Genbank. A phylogenetic tree was constructed using MEGA version 6.0 software.

Genbank accession number

The obtained Hexon gene sequences of the detected FAdVs were submitted to the Genbank database with the accession numbers as in Table 1.

Results

Clinical examination

During 2015–2017 disease outbreaks were recorded periodically; the outbreaks lasted for about 10–18 days in broiler chickens. The birds showed depression and sudden increase in mortality at 18–27 days of age. The *post mortem* lesions in broilers were characterized by haemorrhages and necrosis in the liver. In falcons, the disease persisted for 24–31 days with anorexia, diarrhoea and mortality (Table 1). Macroscopically, livers were necrotic, mottled and slightly enlarged (Figure 1).

Histopathological examination

The hepatocellular architecture was randomly disrupted by multifocal areas of necrosis, characterized by eosinophilic and karyorrhectic debris as shown in Figure 2(A). The neighbouring hepatocytes were either necrotic with hypereosinophilic cytoplasm and pyknotic nuclei, or degenerated with pale vacuolated cytoplasm. Individually, nuclei of some hepatocytes were markedly enlarged with basophilic, smudgy, 10–15 µm intranuclear inclusion bodies seen in Figure 2 (B,C). Multifocally, the portal areas were infiltrated with lymphocytes, macrophages, plasma cells and few heterophils. Mild duct hyperplasia in addition to congestion of the portal vein and hepatic sinusoids are also visible in Figure 2(D).

Molecular characterization of FAdVs

Fifty clinical samples were collected from dead or diseased broiler chickens and falcons displaying one or more IBH-related signs or lesions. Eight samples were positive for FAdVs; five out of 42 (~12%) from broilers and three out of eight (37.5%) samples from falcons (Table 1). The generated PCR products (~890 bp) of the hexon gene were sequenced and aligned with FAdV sequences representing different serotypes (FAdV-1 to 8a and 8b to FAdV11) and 36 different worldwide field strains using Clustal W (DNAstar®Lasergene V.10 Software).

The portions of hexon gene loop-1 sequence from FAdVs from field and reference strains were analysed to classify the eight FAdVs from Saudi Arabia. The phylogenetic analysis classifies these eight FAdVs into two major clades corresponding to the reference species FAdV-D and FAdV-E as shown in Figures 3 and 4. Strains SAF2 and SAF3 (falcon strains) and strains SAC4, SAC19, SAC20, SAC21 (chicken strains) clustered into FAdV-D. However, strain SAF1 (falcon strain) and strain SAC5 (chicken strain) clustered into FAdV-E.

The highest nucleotide identity between the investigated strains and reference strains of the same species was 94.7% and 81.5% with FAdV-2 (FAdV-D) and FAdV-6 (FAdV-E), respectively. Based on the origin of the strains, the highest identity between strains from falcons was 98.2% between SAF2 and SAF3 and the lowest identity was 64.0% between SAF1 and SAF3. Strains originating from chickens showed highest identity (99.2%) between SAC19 and SAC20 and the lowest identity was (67.1%) between SAC5 and SAC4 strains. The percentages of sequence identities are summarized in Supplementary Table 2.

The divergence between field strains was lower in FAdV-D-related strains, with ~94% sequence similarity, while FAdV-E-related strains exhibited slightly higher divergence. The falcon aviadenoviruses from the USA showed more than 50% divergence and clustered into a different clade when aligned with the Saudi strains and reference FAdVs. The strain SAF1 detected

Table 1. Details of samples collected from IBH-positive farms and holdings.

Flock #	Species	Flock size	Age	Mortality	Samples		Strain	Accession no.
					Liver	Faeces		
1	Falcons	3	13 m	0/3	–	+	SAF1	MG029107
2	Falcons	11	11 m	3/11	+	+	SAF2	MG029112
3	Falcons	8	17 m	4/8	+	–	SAF3	MG029108
4	Chickens	10,000	21 d	12%	+	–	SAC4	MG029109
5	Chickens	17,000	18 d	20%	+	–	SAC5	MG029113
19	Chickens	21,000	25 d	18%	+	–	SAC19	MG029110
20	Chickens	18,000	23 d	11%	+	–	SAC20	MG029111
21	Chickens	15,500	27 d	9%	+	–	SAC21	MG029114

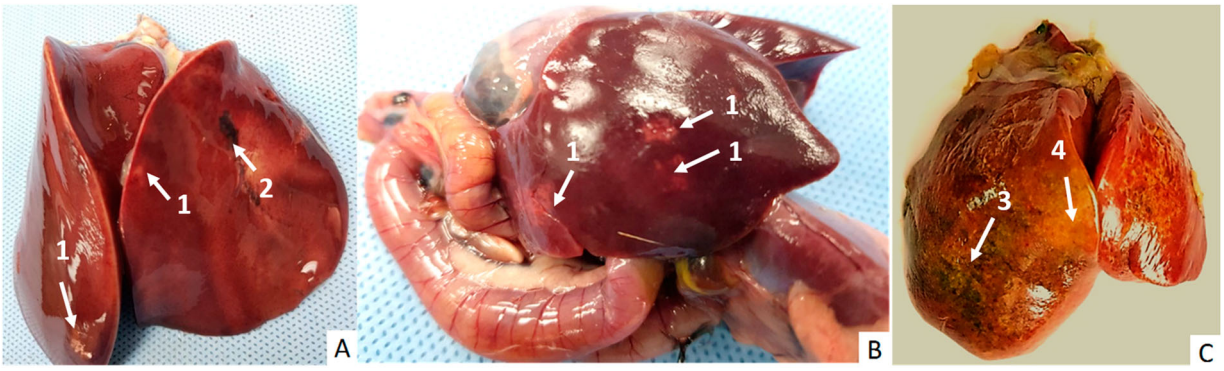

Figure 1. Twenty-seven-day-old broiler liver (A and B): slightly enlarged mottled liver with 1 – multifocal focal necrotic and 2 – haemorrhagic areas. Eight-month-old gyrfalcon liver (C): moderately enlarged congested liver with 3 – reddish, yellowish and greyish mottling and 4 – multiple necrotic areas.

in falcons in 2015 showed high identity (99.9%) with strain 15–2311 detected in chickens during 2015 in Saudi Arabia.

Discussion

Fowl aviadenoviruses have a worldwide distribution and have been commonly reported in many avian species including falcons, chickens and many other wild as well as domestic birds (Hess, 2013). Over the last few years, IBH outbreaks with different serotypes have been reported in different regions of the world (Schachner *et al.*, 2016, 2017). There were no reports of severe IBH in chickens or falcons prior to 2015 in Saudi Arabia. Recently, outbreaks have been reported associated with high mortality rates in both broilers and falcons as well as many other avian species. The hexon gene is routinely used for molecular typing; it is the major capsid protein and contains group-, type- and subgroup-specific antigens (Harrach *et al.*, 2012; Schachner *et al.*, 2016). Based on the molecular characterization, all FAdVs are assigned to five species, FAdV-A to FAdV-E (Harrach *et al.*, 2012). All IBH isolates of chicken origin were assigned to FAdV-D and/or FAdV-E (Schachner *et al.*, 2017), while the falcon adenoviruses (FaAdVs) showed the closest similarity to fowl aviadenovirus seroytpes 1 and 4 but were very different from them (Benko & Harrach, 2003; Schrenzel *et al.*, 2005).

In this study, we demonstrated the presence of IBH-related FAdVs in both falcons and chickens. The observed clinical signs and lesions in both species of

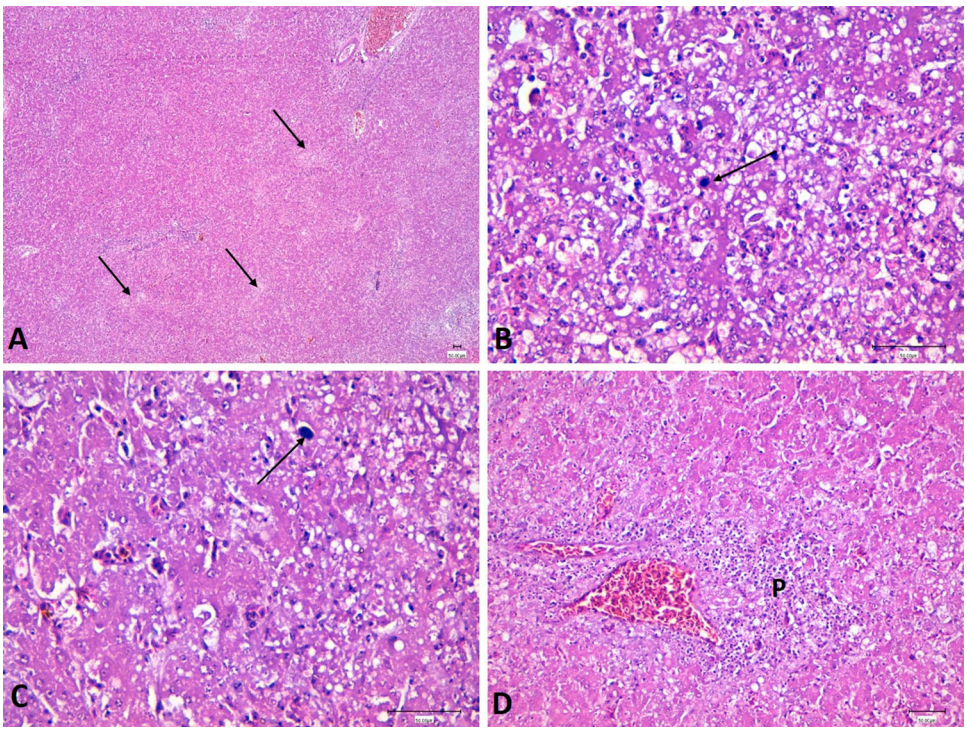

Figure 2. Gyrfalcon liver: (A) multifocal areas of lytic necrosis (arrows) disrupt the hepatic parenchyma. (B and C) large smudgy basophilic intranuclear inclusion body (arrow) is seen within the hepatocytes. (D) portal area **P** is expanded with many inflammatory cells.

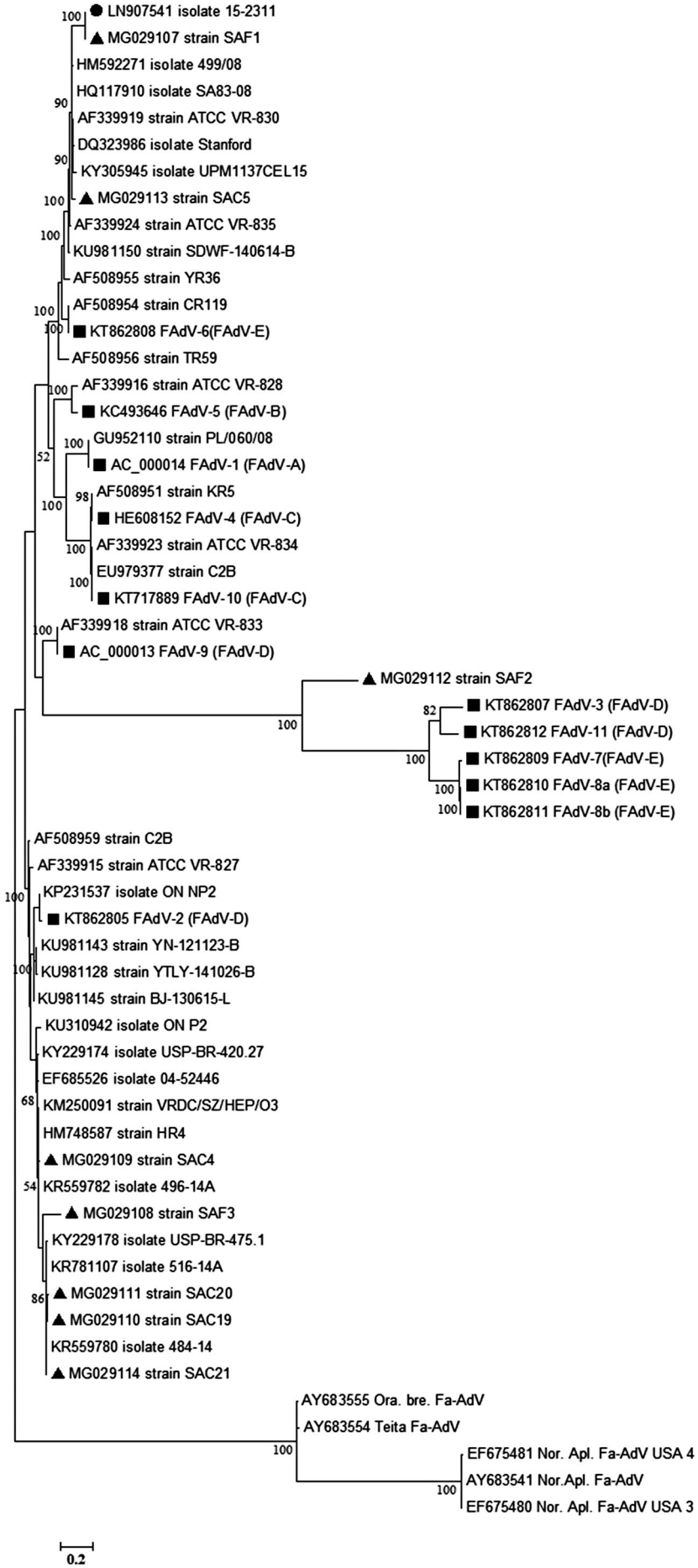

Figure 3. Phylogenetic tree based on 890 bp segment of hexon gene comprising the Hex L1 region showing six isolates clustering into FAdV-2 (species D) and two isolates clustering into FAdV-6 (species E). Falcon adenoviruses (Fa-AdVs) clustered into a separate clade and showed high diversity from FAdVs. The Saudi strain labelled with the black circle was described by Schachner *et al*. (2016). The eight new Saudi isolates are labelled with black triangles and reference FAdVs are labelled with black squares.

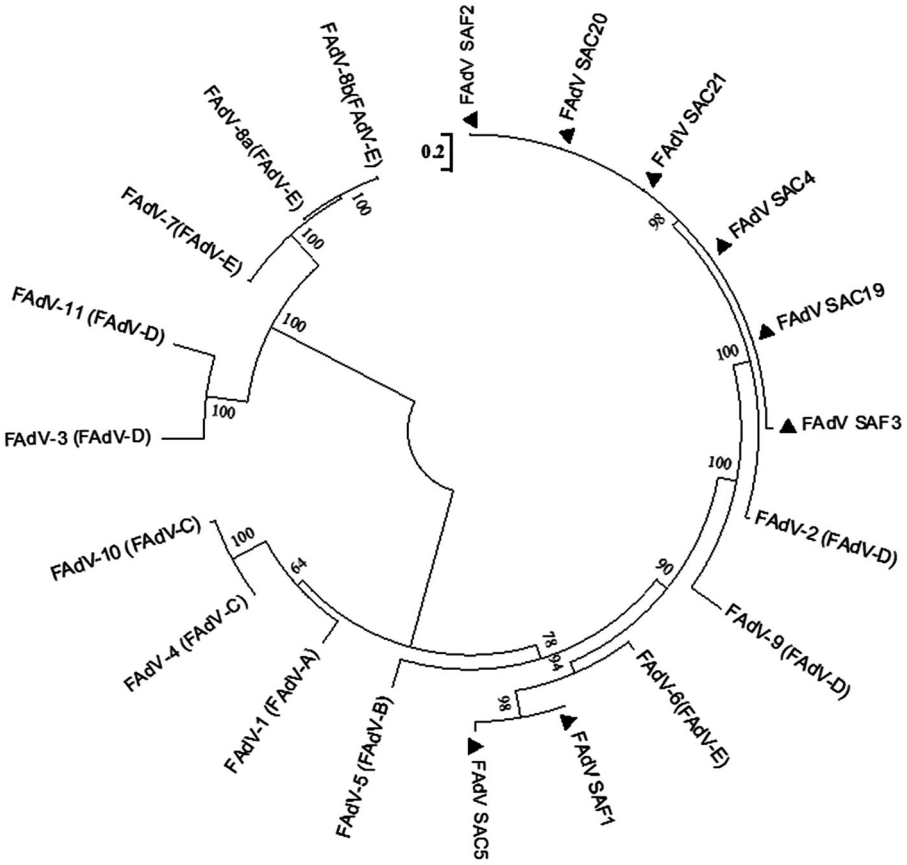

Figure 4. Phylogenetic tree based on the corresponding amino acids of hexon protein of Saudi isolates (black triangles) and FAdV reference strains confirming that both Saudi strains from falcons and chickens clustered within FAdV-2 (species D) and FAdV-6 (species E).

birds were confirmed by histopathological examination of livers, which revealed the presence of large intranuclear inclusion bodies and multifocal necrosis (Steer *et al.*, 2015). We constructed a phylogenetic tree based on the hexon loop L1 sequences, and the results indicated that all eight samples clustered into two FAdV species (FAdV-D and FAdV-E) as reported by Schachner *et al.* (2016).

The results revealed very low differences among strains representing the same serotypes. The percent identity between SAF1 and SAC5 was 94.2% and between SAF3 and SAC4 97.4%. Interestingly, strains SAF1 and SAF3 originated from falcons and strains SAC4 and SAC5 originated from chickens. Surprisingly, the Saudi strains related to FAdV-2 (species D) and FAdV-6 (species E) showed higher divergence, ~30%. In contrast, Marek *et al.* (2013) confirmed a closer genetic relationship between FAdV-D and FAdV-E. The phylogenetic tree revealed that the strains from Saudi Arabia and those from India showed the highest similarity, indicating the possibility of virus transmission through importation of live chicks or contaminated eggs from India, which is previously classified as an endemic area (Kumar *et al.*, 2013). In addition, this supports the hypothesis that differences in hexon gene are related to the serotypes and independent of geographical distance (Schachner *et al.*, 2016).

Inclusion body hepatitis syndrome is a primary disease of falcon caused by Herpesvirus (CoHV-1) or FAdV infections. Lesions are necrosis, splenomegaly and moderate to severe lymphoid atrophy and lymphocyte depletion in the bursa of Fabricius (Schrenzel *et al.*, 2005). In this study, all samples from falcon origin were negative for herpesvirus and positive for FAdVs. The genomic analysis of amino acid sequences revealed 70 amino acid differences between the investigated falcon strains and other FaAdVs available in Genbank. In addition, the phylogenetic analysis of the obtained sequences and other reference strains revealed that the falcon strains were clustered into FAdV-2 (species D) and FAdV-6 (species E) and showed high phylogenetic diversity from other FaAdVs which clustered into separate groups. Moreover, the falcon SAF1 strain showed high identity (99.9%) with the 15–2311 strain isolated from chickens during 2015 in Saudi Arabia (Schachner *et al.*, 2016). Data from the field, epidemiological and genetic data analysis strengthen the hypothesis of cross-species transmission between chickens and falcons and the disease could be transmitted to falcons through feeding on infected chickens; also the role of falcons in dissemination of FAdVs should be further investigated.

In conclusion, the newly emerged and prevalent FAdV-2 (species D) and FAdV-6 (species E) are the

etiological agents of IBH in broiler chickens and falcon flocks from Saudi Arabia and can cause periodical outbreaks. Although falcons and chickens are phylogenetically disparate hosts, they share the susceptibility to closely related FAdV species D and E. Continued molecular and serological surveillance of newly emerged FAdVs in falcons and chicken flocks, as well as other bird species, is preferable for clarifying the relationship and transmission of the disease between different species and provides a valuable reference for the development of an efficacious control strategy. The high divergence percentage between different FAdV species needs more attention during vaccine selection. The falcon raiser should prevent any contact between falcons and infected chickens or chicken by-products.

Disclosure statement

No potential conflict of interest was reported by the authors.

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References

Benko, M. & Harrach, B. (2003). Molecular evolution of adenoviruses. *Current Topics in Microbiology and Immunology*, 272, 33–35.

Dahiya, S., Srivastava, R.N., Hess, M. & Gulati, B.R. (2002). Fowl adenovirus serotype 4 associated with outbreaks of infectious hydropericardium in Haryana, India. *Avian Diseases*, 46, 230–233.

Forbes, N.A., Simpson, G.N., Higgins, R.J. & Gough, R.E. (1997). Adenovirus infection in Mauritius kestrels (*Falco punctatus*). *Journal of Avian Medicine and Surgery*, 11, 31–33.

Gomis, S., Goodhope, R., Ojkic, D. & Willson, P. (2006). Inclusion body hepatitis as a primary disease in broilers in Saskatchewan, Canada. *Avian Diseases*, 50, 550–555.

Harrach, B., Benkö, M., Both, G.W., Brown, M., Davison, A.J., Echavarria, M., Hess, M., Jones, M.S., Kajon, A., Lehmkuhl, H.D., Mautner, V., Mittal, S.K. & Wadell, G. (2012). Family Adenoviridae. In A.M.Q. King, M.J. Adams, E.B. Carstens & E.J. Lefkowitz (Eds.), *Virus Taxonomy: Ninth Report of the International Committtee on Taxonomy of Viruses* (9th ed.) (pp. 125–141). New York: Elsevier Academic Press.

Hess, M. (2000). Detection and differentiation of avian adenoviruses: a review. *Avian Pathology*, 29, 195–206.

Hess, M. (2013). Avidenovirus infections. In D.E. Swayne, J.R. Glisson, L.R. McDougald, L.K. Nolan, D.L. Suarez & V. Nair (Eds.), *Diseases of Poultry* 13th ed (pp. 290–300). Ames: Wiley-Blackwell.

Itakura, C., Yasuba, M. & Goto, M. (1974). Histopatholoical studies on inclusion body hepatitis in broiler chickens. *Japanese Journal of Veterinary Science*, 36, 329–340.

Kiernan, J.A. (2008). *Histological and Histochemical Methods: Theory and Practice* (4th ed.) (pp. 103–131). Bloxham: Scion Publishing.

Kumar, V., Kumar, R., Chandra, R., Bhatt, P. & Dhama, K. (2013). Outbreaks of inclusion body hepatitis (IBH) in chickens; pathological studies and isolation of fowl adenovirus. *Advances in Animal and Veterinary Sciences*, 1, 21–24.

Marek, A., Kosiol, C., Harrach, B., Kaja, G., Schlötterer, C. & Hess, M. (2013). The first whole genome sequence of a fowl adenovirus B strain enables interspecies comparisons within the genus Aviadenovirus. *Veterinary Microbiology*, 166, 250–256.

Mase, M., Mitake, H., Inoue, T. & Imada, T. (2009). Identification of group I–III avian adenovirus by PCR coupled with direct sequencing of the hexon gene. *The Journal of Veterinary Medical Science*, 71, 1239–1242.

Matos, M., Grafl, B., Liebhart, D. & Hess, M. (2016). The outcome of experimentally induced inclusion body hepatitis (IBH) by fowl aviadenoviruses (FAdVs) is crucially influenced by the genetic background of the host. *Veterinary Research*, 47, 69.

McFerran, J.B. & Adair, B.M. (2003). Group I adenovirus infections. In Y.M. Saif (Ed.), *Diseases of Poultry* 11th ed (pp. 213–214). Ames: Iowa State Press.

Meulemans, G., Couvreur, B., Decaesstecker, M., Boschmans, M. & van den Berg, T.P. (2004). Phylogenetic analysis of fowl adenoviruses. *Avian Pathology*, 33, 164–170.

Oaks, J.L., Schrenzel, M., Rideout, B. & Sandfort, C. (2005). Isolation and epidemiology of falcon adenovirus. *Journal of Clinical Microbiology*, 43, 3414–3420.

Raue, R. & Hess, M. (1998). Hexon based PCRs combined with restriction enzyme analysis for rapid detection and differentiation of fowl adenoviruses and egg drop syndrome virus. *Journal of Virological Methods*, 73, 211–217.

Raue, R., Gerlach, H. & Müller, H. (2005). Phylogenetic analysis of the hexon loop 1 region of an adenovirus from psittacine birds supports the existence of a new psittacine adenovirus (PsAdV). *Archives of Virology*, 150, 1933–1943.

Schachner, A., Marek, A., Grafl, B. & Hess, M. (2016). Detailed molecular analyses of the hexon loop-1 and fibers of fowl aviadenoviruses reveal new insights into the antigenic relationship and confirm that specific genotypes are involved in field outbreaks of inclusion body hepatitis. *Veterinary Microbiology*, 186, 13–20.

Schachner, A., Matos, M., Grafl, B. & Hess, M. (2017). Fowl adenovirus-induced diseases and strategies for their control – a review on the current global situation. *Avian Pathology*, 47(2), 111–126.

Schelling, S.H., Garlick, D.S. & Alroy, J. (1989). Adenoviral hepatitis in a merlin (*Falco columbarius*). *Veterinary Pathology*, 26, 529–530.

Schrenzel, M., Oaks, L.J., Rotstein, D., Maalouf, G., Snook, E., Sandfort, C. & Rideout, B. (2005). Characterization of a new species of adenovirus in falcons. *Journal of Clinical Microbiology*, 43, 3402–3413.

Steer, P.A., Sandy, J.R., O'Rourke, D., Scott, P.C., Browning, G.F. & Noormohammadi, A.H. (2015). Chronological analysis of gross and histological lesions induced by field strains of fowl adenovirus serotypes 1, 8b and 11 in one-day-old chickens. *Avian Pathology*, 44, 106–113.

Woźniakowski, G.J., Samorek-Salamonowicz, E., Szymański, P., Wencel, P. & Houszka, M. (2013). Phylogenetic analysis of Columbid herpesvirus-1 in rock pigeons, birds of prey and non-raptorial birds in Poland. *BMC Veterinary Research*, 9, 52.