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Case Report
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Outbreak of fowl adenovirus FAV-E (serotype 8b) in broilers in Libya: A case report

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ABSTRACT

Background: Fowl adenovirus (FAV) is responsible for many disease conditions in poultry, leading to numerous economic impacts, including mortality, drop in egg production, and carcass condemnations. This is the first report on molecular characterization of FAV-E (serotype 8b) in Libya.

Case Description: During January 2023, three broiler flocks had increased mortality up to 25%–50%. Paleness and haemorrhages in the liver appeared in birds that were 23 to 27 days old. The total number of birds was 12,000, 16,000, and 22,000 in the first, second, and third flock, respectively. Ten Samples from the liver and kidneys were collected for histopathological examination from each flock, and liver, spleen, and caecal tonsils were collected for DNA extraction, Real-time polymerase chain reaction, and sequencing. Histopathological examination of the liver revealed hemorrhages, fatty changes, and coagulative necrosis, the presence of intranuclear eosinophilic inclusion bodies in hepatocytes, and karyorrhexis of the nucleus and multiple perivascular infiltration of pleomorphic lymphocytes. The kidney showed subcapsular hemorrhages and infiltration of lymphocytes and inflammatory cells, focal necrosis, and glomeruli enlarged with hyper-cellularity. All tested samples were positive for FAV, and sequencing confirmed the FAV-E (serotype 8b) strain, which is 99.53% identical to some isolates from Turkey.

Conclusion: FAV-E (serotype 8b) strain is circulating in broiler flocks, leading to high mortality. Further research is required to determine the epidemiology of the disease in the country and to investigate the presence of other strains of fowl adenovirus in order to introduce the best vaccine.

Keywords: Broilers, FAV-E (serotype 8b), Libya.

Introduction

The poultry industry faced dramatically increasing viral challenges during the last decade, and fowl adenovirus (FAV) is one of them. FAV is a medium-sized, non-enveloped icosahedral DNA virus known to affect poultry worldwide (Schachner *et al.*, 2018). Initially, there was a doubt about the role of FAV as a primary pathogen in poultry, but over time, its involvement in various well-characterized syndromes has been recognized (Schachner and Hess, 2022). These syndromes include inclusion body hepatitis (IBH), hepatitis hydropericardium syndrome (HHS), gizzard erosion (GE), and ulceration, primarily affecting young broiler chicks.

There are 12 serotypes of FAV described, and classified into five species from FAV A through E. IBH is mainly associated with FAV species D, serotypes 2 and 11 (Schachner *et al.*, 2018; Schachner and Hess, 2022). However, recent reports have also identified FAV species E (FAV serotypes 8a and 8b) as causative agents for the IBH syndrome (Kaján *et al.*, 2013). HHS is associated with species C (FAV serotype 4), and GE is associated with species A (FAV serotype 1). However, FAV serotype D has also been identified in HHS cases (Kaján *et al.*, 2013). These different serotypes and species of FAV contribute to the diversity of the virus and its associated diseases (Harrach *et al.*, 2019).

Determination of the prevalent strains of FAV is crucial for the development of effective control measures, including vaccines, and provides valuable insights into the role of FAV in different disease conditions. This is the first report of FAV infections in Libya based on flock disease history, necropsy and histopathology findings, polymerase chain reaction (PCR) testing, and sequencing. These diagnostic methods help confirm the presence of FAV-E (serotype 8b) and determine its association with the observed clinical signs and lesions in affected birds.

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Case Details

This study reports an outbreak of FAV in three broiler flocks across different farms, which had increased mortality and a change in the colour of the liver to yellow. The first flock, consisting of 12,000 birds, exhibited clinical signs starting at 23 days old. The second flock, comprising of 16,000 birds, experienced clinical signs at 27 days old. The third flock, consisting of 22,000 birds, showed clinical signs at 25 days old. Additional clinical signs observed included loss of appetite, ruffled feathers, and yellowish diarrhoea. The mortality rate during the 7-day infection period ranged from 25% to 50%. The post-mortem examination showed a pale yellow and enlarged liver with haemorrhagic spots on the surface (Fig. 1A). Kidneys were congested and swollen. DNA was extracted from the pooling of five pieces of liver, spleen, and cecal tonsils (from flock number 1 and flock number 3) using ID Gene™ Spin Universal Extraction Kit following the manufacturer's guidelines. The Real-time PCR analysis was performed using the PowerChek™ Fowl Adeno/CAV/MD Triplex Real-time PCR kit (Kogenebiotech, Korea). Each sample underwent a reaction volume of 20 µl. The components include a primer/probe mix, Real-time PCR master mix, and template DNA. The thermal cycling profile involved an initial step of polymerase activation at 95°C for 10 minutes, followed by denaturation at 95°C for 15 seconds, and annealing/extension at 60°C for 1 minute. This cycle was repeated for 40 times. The fluorescence curves obtained during the Real-time PCR analysis were analysed for the detection of specific viruses. The FAM channel is used to read the signal for fowl adenovirus, the HEX channel for chicken infectious anaemia virus, the ROX channel for Marek's disease virus, and the Cy5 channel for internal control. Four controls are included to ensure the validity of the PCR test: positive extraction control, negative extraction control, positive amplification control, and negative amplification control. All pooled samples from the liver, spleen, and cecal tonsils were positive for Fowl Adenovirus using real-time PCR. These samples were then sent to the University of Veterinary Medicine Vienna (Austria) / Clinical Unit of Poultry Medicine for DNA sequencing targeting the hexon gene of FAV. The results are shown in Table 1. Following sequencing, the FASTA file was submitted to BLAST (NCBI) for alignment and to determine the percentage of identity. Phylogenetic analysis was carried out using neighbor joining method in MEGA11 software, as shown in Figure 3. The FAV E (serotype 8b) was diagnosed and was 99.53% identical to some isolates from Turkey (GenBank accession no. MN052902.1). However, submission of the obtained sequence to GenBank was not attempted. Tissue samples from the liver and kidney were fixed in 10% neutral buffered formalin for the standard process of

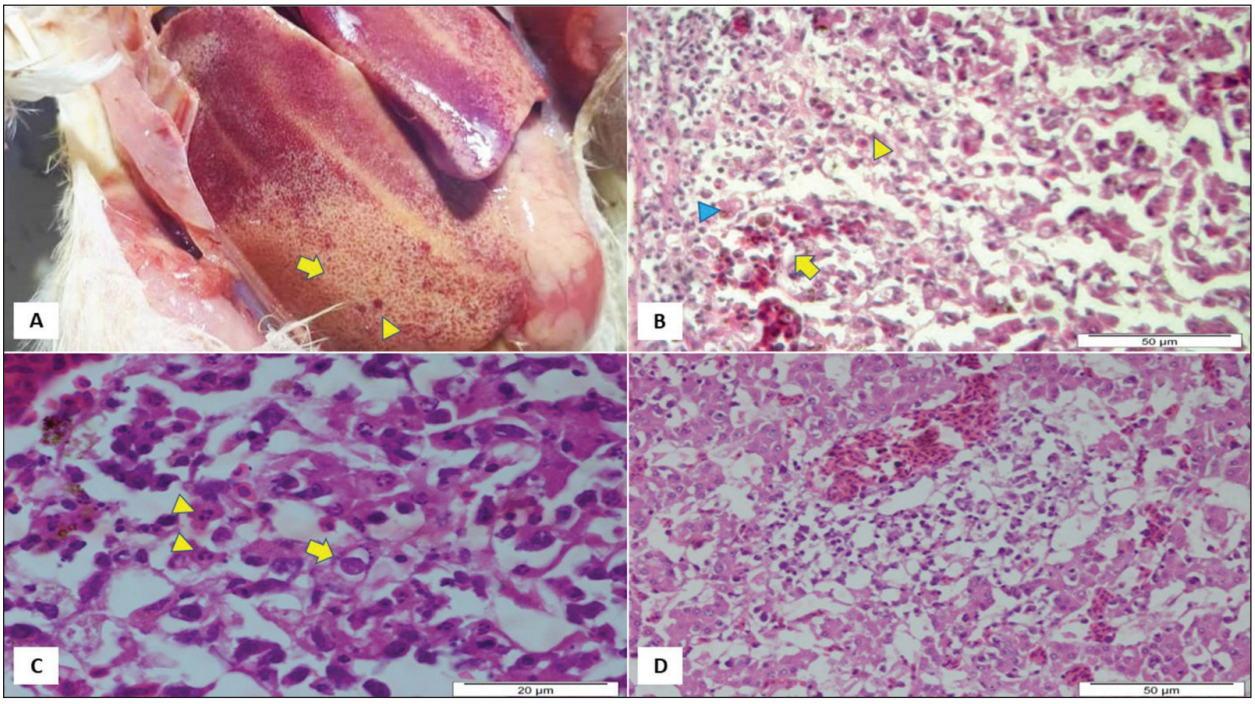

Fig. 1. Gross and histopathological lesions of liver in chicken infected with FAV-E serotype 8b. A: Liver showing paleness, yellowish color (arrow), and presence of hemorrhagic spots (arrow head). B: Areas of hemorrhages (arrow), fatty changes (yellow arrow head), and coagulative necrosis (blue arrow head). C: Intranuclear eosinophilic inclusion bodies (arrow) in hepatocytes and karyorrhexis of the nucleus (arrow heads). D: Multiple perivascular infiltration of pleomorphic lymphocytes.

Table 1. The result of PCR and sequencing.

No.	Sample *	Real time PCR		Sequencing
		Result	Ct	
1	Liver	+	17.5	FAV-E (8b)
2	Spleen	+	21.9	ND
3	CT	+	19.86	FAV-E (8b)
4	Liver	+	16.17	FAV-E (8b)
5	Spleen	+	20.69	FAV-E (8b)
6	CT	+	22.68	FAV-E (8b)

ND = not done because the quality of PCR product was not suitable for sequencing analysis.
*plooling of 5 samples.

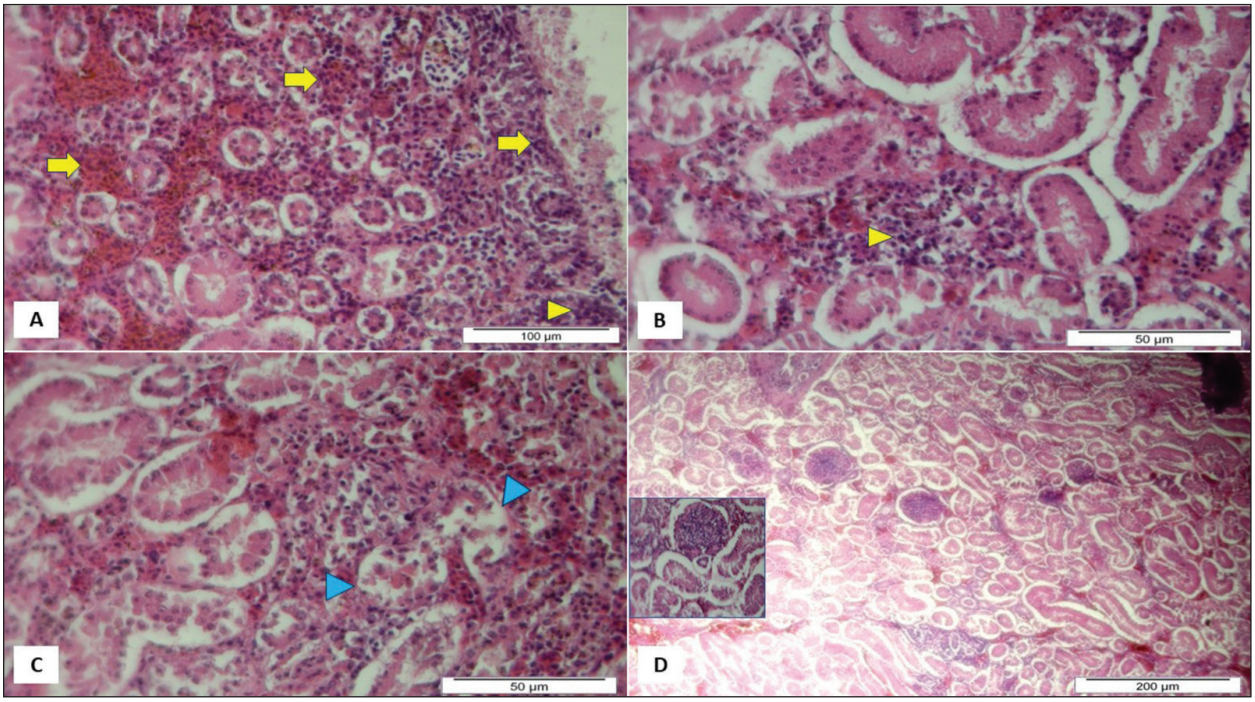

Fig. 2. Sections of kidney infected with FAV-E serotype 8b. A and B: Subcapsular and interstitial hemorrhages (arrows) and infiltration of lymphocytes and inflammatory cells (yellow arrow heads). C: Focal coagulative necrosis (blue arrow head). D: Glomeruli enlarged with hyper-cellularity.

histopathology. The tissues were washed overnight in running water and dehydrated in ascending grades of alcohol, then the tissues were embedded in paraffin, and 5 µm thick sections were cut and stained with haematoxylin and eosin (Slaoui and Fiette, 2011). The histopathological lesions of the infected liver comprised areas of hemorrhages, fatty changes, and coagulative necrosis (Fig. 1B). Intranuclear eosinophilic inclusion bodies were observed in some hepatocytes, and some had karyorrhexis of the nucleus (Fig. 1C). Multiple perivascular infiltration of pleomorphic lymphocytes was also observed (Fig. 1D). The kidney showed subcapsular and interstitial hemorrhages and infiltration of lymphocytes and inflammatory cells (Fig. 2A and B) with focal coagulative necrosis (Fig. 2C). Some glomeruli showed enlargement with hyper-cellularity (Fig. 2D).

Discussion

FAV infections are distributed almost worldwide. Kiss *et al.* (2021)molecularly investigated 365 FAV isolates belonging to 38 countries from 5 continents over a decade. The sequencing and phylogenetic analysis revealed 11% FAV-A, 3% FAV-B, 2% FAV-C, 34% FAV-D, and 50% FAV-E. The majority of concomitant infection from other disease conditions, almost exclusively in boilers of 27 to 42 days of age were caused by FAV-E. This result is similar to our findings

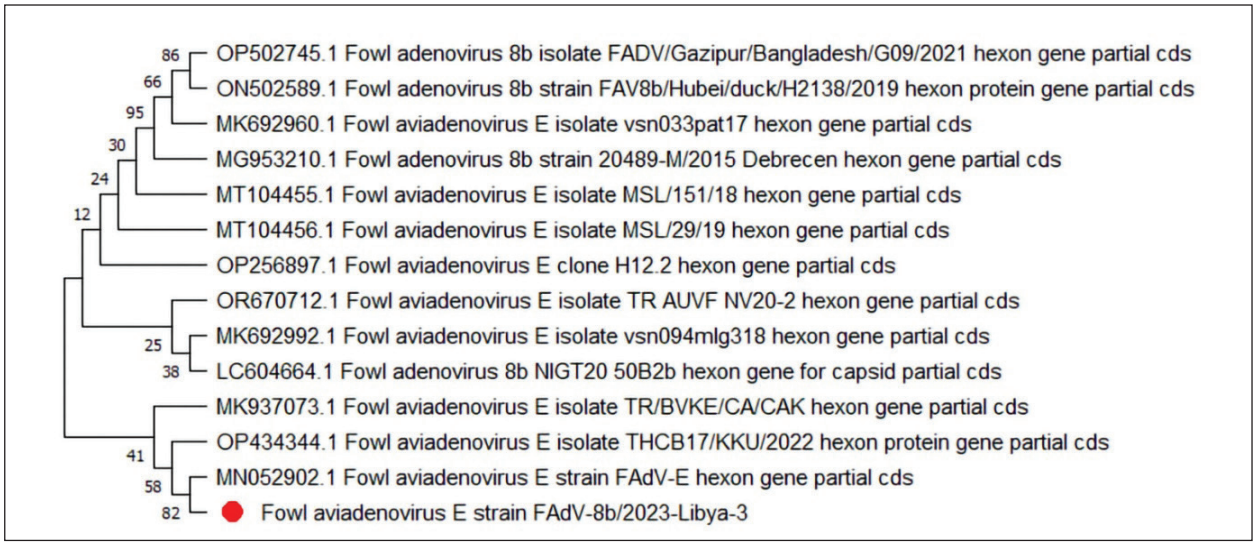

Fig. 3. Phylogenetic analysis of the hexon gene of FAV E (8b) strain in Libya (highlighted in red circle). The phylogenetic tree was constructed in MEGA 11 using the neighbor-joining method with 1,000 bootstrap replicates.

in the current report, although we have not detected other FAV species. The FAV E (serotype 8b) reported in the current study was 99.53% similar to some isolates from Turkey (GenBank accession no. MN052902.1). In Turkey, many studies reported infections with FAV. Bıçakcıoğlu *et al.* (2024) reported that 30.0% of all flocks were FAV positive, with 50.0% (20/40) positivity in nonvaccinated flocks and 10.0% (4/40) in vaccinated flocks. Sequence analysis of the hexon loop-1 gene revealed that all samples were FAV-8b serotype. Cizmecigil *et al.* (2020) also reported an outbreak of fowl adenovirus in broilers in Turkey. Cizmecigil *et al.* (2020) reported clinical signs such as anorexia, depression, ruffled feathers, huddling, and greenish diarrhoea were observed. Mortality started at the eighth day of age and ranged from 10% to 14%. Necropsy showed severe hepatitis, jaundice, and pancreatitis. The main necropsy findings included a pale, enlarged, haemorrhagic, and friable liver, along with swollen and haemorrhagic kidneys and spleen. Our findings are similar to the later onset of disease at 23 to 27 days old. The PCR and sequence analysis revealed the presence of fowl adenovirus serotype 8b (FAV-E). The necropsy and histopathological findings of Cizmecigil *et al.* (2020) and Şahindokuyucu *et al.* (2020) are almost consistent with our findings, in which the liver and kidneys were the most affected organs. The sequence analysis of FAV isolate conducted by Şahindokuyucu *et al.* (2020) showed that FAV-8b and FAV-11 were the circulating serotypes that caused field outbreaks of IBH in the Aegean region of Turkey between January and March, 2019.

FAV infections can occur at any age in poultry, but the severity of lesions is typically related to the age of the birds and the level of maternally derived antibodies. Young birds are particularly susceptible to severe clinical and economic impacts (Haiyilati *et al.*, 2021). The outcome of the infection can also depend on the pathogenicity of the virus strain and the presence of immunosuppressive conditions (Safwat *et al.*, 2022). Vertical transmission of the virus from parent birds to offspring can lead to significant mortality (Schachner *et al.*, 2018).

The outbreak of fowl Adenovirus in Libya is a concerning issue. The possibility to spread rapidly throughout the country and beyond requires rapid and effective interventions and measures to contain and mitigate the spread of fowl Adenovirus. Efforts are essential to prevent the further spread of FAV and protect the poultry industry in Libya. By understanding the prevalent types of FAV and their role in different disease conditions, the development of effective vaccines tailored to the specific strains can be pursued. Furthermore, enhancing biosecurity measures and promoting early detection methods, especially for serotyping, are crucial in preventing and controlling the spread of FAV in Libya. The authorities must collaborate with stakeholders to swiftly develop and implement a strategic plan to combat this outbreak and safeguard the Libyan poultry industry. The cooperation between farmers, breeders, and veterinary authorities is paramount in reporting and addressing the disease.

Vaccination is an important strategy to control FAV infections in poultry. There are registered vaccines available commercially, including live or inactivated vaccines, virus-like particles, subunit vaccines, and autogenous products (De Luca and Hess, 2025). However, the variety of commercially available vaccines is limited. Furthermore, it is worth noting that large vaccine manufacturing companies often do not produce these specific types of vaccines, which may contribute to the limited offerings in the market.

Autogenous vaccines, which are tailored to specific viral strains isolated from a particular farm, are often used to provide a solution for the poultry industry.

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Conflict of interest

The authors declare no conflict of interest regarding this study.

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Authors' contributions

Dr. Abdulatif Asheg was responsible for the sampling and writing the manuscript, and Dr. Abdulwahab Kammon was responsible on histopathological and molecular contribution.

Data availability

The data supporting the findings of this study were available upon reasonable request to the corresponding author.

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