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Short communication

Characterization of fowl adenoviruses isolated between 2007 and 2014 in China

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ABSTRACT

Forty-three fowl adenovirus (FAdV) strains were isolated in China from 2007 to 2014 from poultry and ostriches with inclusion body hepatitis (IBH) and hydropericardium syndrome (HPS). Phylogenetic analysis showed that 28/43 strains clustered into *Fowl aviadenovirus D* (FAdV-D) and 9/43 strains clustered into FAdV-E. FAdV-C included three isolates of ostrich origin, one of goose origin and two of chicken origin. Based on hexon loop 1 gene sequencing analysis, these viruses were genetically related to FAdV-4, FAdV-8a, FAdV-8b and FAdV-11, of which FAdV-11 was dominant. The isolation in 2014 of three FAdV strains belonging to serotype 4 from ostrich flocks is to our knowledge the first finding of FAdV-4 infection and HPS cases in ostriches. Epidemiological analysis showed that FAdV has been circulating in northern and eastern China, where more than 50% of layers and broilers are raised. The hosts of this pathogen included broilers, layers, geese and ostriches. IBH and HPS cases had a sporadic or cluster distribution from 2007 to 2013; however, since 2014 the number of cases has increased sharply. To control FAdV, strict biosecurity protection measures are necessary and a multivalent vaccine may be needed.

1. Introduction

Adenoviruses (AdVs), which are divided into 5 genera, are icosahedral non-enveloped viruses containing double-stranded linear DNA genomes encoding proteins on both strands (Hess, 2000). Chickens can be infected by fowl adenoviruses (FAdVs, genus *Aviadenovirus*), by egg drop syndrome (EDS) virus (duck adenovirus 1, genus *Atadenovirus*) and by turkey hemorrhagic enteritis (HE) virus (turkey adenovirus 3, genus *Siadenovirus*) (Hess, 2000). The most notable diseases associated with FAdV infection in chickens are inclusion body hepatitis (IBH), hydropericardium syndrome (HPS) and gizzard erosion (GE) (Domanska-Blicharz et al., 2011; Nakamura and Yuasa, 1999; Wells and Harrigan, 1974).

Traditionally, serological methods (i.e. virus neutralization [VN]) have been used for the typing of FAdV isolates (Mcferran et al., 1972). FAdVs are divided into 12 serotypes by serum cross-neutralization tests (Meulemans et al., 2004), and are grouped into five species, *Fowl aviadenovirus A* to *E* (FAdV-A to E) based on restriction fragment length polymorphism (RFLP) (Hess, 2000). Characterization of isolated viruses by traditional serological methods is not widely used, because obtaining a full panel of reference FAdV strains is not easy. However, molecular methods could break through the barriers. Analysis of the nucleotide sequences encoding the adenovirus hexon protein, the most abundant viral surface protein that also contains major antigenic determinants, has been used for adenovirus genotyping as an alternative and a complement to classical methods. In the majority of recent surveys in Asia, North America and Europe, novel isolates

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have been studied by molecular methods then classified into the existing serotypes (Choi et al., 2012; Kaján et al., 2013; Meulemans et al., 2004; Ojkic et al., 2008; Steer et al., 2009).

IBH or HPS outbreaks occurred in different continents, resulting in economic losses to the poultry industry (Kaján et al., 2013; Mittal et al., 2014; Ojkić et al., 2008). In China, the clinical cases of IBH have been increasing since 2009, especially since 2014, when there was an unusual outbreak. A number of FAdVs have been isolated from diseased birds in recent years; however, there have been no systemic epidemiological surveys of FAdVs. There is also limited information about the molecular character of FAdVs in China. The objectives of the present study were to characterize FAdVs isolated from IBH and HPS cases in China between 2007 and 2014, using molecular methods.

2. Materials and methods

2.1. Virus isolation

Fowl adenoviruses (Table 1) were isolated from diagnostic material submitted to the Animal Diagnostic Center of the Qingdao Oland-Better Biotechnical Company (Qingdao, China). For virus isolation, liver and kidney tissue from dead birds was removed and homogenized to obtain a 10% suspension. After low-speed centrifugation, the tissue suspensions were screened by PCR using primers specific for FAdVs. The PCR procedure used is described in Part 2.3 below. The FAdV-positive supernatants were inoculated onto the chorioallantoic membrane (CAM) of 10-day-old specific pathogen-free (SPF) embryonated chicken eggs following standard methods (Senne, 1989). Embryonic death within the first 24 h was ascribed to non-specific causes. Embryos that died after 24 h were examined for the presence of gross lesions. The CAMs from dead embryos were harvested under sterile conditions and were blind-passaged three times, as described above. Finally, the CAMs of dead embryos were harvested and prepared as a 10% suspension for further study.

2.2. DNA extraction, PCR and sequencing

Viral genomic DNA was extracted using the High Pure Viral Nucleic Acid Kit (Roche Applied Science, IN, USA). The DNA was tested by PCR with High Fidelity (HiFi) PCR SuperMix II (TransGen

Table 1
Summary of the fowl adenoviruses described in this study.

Strains	Host	Age	Time	Location	Tissues	Species	Serotype	Other positive tests	Accession number
FAdV-YTLY-071107-B	broiler	10d	2007.11.07	SD	Liver/Kidney	D	11	H9	KU981121
FAdV-YTLY-081010-B	broiler	10d	2008.10.10	SD	Liver/Kidney	D	11	–	KU981122
FAdV-YTLY-091107-B	broiler	8d	2009.11.07	SD	Liver/Kidney	D	11	H9	KU981123
FAdV-SDLY-090811-B	broiler	32d	2009.08.11	SD	Liver/Kidney	E	8a	CIA	KU981124
FAdV-JLDH-090814-B	broiler	36d	2009.08.14	JL	Liver/Kidney	D	11	–	KU981125
FAdV-QDPD-090908-B	broiler	13d	2009.09.08	SD	Liver/Kidney	D	11	CIA	KU981126
FAdV-JLDH-091011-B	broiler	22d	2009.10.11	JL	Liver/Kidney	D	11	–	KU981127
FAdV-SDLY-100803-B	broiler	36d	2010.08.03	SD	Liver	E	8a	–	KU981134
FAdV-QDPD-100808-B	broiler	10d	2010.08.08	SD	Liver/Kidney	D	11	H9,CIA	KU981135
FAdV-SDWF-100816-B	broiler	10d	2010.08.16	SD	Liver/Kidney	D	11	–	KU981136
FAdV-HNQX-101017-B	broiler	22d	2010.10.17	SX	Liver	E	8b	–	KU981154
FAdV-LNDL-110703-B	broiler	10d	2011.07.03	LN	Liver	D	11	CIA	KU981138
FAdV-SDWF-110712-B	broiler	20d	2011.07.12	SD	Liver	E	8a	–	KU981142
FAdV-QDLX-111025-B	broiler	65d	2011.10.25	SD	Liver	D	11	H9	KU981141
FAdV-HLJ-111027-B	broiler	uk	2011.10.27	HLJ	Liver	D	11	CIA	KU981146
FAdV-WFCY-120317-B	broiler	uk	2012.03.17	SD	Liver	D	11	–	KU981144
FAdV-HN-120913-B	broiler	uk	2012.09.13	HN	Liver	D	11	CIA	KU981147
FAdV-DL-121011-B	broiler	20d	2012.10.11	LN	Liver	E	8b	*E. coli*	KU981139
FAdV-YN-121123-B	broiler	20d	2012.11.23	YN	Liver	D	11	–	KU981143
FAdV-DLHY-130107-B	broiler	uk	2013.01.07	LN	Liver	D	11	H9	KU981140
FAdV-JL-130131-B	broiler	uk	2013.01.31	JL	Liver	E	8b	CIA	KU981148
FAdV-HNYD-130219a-L[1]	broiler	133d	2013.02.19	HN	Liver	E	8a	–	KX756582
FAdV-HNYD-130219b-L[1]	broiler	133d	2013.02.19	HN	Liver	D	11	–	KX756583
FAdV-BJ-130615-L	layer	uk	2013.06.15	BJ	Liver	D	11	CIA	KU981145
FAdV-QDLX-140224-L	layer	uk	2014.02.24	SD	Liver	D	11	ND	KX756576
FAdV-QDLX-140421-B	broiler	1d	2014.04.21	SD	Liver	D	11	–	KX756577
FAdV-HBHY-140524-B	broiler	uk	2014.05.24	HB	Liver	D	11	–	KX756578
FAdV-SDWF-140614-B	broiler	30d	2014.06.14	SD	Liver/Kidney	E	8b	H9	KU981150
FAdV-SDWF-140620a-B[2]	broiler	33d	2014.06.20	SD	Liver	D	11	CIA	KX756580
FAdV-SDWF-140620b-B[2]	broiler	33d	2014.06.20	SD	Liver	C	4	CIA	KX756581
FAdV-QDLX-140620-B	broiler	10d	2014.06.20	SD	Liver	E	8a	–	KX756579
FAdV-SDRZ-140718-L	broiler	40d	2014.07.18	SD	Liver	C	4	–	KU981149
FAdV-SDLQ-140704-G	goose	uk	2014.07.04	SD	Kidney	C	4	–	KU981153
FAdV-HBHD-140710-O	ostrich	90d	2014.07.10	HB	Liver/Kidney	C	4	*E. coli*	KU981151
FAdV-HBHS-140719-O	ostrich	60d	2014.07.19	HB	Liver/Kidney	C	4	–	KU981152
FAdV-HBSJZ-14094-O	ostrich	10d	2014.09.04	HB	Liver/Kidney	C	4	–	KU981155
FAdV-JLDH-141008-B	broiler	40d	2014.10.08	JL	Liver	D	11	CIA	KU981137
FAdV-YTLY-141026-B	broiler	10d	2014.10.26	SD	Liver/Kidney	D	11	CIA	KU981128
FAdV-YTMP-141028-B	broiler	32d	2014.10.28	SD	Liver	D	11	IB	KU981129
FAdV-LNDL-141030-B	broiler	26d	2014.10.30	LN	Liver/Kidney	D	11	–	KU981130
FAdV-JLPS-141030-B	broiler	30d	2014.10.30	JL	Liver	D	11	CIA	KU981131
FAdV-QDJM-141107-B	broiler	31d	2014.11.07	SD	Liver	D	11	CIA	KU981132
FAdV-QDLX-141120-B	broiler	37d	2014.11.20	SD	Liver	D	11	H9, CIA	KU981133

uk: unknown. SD: Shandong, JL: Jilin, LN: Liaoning, SX: Shanxi, HLJ: Heilongjiang, HN: Henan, YN: Yunnan, BJ: Beijing, HB: Hebei. H9: H9 subtype influenza virus, CIAV: chicken infectious anemia virus, IBV: infectious bronchitis virus, NDV: Newcastle disease virus, *E. coli*, *Escherichia coli*. [1,2] Each strain labeled with an identical footnote number was derived from a mixed isolate.