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Recombinant fiber-2 protein protects Muscovy ducks against duck adenovirus 3 (DAdV-3)

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ABSTRACT

As a novel duck adenovirus 3 (DAdV-3) infection caused significant economic losses to the poultry industry in China, there is an urgent need to develop a safe and effective vaccine. In the research, fiber-1 and fiber-2 proteins were expressed and purified, respectively. To evaluate the immunogenicity of the two recombinant proteins, we investigated the IgY antibodies and virus-neutralizing antibodies in duck sera. The protective efficacy was evaluated by mortality, virus shedding and histopathological examinations after challenged with the DAdV-3. Results showed that the IgY antibody levels of the fiber-2 group was significantly higher than that of the fiber-1 group and inactivated vaccine group. Ducks vaccinated with fiber-2 group provided full protection with no mortality, no virus shedding and no histological lesions, superior to other groups. These results suggest that the fiber-2 protein can be an ideal candidate for subunit vaccine against the disease.

1. Introduction

Adenoviruses (AdVs), family *Adenoviridae*, contain a lot of serotypes, which cause various diseases in humans and poultry. The *Adenoviridae* family is currently divided into five genera, the *Mastadenovirus*, *Aviadenovirus*, *Atadenovirus*, *Siadenovirus*, and *Ichtadenovirus* by the International Committee on Taxonomy of Viruses (ICTV) (Harrach et al., 2011; Marek et al., 2013). Many studies show that AdVs can also infect ducks. Such as, duck adenovirus 1 (DAdV-1, genus *Atadenovirus*) can induce decreased egg production and poor egg quality (Schybli et al., 2014). Duck adenovirus 2 (DAdV-2, genus *Aviadenovirus*) infection has been linked to mortality in Muscovy ducks. In 1977, replacement breeder Muscovy ducks suffered a major outbreak of disease in France. Affected birds became thin, and some birds were lame (Bouquet et al., 1982). In 2014, Marek et al. reported the complete genome sequence of the first isolates of DAdV-2, which was isolated in France (Marek et al., 2014). In the same year, a novel duck adenovirus 3 (DAdV-3, genus *Aviadenovirus*) was isolated from diseased Muscovy ducks that causes pathologically different hydropericardium, fibrosis and hemorrhage of the liver in China. DAdV-3 is severely pathogenic to ducks and poses a significant hazard to poultry industry (Zhang et al., 2016). As no commercial vaccine is available against the novel strain of DAdV-3, the development of an efficacious, safe, and economic vaccine candidate is highly desired for the disease prevention and control.

Adenoviruses (AdVs) are medium-sized, non-enveloped icosahedral virions with linear, non-segmented, double-stranded DNA genomes ranging in size from 26 to 45 kb (Davison et al., 2003). The viral particle of AdVs consist of three major capsid proteins, including hexon, penton base, and fiber (Cusack, 2005; Valentine et al., 2003). The fiber protein is responsible for viral pathogenicity (Pallister et al., 1996) and plays a primary role in the interaction with cellular receptors during viral penetration into the host cells (Nicklin et al., 2005; Russell, 2009). In terms of antigenicity, the fiber-2 is regarded as a protective immunogen because of its ability to induce neutralizing antibodies (Gupta et al., 2017) and provide effective protection from the adenoviral infection (Chen et al., 2018; Schachner et al., 2014). Zhang et al. (2014) showed that the most important distinguishing feature between the DAdV-2 isolate and the DAdV-3 isolate was that the DAdV-2 isolate only contained one fiber gene, whereas DAdV-3 had two fibers (fiber-1 and fiber-2). The fiber-1 protein of DAdV-3 strain was more closely related to the fiber-1 protein of different fowl AdVs (e.g., FAdV-1, FAdV-4) containing two fiber genes. The fiber-2 protein of DAdV-3 strain shared higher amino acid sequence identity with the only fiber protein of the DAdV-2 strain than with its own fiber-1 protein (Zhang et al., 2016).

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In the present study, DAdV-3 *fiber-1* and *fiber-2* gene were expressed in *E. coli* and used them as subunit vaccines. The immunogenicity and protective efficacy of the recombinant proteins (fiber-1 or fiber-2) were evaluated and compared with that of an inactivated vaccine by challenge with a DAdV-3 strain in Muscovy ducks.

2. Material and methods

2.1. Virus

The novel DAdV-3 (CH-GD-12–2014) virus was isolated and propagated on duck embryo fibroblasts (DEFs) as described previously (Zhang et al., 2016), and then used for vaccine production and challenge strain.

2.2. Cloning, expression and purification of recombinant fiber-1 and fiber-2 proteins

Construction of the recombinant plasmids and expression of recombinant proteins were performed as previously described (Chen et al., 2018). In brief, the entire encoding regions for *fiber-1* and *fiber-2* were amplified by PCR from viral DNA (DAdV-3 isolate CH-GD-12–2014, GenBank accession number: KR135164) and cloned into the pSYNO-1 vector (Convenience Biology Corporation in Changzhou, China, product number: CV-001) encoding maltose-binding protein (MBP). The primers used to generate the *fiber-1* fragment (1380 bp) were GAG CTC ATG CTC TGT CCG TTT AGA TTC (forward primer 1) and GCT CGA GTT TAT ACA ATC TTC GCT AGG TA (reverse primer 1). The primers used to generate the *fiber-2* fragment (1443 bp) were GAG CTC ATG AAA CGC ACC AAT CGT AGC (forward primer 2) and GCT CGA GTT TAG TTG ACG TTG CTC GGA TT (reverse primer 2). All the primers were synthesized by Invitrogen Company (Shanghai, China). The resulting plasmids (pSYNO-1-*fiber-1* and pSYNO-1-*fiber-2*) were confirmed with sequencing analysis by Huada Company (Guangzhou, China). To express the fiber-1 and fiber-2 protein, pSYNO-1-*fiber-1* and pSYNO-1-*fiber-2* were transformed into *E. coli* BL21 (DE3) cells respectively, and single colonies were selected and grown in LB medium with kanamycin (50 μg/mL) at 37 ℃ until its OD_{600} raised to 0.5–0.6. To induce protein expression, isopropyl β-D-thiogalactopyranoside (IPTG) was added to a final concentration of 0.1 mM and incubated at 16 ℃ overnight. *E. coli* cells which contain wild type pSYNO-1 vector were processed likewise to serve as a negative control.

Recombinant proteins were purified by nickel-nitrilotriacetate (Ni-NTA) affinity chromatography as previously described (Chen et al., 2018). Birefly, the cells were collected, resuspended in binding buffer (20 mM Tris, 500 mM NaCl, pH 8.0), ultrasonicated, and then centrifuged at 13,000*g* at 4 ℃ for 20 min. The supernatants were loaded on a Ni-NTA agarose column by gravity flow and washed with washing buffer (20 mM Tris, 500 mM NaCl, 50 mM imidazole, pH 8.0), and then eluted with elution buffer (20 mM Tris, 500 mM NaCl, 200 mM imidazole, pH 8.0). Afterwards, the elution fractions containing target proteins were pooled together, with tobaccoetch virus (TEV) enzyme added to remove the N-terminal fusion tags (MBP), and applied to the Ni-NTA agarose column again, and then the target proteins were concentrated and exchanged to phosphate buffered saline (PBS) buffer. The concentration or content of the recombinant fiber-1 or fiber-2 protein was analyzed and determined using SDS-PAGE gel electrophoresis, the results of which were detected by GeneSnap and GeneTools from SynGene software with bovine serum albumin (BSA) as standard.

2.3. SDS-PAGE and western blot

The recombinant fiber-1 and fiber-2 proteins were boiled for 10 min at 100 ℃ in the loading buffer with 1% β-mercaptoethanol and separated on 10% SDS polyacrylamide gels, and then electrophoretically transferred to polyvinylidene difluoride (PVDF) membranes (Millipore, USA). After they were blocked with 3% BSA in TBST (Tris-buffered saline + 0.1% Tween-20) for 1.5 h at room temperature (RT), the PVDF membranes were then incubated with primary duck polyclonal serum (1:500 dilution) overnight at 4 ℃, and subsequently probed with secondary HRP-conjugated goat anti-duck IgY (1:2000; KPL, USA) for 1 h at RT. After washing the membranes four times, the signals of the recombinant proteins were visualized using the commercial ECL kit (Pierce, USA) according to the manufacturer's instructions.

2.4. Production of inactivated DAdV-3 vaccine

The novel DAdV-3 (CH-GD-12–2014) virus was propagated on duck embryo fibroblasts (DEFs) as described previously (Zhang et al., 2016). The median tissue culture infective dose ($TCID_{50}$) of the virus was determined using 96-well plates. Briefly, DEFs were prepared from 14-day-old SPF Shaoxing duck embryos and cultured with DMEM medium (Hyclone, USA), with addition of 10% foetal bovine serum (Gibco, USA). DEFs were inoculated with 10-fold dilutions from 10^{-2} to 10^{-9} of virus stocks and incubated at 37 ℃ with 5% CO_2 for 7 days. Cytopathic effect (CPE) was observed and the $TCID_{50}$ were calculated according to the Reed and Muench method (Reed and Muench, 1938). The titer of the virus was determined as $10^{6.5}$ $TCID_{50}$/mL.

For inactivation of the virus, formaldehyde (0.2% in final product) was added to the culture medium harvested from DAdV-3 infected DEFs. The formaldehyde inactivated antigen solution was emulsified with Montanide™ ISA 563 VG at a 1:1 wt ratio as recommended by the manufacturer (Seppic, France). The immunized dose of the inactivated DAdV-3 vaccine was $10^{5.5}$ $TCID_{50}$ in 0.2 mL per duck.

2.5. Animal immunization and challenge

Animal experiments were performed strictly in accordance with the guidelines of Sun Yat-sen University Institutional Animal Care and Use Committee (approval ID: IACUC-DD-17-1107). The research was conducted in the compliance with the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. Eighty 4-day-old Muscovy ducks were collected from the Experimental Animal Center (Guangdong Wens Dahuanong Biotechnology Co., Ltd., Guangdong, China), randomly divided into four groups (n = 20 ducks/group), and housed in individual isolators under positive pressure. The purified fiber-1 protein, fiber-2 protein and PBS were emulsified with Montanide™ ISA 563 VG at a 1:1 wt ratio (antigens: adjuvant) as recommended by the manufacturer (Seppic, France) respectively and then used in intramuscular (i.m.) injection. Ducks in group I were immunized i.m. with 30 μg fiber-1 protein in 0.2 mL per duck, ducks of group II were immunized i.m. with 30 μg fiber-2 protein in 0.2 mL per duck, while those in group III were immunized i.m. with the final dose $10^{5.5}$ $TCID_{50}$ of the inactivated vaccine in 0.2 mL per bird. Group IV was injected with PBS in 0.2 mL per bird and set up as a challenge control group. Blood was collected at 7, 14 days post-inoculation (dpi) for IgY antibody and neutralizing antibody detection.

14 days after the immunization, animals in groups I to IV were intramuscularly challenged with the novel DAdV-3 (2×10^6 $TCID_{50}$/duck), and observed daily for clinical signs over a 7-day period. Any ducks that died or had to be euthanized during the observation period was immediately necropsied. Cloacal swabs were collected from all groups before challenge (day-0) and at days 3 and 5 post-challenge (dpc) to determine fecal shedding of DAdV-3. All the remaining ducks at the end of the observation period were euthanized to collect the livers and kidneys for pathology at 7 dpc.

2.6. ELISA assay

Sera were collected from the ducks at 7 and 14 dpi to detect DAdV-3-specific antibodies. The purified DAdV-3 viruses were produced following the procedures as previously described (Zhang et al., 2016) and

used as antigens to detect the DAdV-3-specific antibodies through indirect ELISA. Briefly, plates were coated with the purified DAdV-3 viruses at a concentration of 0.27 μg/well and incubated at 4 °C overnight. Sera were added to each well at a dilution of 1:200 (37 °C for 1 h). The secondary HRP-conjugated goat anti-duck IgY antibodies (KPL, USA) were used at a 1:500 dilution. The optical density was measured at 450 nm on a microplate reader (model ELx800, Bio-Tek). The cut-off value was set as 0.290, which was determined on basis of the negative control sera by adding three times the standard deviation to the arithmetic mean OD 450 value of the 50 negative serum samples.

2.7. Neutralizing test

The neutralization tests (NT) were performed on DEFs with a few modifications in the protocol as previously described (Wolf et al., 1974). Briefly, serum samples were inactivated at 56 °C for 30 min and serially diluted two-fold. The DAdV-3 strain at a concentration of 100 $TCID_{50}$/0.1 mL was mixed with an equal volume of diluted serum, and the mixture was incubated at 37 °C for 1 h. Subsequently, DEF monolayers on 96-well plates were inoculated with the mixture, and the plates were incubated for 7 days at 37 °C with 5% CO_2. The SN titers were determined based on the reciprocals of the highest serum dilution that caused inhibition of the CPE.

2.8. Virus isolation

The virus isolation was performed on DEFs with some modifications in the protocol as previously described (Matczuk et al., 2017; Grafl et al., 2013). Briefly, cloacal swabs were collected and placed in 1 mL antibiotic (1:100, Gibco, USA)-PBS solution. The cloacal swabs in PBS were vortexed and centrifuged at 12,000*g* at 4 °C for 10 min. The supernatants were filter sterilized using 0.22 μm filter (Millipore, Germany). Subsequently, DEF monolayers on 96-well plates were inoculated with filtrated samples for 7 days at 37 °C in 5% CO_2 atmosphere during which cells were examined for CPE. A sample was considered negative when no CPE was noticed after three blind passages.

2.9. Histopathology

Livers and kidneys of ducks in different groups were collected 7 dpc and fixed in 10% formalin for 48 h at RT. Those tissues were embedded in paraffin wax, cut into 4 μm sections, stained with hematoxylin and eosin (HE), and then examined using light microscopy.

2.10. Statistics

The statistics analyses of IgY antibody and neutralizing antibody were conducted using two-way ANOVA by Prism 6 (Graphpad). Statistics analyses for other experiments were carried out through an unpaired *t*-test. Differences were considered to be significant at $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ or $^{****}p < 0.0001$.

3. Results

3.1. Expression of protein

To check whether fiber-1 and fiber-2 protein could be expressed in *E coli.* cells, we analyzed expression of proteins in collected cell lysates of the recombinant *E coli.* cells by reducing SDS-PAGE and western blot. The results of western blot showed that the recombinant proteins could be detected as a clear band with molecular weight of 52 kDa (fiber-1 protein) and 55 kDa (fiber-2 protein) respectively, and could be detected using polyclonal sera against DAdV-3 strain CH-GD-12-2014 (Fig. 1 A). This result was further confirmed by SDS-PAGE using purified proteins (Fig. 1B), and the purified recombinant proteins were prepared for the vaccination procedure later in our study.

3.2. IgY antibodies

DAdV-3 specific antibodies in the serum of the vaccinated ducks were detected by indirect ELISA, which had a cut-off value of 0.290. In general, the IgY antibody levels in ducks immunized with the inactivated vaccine were significantly increased, comparing to the PBS group at 7 dpi ($p < 0.05$) and 14 dpi ($p < 0.0001$). Fiber-1 group developed a higher level of antibody than the PBS group at 14 dpi ($p < 0.0001$). Ducks vaccinated with fiber-2 showed significantly higher antibody titers than animals in the fiber-1 and inactivated vaccine group at 7 dpi ($p < 0.0001$) and 14 dpi ($p < 0.0001$) (Fig. 2 A). These data indicate that the fiber-2 protein can induce positive immune responses, resulting in a significantly increased antibody level.

3.3. Neutralizing antibodies

Serum-neutralizing antibodies at 7 and 14 dpi were detected in each group. As shown in Fig. 2B, compared to the control group, the neutralizing antibody levels in the fiber-2 group ($p < 0.01$), the fiber-1 group ($p < 0.0001$) and the inactivated vaccine group ($p < 0.0001$) were significantly increased. There was no significant difference between the fiber-1 group, fiber-2 group and the inactivated vaccine group at 7 dpi. In addition, chickens vaccinated with the inactivated vaccine had a significantly higher level of neutralizing antibodies than animals in the fiber-1 group ($p < 0.05$), the fiber-2 group ($p < 0.0001$) and the control group ($p < 0.0001$) at 14 dpi. The neutralizing antibody levels in chickens immunized with fiber-1 and fiber-2 were significantly increased, comparing to the PBS group at 14 dpi ($p < 0.0001$), and there was no significant difference between the fiber-1 group and fiber-2 group at 7 and 14 dpi.

3.4. Histopathology in different tissues

Livers and kidneys of ducks in different groups were collected 7 dpc and fixed, sectioned and stained with HE (Fig. 3). For ducks in the PBS group, massive pathological damages were observed in various tissues. In the liver, inflammatory cells were clustered and hepatic cells were denatured with signs of fatty degeneration (Fig. 3 A). Hemorrhagic spots and inflammatory cells were evident in the kidney (Fig. 3E). The proportion of ducks in each group with histological lesions in tissues examined are summarized in Table 1. Generally, there was no significant difference among the fiber-1 group (Figs. 3B, 3F), fiber-2 group (Figure 3C, 3G) and inactivated vaccine (Figs. 3D, 3H).

3.5. Viral shedding and protection

Cloacal swabs were collected from each group before challenge (day-0) and at days 3 and 5 dpc to determine fecal shedding of DAdV-3. The proportion of ducks in each experimental group with viral shedding are summarized in Table 2. Peak viral shedding was observed at 3 dpc with the highest proportion in the PBS group (16/16), followed by the fiber-1 group (8/16), and inactivated vaccine (2/16). The proportion of ducks with viral shedding declined at 5 dpc in the fiber-1 group (5/16) and inactivated vaccine (1/16), but not in PBS group (16/16). No fecal DAdV-3 shedding was detected in the fiber-2 group at 3 and 5 dpc. The proportion of ducks with viral shedding in the fiber-2 group and inactivated vaccine were significantly lower than animals in the fiber-1 and PBS group at 3 dpc ($p < 0.05$). The proportion of ducks with viral shedding in the fiber-2 group, the fiber-1 group and inactivated vaccine were significantly lower than animals in the PBS group at 5 dpc ($p < 0.05$). In addition, the survival rates after challenge are summarized in Fig. 4. Ducks in the fiber-1 group, fiber-2 group and inactivated vaccine group showed 100% protection with no mortality. However, ducks of the PBS group can not be protected, with 2 ducks

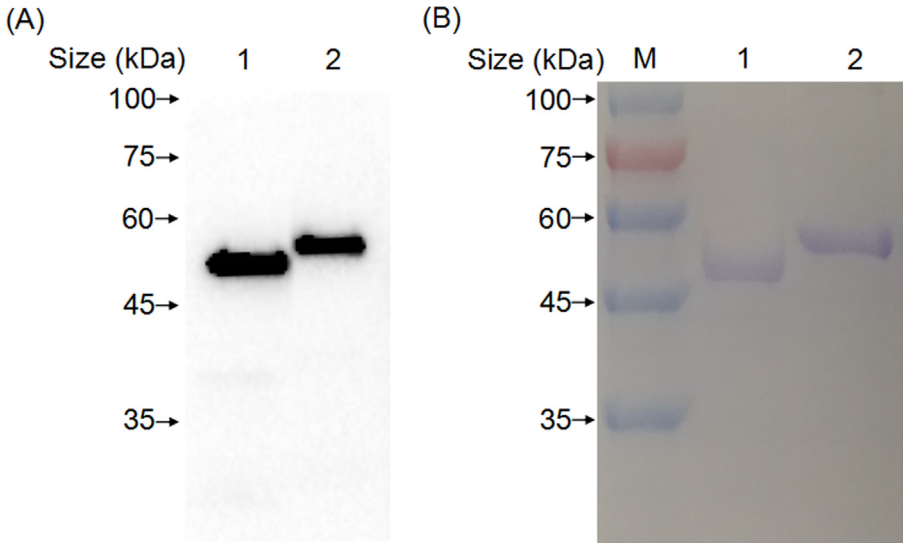

Fig. 1. Analyses of protein expression of the recombinant fiber-1 and fiber-2 protein in *E. coli.* M: protein marker, 1: the fiber-1 protein predicted as 52 kDa, 2: the fiber-2 protein predicted as 55 kDa. (A) Western blot of the recombinant proteins probed by duck polyclonal sera. (B) SDS-PAGE of the purified recombinant proteins.

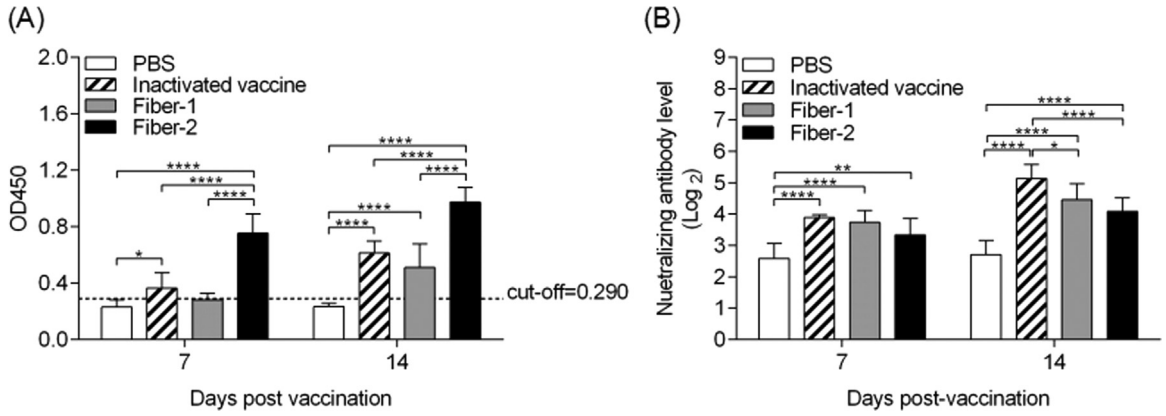

Fig. 2. (A) IgY antibody levels (Mean OD 450 + SD) of ducks at 7 and 14 dpi in each group. (B) Serum neutralizing antibody titers (Mean + SD) of ducks at 7 and 14 dpi. n = 16 ducks per group.

died at 3 dpc.

4. Discussion

Adenoviruses infection prevails among wild and domestic birds, and induces huge economic losses for poultry production (Schachner et al., 2018). Such as, FAdVs are implicated in a wide range of avian diseases, including hepatitis-hydropericardium syndrome (HHS), inclusion body hepatitis (IBH) and gizzard erosion (GE). Among these FAdVs, FAdV serotype 4 (FAdV-4) can cause straw-colored fluid accumulation in the

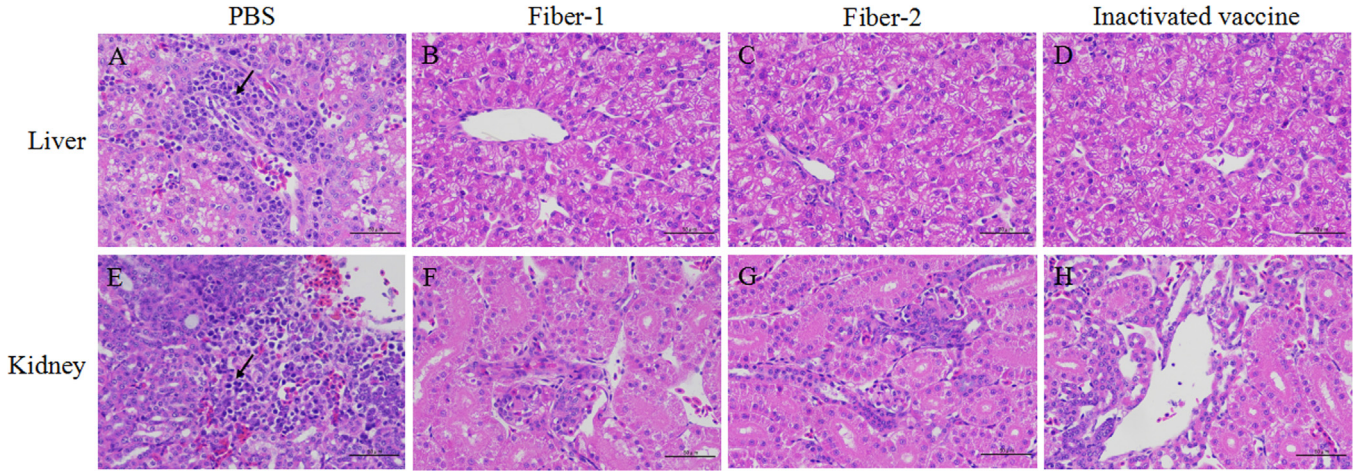

Fig. 3. Histopathology in tissues stained with HE (magnification, 400 ×) at 7 dpc liver (A, B, C and D) and kidney (E, F, G and H).

Table 1
Histological lesions in livers and kidneys of ducks in each group following challenge with the novel DAdV-3.

Groups	Histological lesions	
	Liver	Kidney
PBS	16/16* a	16/16 a
Inactivated vaccine	0/16 b	0/16 b
Fiber-1	2/16 b	0/16 b
Fiber-2	0/16 b	0/16 b

Data with the same letter (a–b) are not significantly different ($P > 0.05$).
* Number of ducks positive for histological lesions/number of ducks examined (n = 16).

Table 2
Detection of live virus in cloacal swabs by virus isolation.

Groups	Days post-challenge		
	0	3	5
PBS	0/16#	16/16 a	16/16 a
Inactivated vaccine	0/16	2/16 b	1/16 b
Fiber-1	0/16	8/16c	5/16 b
Fiber-2	0/16	0/16 b	0/16 b

Data with the same letter (a–c) are not significantly different ($P > 0.05$).
Number of ducks positive for virus isolation/number of ducks examined (n = 16).

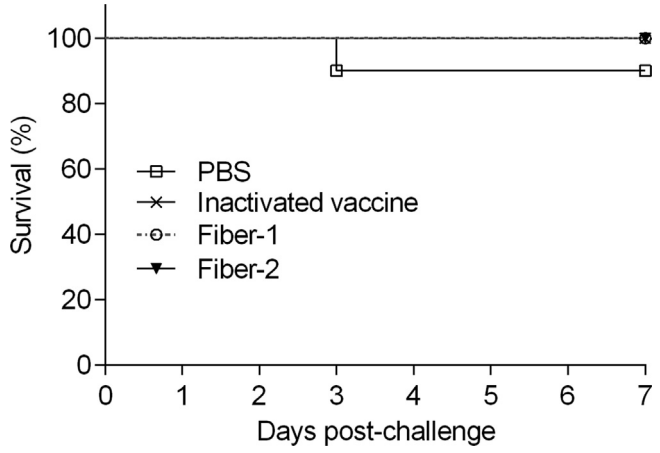

Fig. 4. Survival of ducks following challenge with the novel DAdV-3.

pericardial sac; friable, discolored and swollen livers with focal necroses and petechial haemorrhages; congested lungs and kidneys (Schachner et al., 2018). In addition, such viruses are noticed in healthy animals as well (Fitzgerald, 2013).

Since 2014, a novel duck AdV (DAdV-3) has struck many poultry farms in China, leading to major economic losses. The fatality rate was 20% or so, but the positive proportion of DAdV-3 infection was very high in ducks, which could be easily influenced by secondary infections (Zhang et al., 2016). Until now, no vaccine has been developed and evaluated for the novel DAdV-3 strain. Thus, there is an urgent need to develop a safe and effective vaccine for the poultry industry. In the current study, the fiber-1 and fiber-2 protein of the novel DAdV-3 were cloned, expressed in *Escherichia coli* and used as subunit vaccines in ducks. The protective efficacy of the recombinant proteins were evaluated through challenging ducks in four different groups with the novel virus. These results suggest that the recombinant fiber-2 protein is an attractive candidate for subunit vaccines against the novel strain of the virus.

It is generally known that humoral immunity is critical for disease recovery and virus elimination (Coico and Sunshine, 2015). The current study showed that IgY antibody titers induced by the recombinant fiber-1 protein, the recombinant fiber-2 protein and the inactivated vaccine were significantly higher than that in the PBS group at 14 dpi. And the levels of IgY antibody induced by fiber-2 were significantly higher than those in the fiber-1 and the inactivated vaccine group. In addition, the neutralizing activity of sera in the fiber-2 group is consistent with the observations of weak neutralization capacity of sera comprising only anti-fiber antibodies as previously reported (Kjellen and Pereira, 1968; Fingerut et al., 2003; Gahery-Segard et al., 1998; Schachner et al., 2014). In contrast, the neutralizing activity of sera was detectable in the DAdV-3 inactivated vaccine group, which might be explained by in vitro investigations suggesting that neutralizing reaction is mainly a consequence of interactions between antibodies and various major adenovirus capsid components (Russell, 2009), and the major neutralizing antigen of adenovirus is hexon (Bradley et al., 2012; Roy et al., 2005; Sumida et al., 2005).

Furthermore, the protective efficacy was evaluated by mortality, virus shedding and histopathological examinations after challenged with the DAdV-3. Ducks of group II (fiber-2 immunized) showed 100% protection with no mortality, no virus shedding and no histological lesions. Ducks of group III (inactivated vaccine immunized) also showed full protection with no mortality and no histological lesions, but 2 out of 16 ducks (13%) shed virus at 3 dpc, and 1 out of 16 ducks (6%) shed virus at 5 dpc. Ducks of group I (fiber-1 immunized) showed full protection with no mortality, but 2 out of 16 ducks showed histological lesions, 8 out of 16 ducks (50%) shed virus at 3 dpc, and 5 out of 16 ducks (31%) shed virus at 5 dpc. Ducks vaccinated with PBS can not be protected, with 2 ducks died at 3 dpc and all ducks showed histological lesions and shed virus at 3 and 5 dpc. The results of the current study has been consistent with the study about FAdV-4 by Schachner et al. and they showed that the fiber-2 vaccinated group displayed high resistance against the adverse effects of the challenge with 96% protection, whereas the fiber-1 vaccinated group was not effectively protected with 62% protection (Schachner et al., 2014). In fact, the protective effect of a recombinant fiber in birds was first demonstrated by Pitcovski et al. in 2005 using Turkey adenovirus 3 (TAdV-3), a virus with a single fiber gene and a much smaller genome (Pitcovski et al., 2005). Later on, Schachner et al., 2014 demonstrated the potential of fiber-2 protein of FAdV-4, a virus with two fiber genes, to protect chickens (Schachner et al., 2014). Altogether, the genomic and morphological relationship of DAdV-3 with FAdV-4 resembles certain similarities.

In summary, this study presents a suitable vaccine candidate (the fiber-2 protein) against the novel DAdV-3. As the recombinant fiber-2 protein increased levels of humoral antibody and provided full protection against the challenge virus, it can be inferred that it is an ideal vaccine candidate for the development of vaccines against the novel DAdV-3 that has been leading a severe hazard to poultry industry in China.

Conflict of interest

The authors declare no conflict of interest.

References

Bouquet, J.F., Moreau, Y., McFerran, J.B., Connor, T.J., 1982. Isolation and characterisation of an adenovirus isolated from Muscovy ducks. Avian Pathol. 11, 301–307.
Bradley, R.R., Lynch, D.M., Iampietro, M.J., Borducchi, E.N., Barouch, D.H., 2012. Adenovirus serotype 5 neutralizing antibodies target both hexon and fiber following vaccination and natural infection. J. Virol. 86, 625–629.
Chen, L., Yin, L., Zhou, Q., Li, Q., Luo, Y., Xu, Z., Zhang, Y., Xue, C., Cao, Y., 2018. Immunogenicity and protective efficacy of recombinant fiber-2 protein in protecting SPF chickens against fowl adenovirus 4. Vaccine 36, 1203–1208.
Coico, R., Sunshine, G., 2015. Immunology: A Short Course, 7th ed. John Wiley & Sons Ltd, Chichester.
Cusack, S., 2005. Adenovirus complex structures. Curr. Opin. Struct. Biol. 15, 237–243.

Davison, A.J., Benko, M., Harrach, B., 2003. Genetic content and evolution of adenoviruses. J. Gen. Virol. 84, 2895–2908.
Fingerut, E., Gutter, B., Gallili, G., Michael, A., Pitcovski, J., 2003. A subunit vaccine against the adenovirus egg-drop syndrome using part of its fiber protein. Vaccine 21, 2761–2766.
Fitzgerald, S.D., 2013. Adenovirus infections. In: Swayne, D.E., Glisson, J.R., McDougald, L.R., Nolan, L.K., Suarez, D.L., Nair, V.L. (Eds.), Diseases of Poultry. Blackwell Publishing Professional, Ames, Iowa, pp. 289–332.
Gahery-Segard, H., Farace, F., Godfrin, D., Gaston, J., Lengagne, R., Tursz, T., Boulanger, P., Guillet, J.G., 1998. Immune response to recombinant capsid proteins of adenovirus in humans: antifiber and anti-penton base antibodies have a synergistic effect on neutralizing activity. J. Virol. 72, 2388–2397.
Grafl, B., Liebhart, D., Gunes, A., Wernsdorf, P., Aigner, F., Bachmeier, J., Hess, M., 2013. Quantity of virulent fowl adenovirus serotype 1 correlates with clinical signs, macroscopical and pathohistological lesions in gizzards following experimental induction of gizzard erosion in broilers. Vet. Res. 44, 38.
Gupta, A., Ahmed, K.A., Ayalew, L.E., Popowich, S., Kurukulasuriya, S., Goonewardene, K., Gunawardana, T., Karunarathna, R., Ojkic, D., Tikoo, S.K., Willson, P., Gomis, S., 2017. Immunogenicity and protective efficacy of virus-like particles and recombinant fiber proteins in broiler-breeder vaccination against fowl adenovirus (FAdV)-8b. Vaccine 35, 2716–2722.
Harrach, B., Benkő, M., Both, G.W., Brown, M., Davison, A.J., Echavarra, M., Hess, M., Jones, M.S., Kajon, A., Lehmkuhl, H.D., Mautner, V., Mittal, S.K., Wadell, G., 2011. Family adenoviridae. In: King, A.M.Q., Adams, M.J., Lefkowitz, E.J. (Eds.), Virus Taxonomy: Classification and Nomenclature of Viruses. Ninth Report of the International Committee on Taxonomy of Viruses. Elsevier, San Diego, pp. 25–41.
Kjellen, L., Pereira, H.G., 1968. Role of adenovirus antigens in the induction of virus neutralizing antibody. J. Gen. Virol. 2, 177–185.
Marek, A., Kajan, G.L., Kosiol, C., Harrach, B., Schloetterer, C., Hess, M., 2014. Complete genome sequences of pigeon adenovirus 1 and duck adenovirus 2 extend the number of species within the genus Aviadenovirus. Virology 462, 107–114.
Marek, A., Kosiol, C., Harrach, B., Kajan, G.L., Schloetterer, C., Hess, M., 2013. The first whole genome sequence of a Fowl adenovirus B strain enables interspecies comparisons within the genus Aviadenovirus. Vet. Microbiol. 166, 250–256.
Matczuk, A.K., Niczyporuk, J.S., Kuczkowski, M., Wozniakowski, G., Nowak, M., Wieliczko, A., 2017. Whole genome sequencing of Fowl aviadenovirus A - a causative agent of gizzard erosion and ulceration, in adult laying hens. Infect. Genet. Evol. 48, 47–53.
Nicklin, S.A., Wu, E., Nemerow, G.R., Baker, A.H., 2005. The influence of adenovirus fiber structure and function on vector development for gene therapy. Mol. Ther. 12, 384–393.
Pallister, J., Wright, P.J., Sheppard, M., 1996. A single gene encoding the fiber is responsible for variations in virulence in the fowl adenoviruses. J. Virol. 70, 5115–5122.
Pitcovski, J., Fingerut, E., Gallili, G., Eliahu, D., Finger, A., Gutter, B., 2005. A subunit vaccine against hemorrhagic enteritis adenovirus. Vaccine 23, 4697–4702.
Reed, L.J., Muench, H., 1938. A simple method of estimating fifty percent endpoints. Am. J. Hyg. 27, 493–497.
Roy, S., Clawson, D.S., Calcedo, R., Lebherz, C., Sanmiguel, J., Wu, D., Wilson, J.M., 2005. Use of chimeric adenoviral vectors to assess capsid neutralization determinants. Virology 333, 207–214.
Russell, W.C., 2009. Adenoviruses: update on structure and function. J. Gen. Virol. 90, 1–20.
Schachner, A., Marek, A., Jaskulska, B., Bilic, I., Hess, M., 2014. Recombinant FAdV-4 fiber-2 protein protects chickens against hepatitis-hydropericardium syndrome (HHS). Vaccine 32, 1086–1092.
Schachner, A., Matos, M., Grafl, B., Hess, M., 2018. Fowl adenovirus-induced diseases and strategies for their control - a review on the current global situation. Avian Pathol. 47, 111–126.
Schybli, M., Sigrist, B., Hess, M., van Leerdam, B., Hoop, R.K., Voegtlin, A., 2014. Development of a new real-time polymerase chain reaction assay to detect Duck adenovirus A DNA and application to samples from Swiss poultry flocks. J. Vet. Diagn. Investig. 26, 189–194.
Sumida, S.M., Truitt, D.M., Lemckert, A.A., Vogels, R., Custers, J.H., Addo, M.M., Lockman, S., Peter, T., Peyerl, F.W., Kishko, M.G., Jackson, S.S., Gorgone, D.A., Lifton, M.A., Essex, M., Walker, B.D., Goudsmit, J., Havenga, M.J., Barouch, D.H., 2005. Neutralizing antibodies to adenovirus serotype 5 vaccine vectors are directed primarily against the adenovirus hexon protein. J. Immunol. 174, 7179–7185.
Valentine, R.C., Pereira, H.G., Russell, W.C., 2003. Antigens and structure of the adenovirus. Rev. Med. Virol. 13, 71–83.
Wolf, K., Burke, C.N., Quimby, M.C., 1974. Duck viral enteritis: microtiter plate isolation and neutralization test using the duck embryo fibroblast cell line. Avian Dis. 18, 427–434.
Zhang, X., Zhong, Y., Zhou, Z., Liu, Y., Zhang, H., Chen, F., Chen, W., Xie, Q., 2016. Molecular characterization, phylogeny analysis and pathogenicity of a Muscovy duck adenovirus strain isolated in China in 2014. Virology 493, 12–21.