CASE REPORT

A CASE OF FOWL ADENOVIRUS-8B INFECTION IN A TUNNEL VENTILATED BROILER FARM IN CENTRAL LUZON, PHILIPPINES

Jo Vicente M. Mapa, DVM[1] and Patrick Linus D. delos Reyes, DVM[2*]

[1]Philippine College of Poultry Practitioners Taytay, Rizal, 1920, Philippines
[2]Abba Farm, Inc. Consolacion, Cebu, 6000, Philippines

ABSTRACT

Fowl adenovirus (FAdV), a non-enveloped, double stranded DNA virus, causes varying clinical presentations in commercial broiler flocks which include gizzard erosion, hepatitis, and hydropericardium. A commercial broiler farm in Tarlac, Central Luzon, Philippines showed clinical signs indicative of inclusion body hepatitis which also manifested through increased numbers of underweight birds. Serological and molecular diagnostic procedures were carried out to confirm the disease. Serological results showed tentative diagnoses with titers indicative of Infectious Bronchitis, Infectious Bursal Disease, Fowl Adenovirus, and Newcastle Disease. Polymerase chain reaction and sequencing of genes of interest showed positive results for IB B48 strain of the Massachusetts serotype (vaccine strain) and FAdV-8b serotype (field strain) of the species E. Medication program with consideration to impaired liver function, hypovitaminosis, and impaired metabolism was recommended for the succeeding grows. Furthermore, procedures for routine monitoring of the disease and vaccination of the parent stock sources with an inactivated FAdV-4/8 bivalent vaccine were recommended. To the knowledge of the authors, this is the first report of FAdV-8b infection in the Philippines.

Keywords: *adenovirus, broiler, inclusion body hepatitis, serology, polymerase chain reaction*

Philipp. J. Vet. Med., 59(1): 74-79, 2022

INTRODUCTION

Adenoviral infections that cause disease in poultry belong to three genera under the family adenoviridae: Aviadenovirus (group I), Siadenovirus (group II), and Atadenovirus (group III). Adenoviruses are non-enveloped, icosahedral, linear, double-stranded DNA viruses. Of the three groups, aviadenovirus causes most severe lesions in poultry with its different presentations (Steer *et al.*, 2009). It is further divided into species (A-E) and different serotypes, based on the ICTV, US, or EU classification.

Different serotypes are associated with different clinical presentations. The disease in domestic chicken usually includes the liver but can also present gizzard, heart, and kidney lesions. Adenoviral Gizzard Erosion (AGE) or Gizzard Erosion and Ulceration (GEU) is associated with FAdV-1 serotype infections. More severe disease involving the liver which is described as Inclusion Body Hepatitis (IBH) is caused by serotypes from species D and E. In even more severe cases, infections involve the heart and cause the Hydropericardium-Hepatitis Syndrome (HHS) which is associated with FAdV-4 infection (Mirzazadeh *et al.,* 2021; Schachner *et al.,* 2018)

Because of the many clinical presentations, it may cause economic losses due to mortalities and condemnations. Growth retardation brought about by impaired liver function, gizzard erosion, and high mortalities due to impaired cardiac function cause s ubstantial economic losses (Schacner *et al.,* 2018). The inherent nature of the virus to resist physical and chemical agents also pose a significant concern to its control in farm operations.

CASE PRESENTATION

The farm of interest is located in Tarlac, Central Luzon, Philippines which is a tunnel ventilated broiler operation with four (4) buildings with Cobb500™ strain but from different breeder sources of 35 to 55 weeks of age.

***FOR CORRESPONDENCE:**
(e-mail: plddr.dvm@gmail.com)

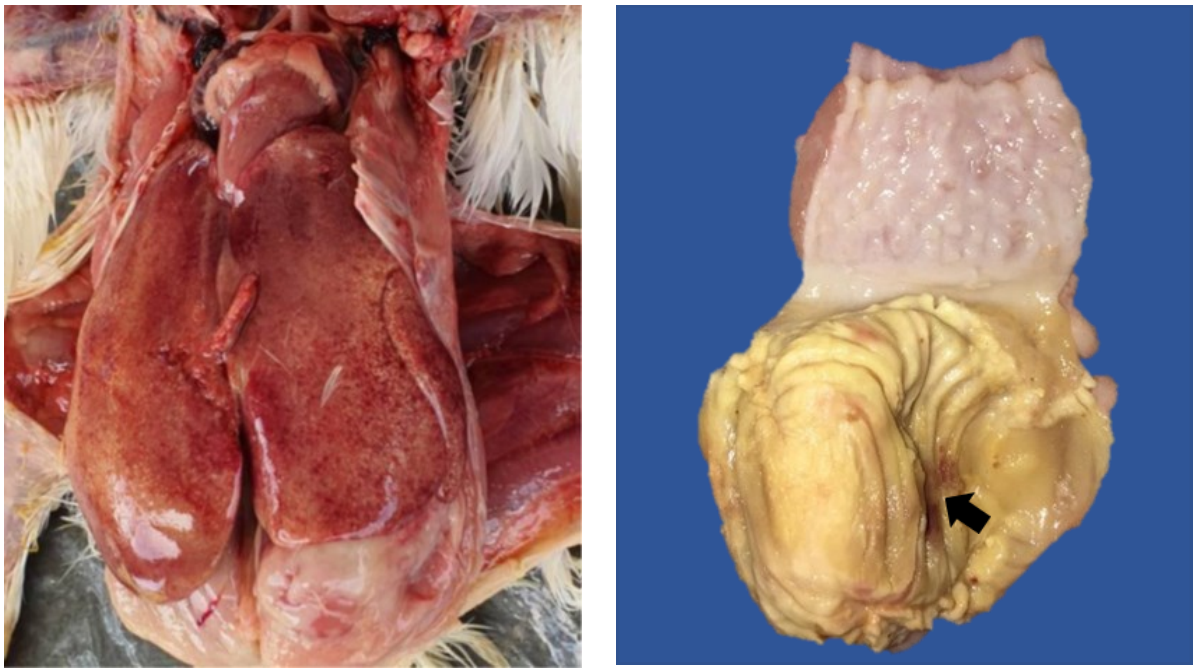
Flooring for all buildings were all slat. Feeds were commercial and were formulated specifically based on Cobb 500 requirements.

Birds were vaccinated with live Infectious Bronchitis (Day 1: 1/96; Day 7: H120), immune complex Infectious Bursal Disease (Day 1: Winterfield 2512 strain), and live/vectored Newcastle Disease (Day 1: HVT Vectored; Day 7: PHY.LMV.42; Day 7 and 22: Clone 30; Day 15: Lasota).

Two houses were visited: building 1 at Day 26 and building 4 at day 21/22. Building 1 was reported to have had concerns with coccidiosis, tremors, and a previous diagnosis of Infectious Bursal Disease (IBD) by a third party. No reports of any disease for building 4 were relayed by the management. According to the management, chicks were apparently normal upon arrival. The farm used feed brand A for their booster ration and cases of coccidiosis were reported. After shifting to feed brand B for starter, the flock at building 1 experienced tremors prompting the farm to shiftimmediately to another feed brand. Feed shifting takes three days based on farm practice with 1:3, 1:1, and 3:1 ratio to avoid abrupt changes and subsequent decreased feed intake.

As early as the first two weeks, the birds were scratching feeders and selecting feed particles to feed on. Upon observation, poor uniformity was observed with the flock in building 1. Additionally, the segregation pen contained approximately 10,000 birds which was 25% of the total house population. Other clinical signs observed in this building were mild respiratory signs and isolated cases of tremors. For building 4, mild respiratory signs were also noticed.

Twelve (12) birds, both morbid birds and mortalities, were necropsied. Birds showed varying presentations of intestinal conditions: thin intestinal mucosa, hyperemia, enteritis, and sloughing off of the intestinal lining. All the birds opened had swollen, pale, and/or hemorrhagic livers (Figure 1A). Additionally, grade 1 gizzard erosions were observed in six (6) birds (Figure 1B).



(A) **(B)**

Figure 1. Gross Lesions from FAdV-8b infected broieler. (A) Liver from bird showing signs of inclusion body hepatitis. Edges are rounded with palor and numerous diffuse petecchial hemorrages. (B) Gizzard of bird infected with fowladenovirus. Koilin layer is discolored and with mild erosion (arrow).

Terminal (35 days) blood samples were collected to evaluate titers for Fowl Adenovirus Grp 1 (FAdV Grp-1), Infectious Bronchitis (IB), Infectious Bursal Disease (IBD), and Newcastle Disease. Biochek® ELISA Test Kit measured the amount of antibodies for group 1 FAdV, IBV, and NDV. The test determined the presence of antibodies in the serum of chickens. Serum dilution is 1:100 with test incubation of 30-30-15 minutes that was read at 405 nm wavelength. Results are illustrated in Table 1. Antibody titers from Biochek® ELISA showed that despite vaccination against IB, IBD, and ND, outliers were still observed. Additionally, only IBD CV (%) was below the desirable value of 40%. Titers also showed that 19 out of 19 birds tested positive for FAdV Grp-1 despite not vaccinating for the disease. The CV (%) for this titer result also showed poor uniformity in flock response.

Table 1. Antibody titer results for FAdV, IBV, IBDV, and NDV.

Disease Agent	Mean Titer	Min-Max Titer	CV (%)	Suspect Titer Infection
FAdV Grp-1	5872	1104-15684	80	Positive
IBV	6861	1226-12243	44	>3000
IBDV	13623	1137-18271	32	>14000
NDV	5850	1142-15580	67	>12000

For a more confirmatory diagnosis, tissue samples from the cecal tonsils, kidney, liver, spleen, and trachea were collected and subjected to polymerase chain reaction (PCR) and were sequenced to determine the strains or serotypes (Table 2). Primer probes used for targeted genes were based from literature. The flock exhibited positive results for IBV B48 strain of the Massachusetts serotype and FAdV-8b serotype.

Table 2. Polymerase chain reaction (PCR) and sequencing results.

Virus	Target gene	Result	Strain/Serotype	Interpretation
Fowl Adenovirus	Hexon gene	Positive	FAdV-8b	Field Challenge
Infectious Bronchitis virus	Spike gene	Positive	B48	Vaccine strain
Newcastle Disease virus	Fusion gene	Negative	-	-

DISCUSSION

Despite having unsatisfactory titer results for IB, IBD, and ND, the flock did not display specific signs for these diseases. Outliers in titer readings may indicate poor vaccination administration or possibly host immune response to vaccination which may be caused by an immunosuppressive disease agent such as FAdV.

Necropsy findings were more indicative of Inclusion Body Hepatitis (IBH) which is caused by adenovirus under the Group 1 adenoviruses or Aviadenovirus. This was supported by the ELISA results which tested positive for all samples. The CV (%) of 80 was also indicative that the flock responded uniformly to the suspected field challenge.

Other clinical signs and gross lesions that needed be ruled out are mycotoxicosis, feed formulation anomaly, liver damage due to medication overdose, pasteurellosis, colibacillosis, and mycoplasmosis. A complete panel of tests may be ideal but not economical for a commercial operation. Conducted tests (ELIA, PCR, and sequencing) were based on farm history of management and disease, clinical signs, and gross lesions observed.

Liver damage due to overdosing of medication was ruled out because the farm practices prudent use of antibiotics and administers liver tonics in cases of antibacterial therapy. As for other bacterial diseases such as colibacillosis and mycoplasmosis, lesions were more of polyserositis (perihepatitis) with fibrinosuppurative exudates (Armour, 2020; Nolan *et al.*, 2020).

Mycotoxicosis can be a complicating factor but too unlikely present in this case as the feeds are regularly delivered in one to three days after production and no storage of feeds exceeding one week is being practiced. Pasteurellosis in broilers present the acute form which does not have liver lesions. This form is characterized by fever, anorexia, diarrhea, and sudden death (Blackall and Hofacre, 2020) which were not observed in the case.

Results from PCR and sequencing confirmed adenoviral infection of serotype

FAdV-8b from the species E and a concurrent infection with IBV B48 strain. The infectious bronchitis from the sequencing was a vaccine strain that could have circulated in the farm due to historical vaccination with a B48 strain. In this case, the hexon gene was targeted for molecular diagnosis. Hexon is the major capsid protein with fiber and penton being the other two structural components (Wang and Zhao, 2019). It is a relatively conserved gene among aviadenoviruses (Kajan *et al*., 2013) with type group-specific antigen epitope and a neutralizing antigen epitope (Mittal *et al.,* 2013). Studies showed that substitution of the fiber-2 or hexon gene in highly pathogenic FAdV resulted in decreased virulence. This suggests that virulence of FAdV is associated with fiber-2 and hexon genes (Zhang *et al.*, 2018).

The fowl adenovirus can be transmitted both vertically and horizontally through feco-oral route (Gomis *et al.,* 200). Horizontal transmission plays an important aspect in the persistence of the virus in the farm due to its inherent resistance to physical and chemical agents (Kanwar, 2010). The only disinfectant recommended to inactivate the virus is formaldehyde at a concentration of 1:1000. Research regarding the use of divalent cations in inactivating FAdVs have inconsistent results. Some strains of FAdVs can also survive temperatures of 60-70°C for 30 minutes (Hess, 2020). Locally, formalin prillls are available and are used mostly in hatchery operations. For commercial poultry buildings, 30 minutes to 1 hour by fumigation using a heater then subsequent ventilation until odor has dispersed (may take up to 24 hours) is recommended. This will be the terminal disinfection after two rounds of other disinfectants such as oxidizing agents, phenolic, and quaternary ammonium compounds.

Cases of FAdV infections are usually more observed in broilers up to five weeks of age than in layers and breeders (Schachner *et al.,* 2018). Observed cases within the first days of the first week may indicate vertical transmission. The incubation period may be as short as 24-48 hours (Li *et al*., 2017) which may cause signs in flocks in the latter part of the first week. For this particular case, a horizontally transmitted infection was concluded since the signs were observed later than the first week which was more evident during the third week.

Infections with FAdVs show varying clinical signs and effects which depends on the observed syndrome. There are three (3) presentations of FAdV infections: hepatitis-hydropericardium syndrome (HHS), inclusion body hepatitis (IBH), and adenoviral gizzard erosion (AGE) (Schachner *et al.,* 2018). Mortalities are more severe in cases of HHS than IBH and AGE. Field records show mortalities of 10-30% for IBH, 20-70% for HHS, and 6-20% for AGE (Mirzazadeh *et al.,* 2021; Schachner *et al.,* 2018). Other mortalities and negative effects in production in cases of HHS and IBH are attributed to compromised liver function which was the case for this operation. Macro- and micronutrient metabolism may be impaired in such cases which may result to high condemnations due to poor average live weight (ALW) and uniformity. Signs of mineral and vitamin deficiencies such hypovitaminosis E, which explain tremors, may also be observed in hepatitis cases (Schachner *et al.,* 2021). Although not observed during the necropsy, coccidiosis as reported by a third-party diagnostician, could have had an effect on nutrient absorption due to damaged intestinal villi. As for AGE, growth retardation and poor uniformity will be observed in the flock because of the damage in the koilin layer of the gizzard that impairs its ability to mechanically digest food (Mirzazadeh *et al.,* 2021).

Because FAdV infections are immunosuppressive diseases, they exacerbate existing or permit the introduction of other diseases in poultry flocks (Hoerr *et al.,* 2010). Common presentations may include cases of IB, IBD, and ND even in vaccinated flocks (Xu *et al.,* 2021;). Titers for IB, IBD, and ND showed outliers which indicated that some birds poorly responded to vaccination.

The role of maternal antibody protection is satisfactory for IBH and HHS while minimal protection is offered in cases of AGE (Wang and Zhao, 2019). After infection, birds develop type-specific neutralizing antibodies that are detectable after one week and peak after three weeks (Hess, 2020). It is common to observe multi-serotype infection in field cases (Jordan *et al.,* 2019).

Aviadenoviruses have a group-specific antigen that differentiate them from Siadenoviruses and Atadenoviruses. Because of this, there have been no proven cross-protection across groups. For cross-protection across species, there is little evidence. Fortunately, serotypes belonging to the same species have satisfactory cross-protection (Redondo *et al.,* 2018; Kim *et al.,* 2013; Dar *et al.,* 2012).

There are several control points that are essential to managing IBH cases in commercial poultry production. Several factors must also be considered such as mode of management, diagnostic or serological monitoring, and health and biosecurity. Co-infections and sequelae must also be taken into account in formulating

medication program in farms with existing cases.

In active IBH cases, the first intervention strategy must be focused on the management aspect of the operation. It is best to segregate and cull early as to decrease the viral load in the flock. A regular monitoring of ALW and uniformity must also be routinely done as to identify any effects early in production.

Since FAdV-8b is both vertically and horizontally transmitted, the first control point must be in the breeder farm. If stocks are being sourced from multiple breeder sources, proper recording of breeder source and serology of progeny flock may localize the infection into specific breeder sources. This, however, may be difficult if horizontal transmission is already established in the farm. Additionally, the commercial broiler grower may request, if permitted, a vaccination record to check if breeder stocks are protected against fowl adenoviruses. Furthermore, antibody titer monitoring of breeders and DOCs may be done to determine if infection is vertical, horizontal, or both. Serological monitoring of several flocks in the farm over several cycles may also be necessary to determine if the virus is still present even after intervention strategies. In highly suspected cases, tissue samples of the liver may be collected for histopathology to microscopically confirm presence of inclusion bodies. Furthermore, as is with the case, samples of the affected tissues may be collected for PCR and sequencing to determine the specific serotype and or strain.

Current breeder broiler vaccination programs in the Philippines include a different adenovirus of the Atadenovirus group which is Egg Drop Syndrome '76 (EDS '76) which does not protect against FAdVs. Both live and inactivated vaccines have been developed to protect birds from aviadenoviral infections which can offer up to 98-100% protection (Gupta *et al.*, 2017). Based on several studies, a bivalent killed vaccine with the 8b and 11 serotype offered best protection across FAdV species (Du *et al,* 2017) while 8a produced inconsistent results (De Luca *et al.*, 2020).

While it is essential to directly monitor and control IBH in farm operations, its many clinical presentations due to co-infections and metabolic disorders must also be a point of control. The effect in performance of broilers in IBH cases is mainly due to the liver insult. Because of this, it is best to formulate a therapeutic program that is aimed to restore liver function and boost the immune system. Additionally, since the liver is the main organ for metabolism of macromolecules and other nutrients, supplements such as vitamins and amino acids or peptides were given in this particular case. Regular monitoring for other common diseases such as IB, IBD, ILT, and NCD must also be done considering that FAdV is an immunosuppressive pathogen that may affect vaccine efficacy for other pathogens.

In summary, controlling active cases of IBH must be a holistic approach which includes refining of management procedures, disease agent monitoring, prevention through vaccination and biosecurity, and a well-formulated therapeutic program based on clinical presentations present in the case. To the knowledge of the authors, this study is the first report of FAdV-8b infection in the Philippines.

STATEMENT ON COMPETING INTEREST

The authors declare that they have no competing interests.

AUTHOR'S CONTRIBUTIONS

JVM performed initial field investigation of the case and interpreted results for further analysis of PLD with existing literature on the topic. Both JVM and PLD formulated recommendations for the case. Furthermore, PLD drafted the manuscript which was subjected to final editing, review, and approval of JVM

REFERENCES

Armour NK. 2020. *Mycoplasma gallisepticum* infection. In Swayne DE, Boulianne M, Logue CM, McDougald LR, Nair V, and Suarez DL (Eds.). *Diseases of Poultry*. 14th ed. New Jersey: John and Wiley Sons, Inc., pp. 911-923.

Blackall PJ and Hofacre CL. 2020. Fowl cholera. In Swayne DE, Boulianne M, Logue CM, McDougald LR, Nair V, and Suarez DL (Eds.). *Diseases of Poultry*. 14th ed. New Jersey: John and Wiley Sons, Inc., pp. 831-846.

Dar A, Gomis S, Shirley I, Mutwiri G, Brownlie R, Potter A, Gerdts V, and Tikoo SK. 2012. Pathotypic and molecular characterization of a fowl adenovirus associated with inclusion body hepatitis in Saskatchewan chickens. *Avian Diseases* 56:73-81.

De Luca C, Schachner A, Mitra T, Heidl S, Liebhart D, and Hess M. 2020. Fowl adenovirus (FAdV) fiber-based vaccine against inclusion body hepatitis (IBH) provides type-specific protection guided by humoral immunity and regulation of B and

T cell response. *Veterinary Research* 51:143-158.

Gomis S, Goodhope R, Ojkic D, and Willson P. 2006. Inclusionn body hepatitis as a primary disease in broilers in Saskatchewan, Canada. *Avian Diseases* 50 (4):550-555.

Gupta A, Popowich, Ojkic D, Kurukulasuriya S, Chow-Lockerbie B, Gunawardana T, Goonewardene K, Karunnarathna R, Ayalew LE, Ahmed KA, Tikoo SK, Willsonn P, and Gomis S. 2017. Inactivated and live bivalent fowl adenovirus (FAV8b + FAdV11) breeder vaccines provide broad-spectrum protection in chicks against inclusion body hepatitis (IBH). *Vaccine*, 32(28):3564-3568.

Hess, M. 2020. Aviadenovirus infections. In Swayne DE, Boulianne M, Logue CM, McDougald LR, Nair V, and Suarez DL (Eds.). *Diseases of Poultry*. 14th ed. New Jersey: John and Wiley Sons, Inc., pp. 322-332.

Hoerr HJ. 2010. Clinical aspects of immunosuppression in poultry. *Avian Diseases* 54:2-15.

Jordan AB, Blake L, Bisnath J, Ramgattie C, Carrington CV, and Oura CAL. 2018. Identification of four serotypes of fowl adenovirus in clinically affected commercial poultry co-infected with chicken infectious aneamia virus inn Trinidad and Tobago. *Transboundary Emerging Diseases* 66:1341-1348.

Kajan GL, Sameti S, Benko M. 2013. Partial sequence of the DNA-dependent DNA polymerase gene of fowl adenoviruses: a reference panel for a general diagnostic PCR in poultry. *Acta Veterinaria Hungarica* 59 (2): 279-285.

Kajan GL, Kecskemeti S, Harrarch B, Benko M. 2013. Molecular typing of fowl adenoviruses, isolated in Hungary recently, reveals high diversity. *Vet Microbiology* 167(3-4):357-363.

Kanwar P, Sharma M, Dhar P, Katoch V, and Asrani RK. 2010. Physiochemical characterization of fowl adenovirus serotype 4 isolate from Himachal Pradesh. *Indian Journal of Animal Sciences* 80(8):719-720.

Li PH, Zheng PP, Zhang TF, Wen GY, Shao HB, and Luo QP. Fowl adenovirus serotype 4: epidemiology, pathogenesis, diagnostic detection, and vaccine strategies. *Poultry Science* 96:2630-2640.

Mirzazadeh A, Grafl B, Abbasnia M, Emadi-Jamali S, Abdi-Hachesoo B, SSchachner A, and Hess M. 2021. Reduced performance due to adenoviral gizzard erosion in 16-day-old commercial broiler chickens in Iran, confirmed experimentally. *Frontiers in Veterinary Science* 8:1-8.

Mittal D, Jindal N, Tiwari AK, Khokhar RS. 2014. Characterization of fowl adenovirus associated with hydropericardium syndrome and inclusion body hepatitis in broiler chickens. *Virus Disease,* 25(1):114-119.

Nolan LK, Vaillancourt JP, Barbieri NL, Logue CM. 2020. Colibacillosis. In Swayne DE, Boulianne M, Logue CM, McDougald LR, Nair V, and Suarez DL (Eds.). *Diseases of Poultry*. 14th ed. New Jersey: John and Wiley Sons, Inc., pp. 771-808.

Redondo H, Sarabia F, Tahala MA, Bensassi Y, Gil I, Elbachir E, Rodriguez MJ, and Moreno JCA. 2018. Characterization of strain of fowl adenovirus circulating in Morocco. *Poultry Sciencei* 97:4057-4062.

Schacner A, Matos M, Grafl B, and Hess M. 2018. Fowl adenovirus-induced diseases and strategies for their control – a review on the current global situation. *Avian Pathology* 47 (2):111-126.

Steer PA, Kirkpatrick NC, O'Rourke D, and Noormohammadi AH. 2009. Classification of fowl adenovirus serotypes by use of high-resolution melting-cure analysis of the hexon gene region. *Journal of Clinical Microbiology* 47(2):311-321.

Wang Z and Zhao J. 2019. Pathogenesis of hypervirulent fowl adenovirus serotype 4: the contributions of viral and host factors. *Viruses* 11:741-751.

Wellehan JFx, Johnson AJ, Harrach B, Benko M, Pessier AP, Johnson CM, Garner MM, Childress A, Jacobson ER. 2004. Detection and analysis of six lizard adenoviruses by consensus primer PCR provides furter evidence of a reptilian origin for atadenoviruses.

Zhang Y, Liu R, Tian K, Wang Z, Yang X, Gao D, Zhang Y, Fu J, Wang H, Zhao J. 2018. Fiber 2 and hexon genes are closely associated with the virulence of the emerging and highly pathogenic fowl adenovirus 4. *Emerging Microbes & Infections* 7:199-209.