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Emergence of fowl aviadenovirus C-4 in a backyard chicken flock in California

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Abstract. Fowl aviadenovirus (FAdV) species D and E are associated with inclusion body hepatitis (IBH); species C, serotype 4 (hereafter, FAdV4) is associated with hepatitis–hydropericardium syndrome (HHS) in young chickens. Outbreaks of HHS have led to significant losses in the poultry industry in several countries, predominantly in China. In April 2020, FAdV4 was detected in a remote backyard flock in California. In a mixed flock of chickens of various breeds and ages (6 mo to 2 y old), 7 of 30 were found dead within a week without premonitory signs. One additional bird died after the flock was relocated to fresh pasture, bringing the total mortality to 8 of 30 (27%). Postmortem examination of 3 birds revealed good body condition scores and active laying. One chicken had subtle hemorrhages throughout the liver, and the other 2 had diffusely dark mahogany livers. On histopathology, 2 chickens had hepatic necrosis with hepatocytes containing large, mostly basophilic, intranuclear inclusion bodies, identified by electron microscopy as 82.2-nm diameter adenoviral particles. Virus isolation and genomic sequencing performed on a liver sample revealed strains with 99.9% homology to FAdV4 isolates reported from China. To our knowledge, FAdV4 has not been reported in the United States to date. Furthermore, the chickens affected here were all adults and exhibited a variation of serotype 4 disease in which IBH was present but not hydropericardium.

Keywords: backyard chickens; FAdV C-4; fowl aviadenovirus; hydropericardium syndrome; inclusion body hepatitis; serotype 4.

Fowl aviadenoviruses (FAdVs) cause a triad of diseases in chickens: inclusion body hepatitis (IBH), hepatitis–hydropericardium syndrome (HHS), and adenoviral gizzard erosion (AGE).[13] Of these, IBH is the best described given the frequent and globally widespread outbreaks, followed by HHS. FAdV species D and E are the species detected most commonly in IBH outbreaks. Serotype 4 of FAdV species C (hereafter, FAdV4) is associated with HHS. Both IBH and HHS affect young chickens primarily. Among commercial poultry, outbreaks in broiler chickens are the most common, followed by layers and broiler breeders. Reduced performance and reproduction are the primary cause for economic losses, although mortality can reach 30% with IBH and higher with HHS.[13]

The lesions of IBH are characteristic wherein the liver lobes are variably swollen, friable, pale, occasionally yellow-tinged, and contain prominent hemorrhagic foci that correspond to acute, multifocal-to-coalescing necrosis, and there are distinctive large, intranuclear adenoviral inclusion bodies (INIBs) within hepatocytes.[14] The pancreas and kidneys may have pathologic changes in some cases.[19] In HHS, hepatic lesions similar to those in IBH are accompanied by hydropericardium as a pathognomonic feature.[3,4]

FAdV4 strains have caused epidemics in China since 2015, with severe losses as a result of HHS outbreaks in commercial flocks.[4] To our knowledge, FAdV4-associated outbreaks have not been reported in the U.S. commercial poultry industry. Furthermore, studies and surveillance conducted over the past 10 y in California, and over the past 5 y in 7 additional states throughout the United States have not reported IBH or HHS in backyard chickens.[1,5] The tremendous increase in small backyard poultry flocks throughout the United States has introduced an unpredictable interface with commercial facilities, and we are constantly monitoring the disease dynamics between these 2 entities.

In April 2020, a California Department of Food & Agriculture veterinarian (M. Mott) was called to investigate increased mortality in a backyard flock of 30 hens located in Humboldt County, CA. The flock consisted of multiple breeds of laying hens from 6 mo to 2 y old. The poultry were housed on pasture in a mobile coop with access to a 30 × 7 m (100 × 20 ft) fenced outdoor enclosure. Seven sudden deaths without premonitory signs were documented April 19–26, 2020, and 3 of these mortalities were collected at random for testing. The chicken coop was then cleaned and relocated to adjacent fresh pasture. An eighth chicken died within several days of relocation, then no further mortalities were reported.

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Autopsy of the freshest carcass (chicken 1) was performed on-site. Two other chickens (chickens 2 and 3) were shipped to the California Animal Health and Food Safety laboratory system, Davis branch for diagnostic investigation. Chicken 1 (postmortem interval < 24 h) had good body condition with abundant adipose and active ova with a shelled egg in the uterus. The tissues appeared dark, the lungs were edematous, and the liver had generalized subtle petechiation (Fig. 1 inset). Chickens 2 and 3 were in poor postmortem condition (postmortem interval > 48 h). Both chickens had good body condition and reproductive activity, with overall diffusely darkened organs. The spleens of both birds were prominent, measuring 1 × 2 × 1 cm. The lungs were edematous. The kidneys were swollen and had subtle, pale, tubular highlights. There were minimal crop and gastric feed contents. The koilin layer of the ventriculus peeled easily and was partially detached. One hen (chicken 3) had mild egg yolk peritonitis. Brain, peripheral nerves, heart, lung, trachea, liver, kidney, spleen, ova, adrenal gland, skeletal muscle, pancreas, and gastrointestinal tract tissues from chickens 2 and 3 were collected, processed routinely for histology, and sections stained with hematoxylin and eosin.

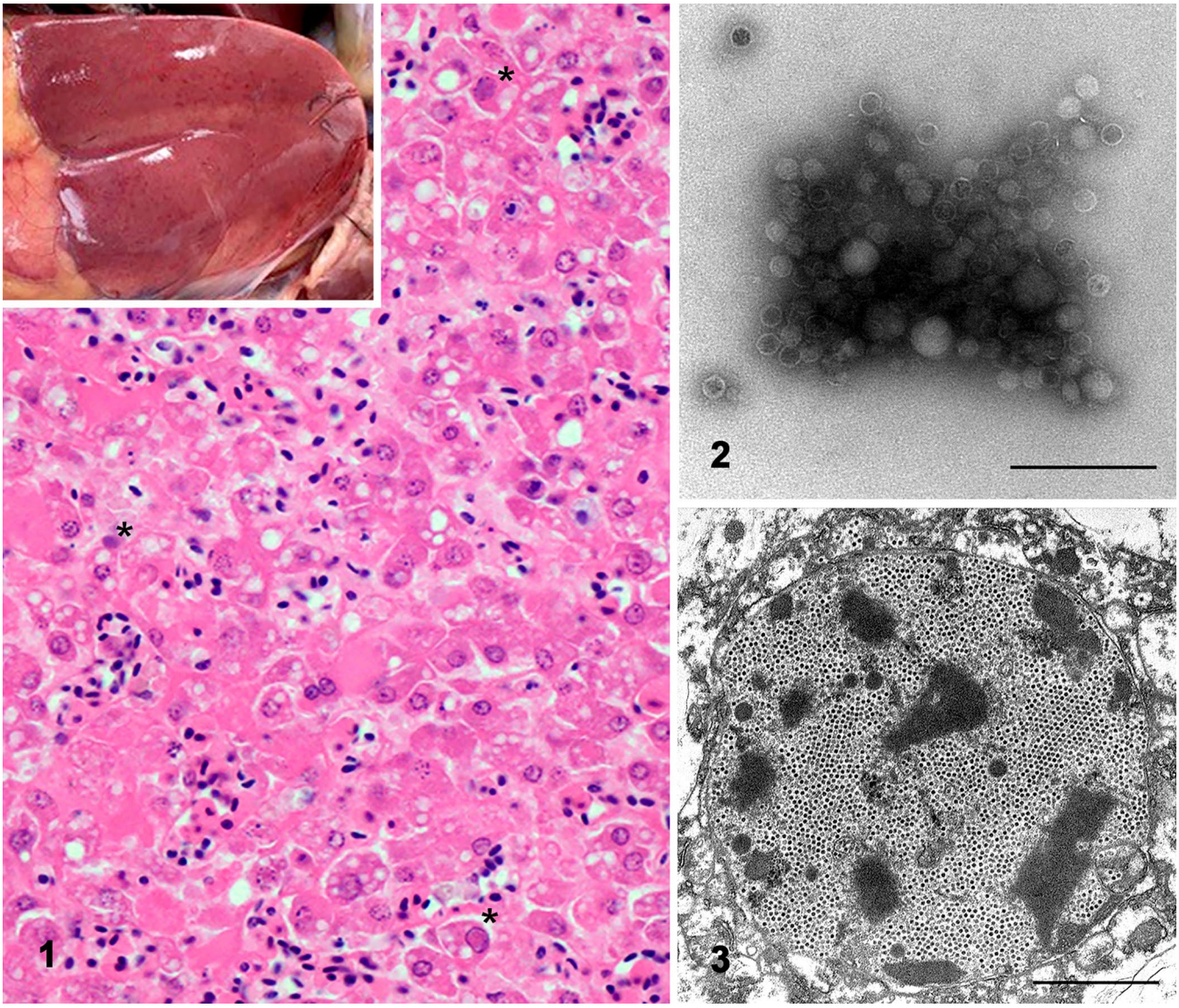
Histopathologic findings in the livers of both birds consisted of extensive acute hepatic necrosis with hemorrhages and fibrin exudation (Fig. 1). Hepatocellular nuclei were frequently obliterated by large basophilic INIBs or occasionally by eosinophilic INIBs and peripheralized chromatin (Fig. 1). Pulmonary capillaries had fibrin thrombi, predominantly in chicken 2. The koilin was lifted off, had segmental degeneration, and there was mild heterophilic (chicken 2) or lymphocytic (chicken 3) ventriculitis. The kidneys in both birds had moderate edema, tubular degeneration with proteinaceous luminal material, and urate stasis. In the spleen, there was mild-to-moderate lymphoid depletion, fibrin exudation, and histiocytosis.

Liver samples frozen at −20°C and formalin-fixed livers from chickens 2 and 3 were analyzed by transmission electron microscopy. On the liver homogenates prepared from both chickens, contrasted phosphotungstic acid detected nonenveloped, icosahedral, hexagonal, 82.2 (SD 5.5) nm diameter virus particles. Non-vertex capsomeres measured ~1.0 (SD 1.3) nm. Double-vertex fibers were observed infrequently. Density of adenoviral particles of chicken 2 was calculated to be 7,780 per μm^2. On examining liver tissue embedded in plastic, hepatocellular intranuclear virus replication and assembly complexes were composed of electron-dense viroplasm and maturating and mature virions displaying loose-to-compact aggregation designated as paracrystalline arrays. Denatured nuclear chromatin formed asymmetric electron-dense amorphous aggregates (Figs. 2, 3).

PCR was performed on liver from chickens 2 and 3 and virus isolation from chicken 2. FAdV4 was confirmed in both samples by quantitative PCR amplification of a 143-bp region of the *hexon* gene using primers published previously[2] and a newly designed probe (FAM-CAGATGWCTGACGC SGASTAC-BHQ1). For virus isolation, the liver suspension was inoculated on chicken embryo liver cell culture. Cytopathic effect was observed after 5 d of incubation at 37°C. Following ultracentrifugation, total nucleic acid was extracted (MagMAX pathogen RNA/DNA kit; Thermo Fisher). A DNA library was constructed (Ligation sequencing SQK-LSK109 kit; Oxford Nanopore Technologies [ONT]). The library was loaded onto a Flongle flow cell (ONT) and run on a sequencer (MinION; ONT) using default parameters for 24 h. Base-called FASTQ files containing "pass" reads (Q-score ≥ 7) were loaded into Geneious Prime (v.2020.0.5; Biomatters) and mapped to a reference FAdV4 genome (MH454598). A consensus sequence corresponding to the entire FAdV4 genome (43,717 bp) at a coverage of 200× was generated. On BLASTN, the sequence identified 99.99% homology with GenBank accession MG547384, corresponding to a C-4 isolate from China (Fig. 4). We submitted our isolate to GenBank (accession MT813039).

There are 12 known serotypes of FAdVs, belonging to 5 distinct species.[13] Serotypes isolated from field outbreaks in the poultry industry throughout the world show a predominance of serotype 8 in IBH cases and serotype 4 in HHS cases.[6,9,12,20] Although the hepatic lesions are equivalent in IBH and HHS, the distinguishing feature between these 2 conditions is the presence of hydropericardium and cardiac lesions described with HHS.[8] In our FAdV C-4–associated deaths in a flock of adult backyard hens, none of the 3 examined chickens had classical gross lesions described in commercial birds with adenoviral hepatitis, with the exception of the petechiation in the liver of chicken 1. All 3 birds appeared to have relatively normal-sized livers, and 2 had diffusely dark mahogany livers. Although some lesions were most certainly obscured by the prolonged postmortem intervals, these birds also did not have hydropericardium. In a report from Peru of C-4 isolates from outbreaks of adenoviral hepatitis consistent with IBH in broilers, hydropericardium was absent.[11] The 789-bp sequences analyzed in Peru are considerably different from the C-4 sequenced from our cases, and further comparisons cannot be made. Nevertheless, the serotype identified in the backyard chickens described here is identical to the strains identified in HHS outbreaks in China, and we cannot account for this discrepancy.[3] Our outbreak of C-4 in a backyard flock of adult hens with classical IBH lesions, but not HHS, and with mortality close to what is expected (30%) in HHS outbreaks, adds to the complexity of categorizing FAdV-associated diseases.[18]

It is not clear how this flock of backyard hens in rural northern California became exposed to FAdV. The farm did not allow visitors access to the poultry, and the owners had neither a travel history nor any connection to commercial poultry operations. It is known that ~60 d before the FAdV disease outbreak, a group of 15 mature laying hens had been introduced to the existing flock, doubling the size of the flock. Whereas the original birds had been sourced from a National Poultry Improvement Plan (NPIP; www.poultryimprovement.org/)-approved hatchery, the new arrivals came from a neighborhood eclectic backyard



Figures 1–3. Fowl aviadenovirus C-4 infection in backyard chickens. **Figure 1**. Coalescing areas of severe, acute hepatic necrosis with fibrin accumulation in chickens 2 and 3. Large, basophilic and eosinophilic adenoviral inclusion bodies were frequent in hepatocellular nuclei (*). H&E. Inset: gross appearance of chicken 1 liver; mahogany tissue with generalized, 1–3 mm diameter hemorrhages. **Figure 2**. Cluster of adenovirus particles contrasted negatively. Transmission electron microscopy (TEM). Bar = 0.5 µm. **Figure 3**. Intranuclear adenoviral particles contrasted positively, in a paracrystalline array. TEM. Bar = 2 µm.

flock with many potential sources. The outdoor housing environment also allowed for ample exposure to wildlife, and wild birds such as falcons or pigeons have been suggested as carriers of FAdVs, with possible cross-species transmission of FAdV-D and -E from falcons.[7,10] Furthermore, although FAdVs are now recognized as primary pathogens, we cannot rule out coinfections with immunosuppressive agents, such as Marek disease virus (*Mardivirus*), chicken anemia virus (CAV), or infectious bursal disease virus,[13] that may have predisposed to or exacerbated the FAdV infection in the adult chickens that we have described here. With the exception of the single hen that died shortly after laboratory submissions were received for this case, the rest of the laying flock, as well as an NPIP-sourced broiler flock housed separately on the same premises, have remained healthy through the time of our report, although it is unknown if there has been a change in the growth or production rates.

The FAdV C-4 strain isolated here lies in a cluster of Chinese strains that are 99.99% identical, differing in only 6 bp in the genome, and with no amino acid changes: F-Vac, JSJ13, NIVD2, HLJFAd15, and ZJ2015.[17] Of these, F-Vac is the strain isolated from a contaminated Newcastle disease (*Avian orthoavulavirus 1*) viral vaccine; the remainder are wild strains isolated from the outbreaks in China.[17] The role of contaminated attenuated live Newcastle disease viral vaccines has been documented, and these vaccines are the

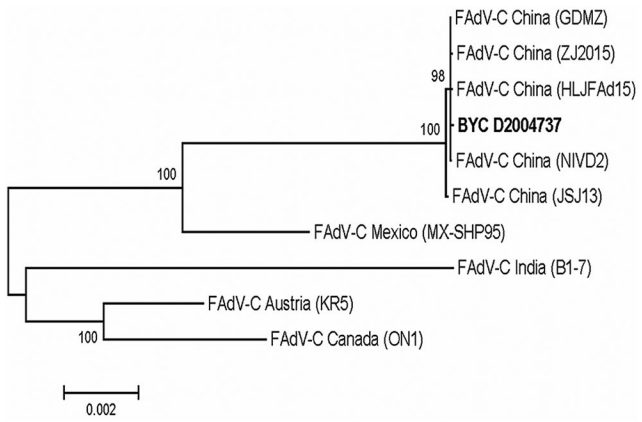


Figure 4. Backyard chicken fowl aviadenovirus C-4 isolate (BYC D2004737) whole genome BLASTN alignment with identical strains. The 5 strains with 99.99% homology are all from China and were associated with hepatitis–hydropericardium syndrome outbreaks.

suggested cause of the rapid and geographically distinct spread of FAdV outbreaks in China.[15] The spread of the outbreaks was exacerbated mostly by contamination by both FAdV and CAV.[16] The suggested vaccine associated with these outbreaks is in use in the United States, although we are not aware of any contamination issues. The spread of FAdV through contaminated vaccines does not seem likely to be a primary route of exposure for backyard chickens, given that small hobby flocks tend to have minimal vaccination strategies. If vaccinated at all, backyard birds are vaccinated against Marek disease, but rarely against other diseases such as Newcastle disease or adenoviral infections. Commercial California poultry operations may, however, be at risk.

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Declaration of conflicting interests

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