

## Comparative immune efficacy of recombinant penton base and fibre proteins against fowl adenovirus - 2/11 infection in chickens

Raj Narayan Trivedi<sup>1</sup>, Rajesh Kumar<sup>1\*</sup>, Mamta Metwal<sup>1</sup>, Anupama Mishra<sup>2</sup>  
and Prakash Bhatt<sup>3</sup>

<sup>1</sup>Department of Veterinary Microbiology, College of Veterinary and Animal Sciences, G. B. Pant University of Agriculture and Technology, Pantnagar, India

<sup>2</sup>College of Community Sciences Tura (Meghalaya), Central Agricultural University, Imphal, Manipur, India

<sup>3</sup>Department of Veterinary Clinics, College of Veterinary and Animal Sciences, G. B. Pant University of Agriculture and Technology, Pantnagar, India

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### ABSTRACT

Fowl adenovirus (FAdV) has 12 serotypes. However, recently hybrid types have also been reported. FAdV-2/11 has emerged as the most frequently isolated FAdV type from Inclusion Body Hepatitis (IBH) and Hepatitis–Hydropericardium Syndrome (HHS) cases in chickens, causing severe economic impact worldwide. In an attempt to develop a subunit vaccine against FAdV-2/11 infection, viral capsid proteins, penton base and fibre, were expressed in *E. coli* and their immune potential was evaluated in domestic chicken. Purified recombinant proteins were administered on day 14 to specific pathogen-free (SPF) chickens followed by a challenge with the virulent virus on day 35 of life. The fibre induced the best immune response against the homologous challenge, with 80% protection. A moderate protective effect, resulting in 66% protection, was observed for penton base + fibre followed by the penton base vaccinated group manifesting 56% protection. Faecal excretion of the virus in immunized birds following the challenge was also studied. The study concludes that recombinant fibre protein may be a potential candidate for a subunit vaccine against FAdV-2/11 infection in chickens.

**Key words:** fowl adenovirus; recombinant protein; penton base; fibre; subunit vaccine

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### Introduction

Fowl adenoviruses (FAdVs) belong to the genus *Aviadenovirus* of the *Adenoviridae* family. They are distributed worldwide and are ubiquitous in poultry farms (McFERRAN and SMYTH, 2000). Some FAdVs can cause clinical diseases, such as

inclusion body hepatitis (IBH), hydropericardium hepatitis syndrome (HHS) respiratory disease, gizzard erosions and ulceration, tenosynovitis, impaired growth, reduced egg production, aplastic anaemia, atrophy of bursa and thymus

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\*Corresponding author:

Dr. Rajesh Kumar, Department of Veterinary Microbiology, College of Veterinary and Animal Sciences, G. B. Pant University of Agriculture and Technology, Pantnagar-263145, India, e-mail: rajeshvet@rediffmail.com

enteritis, and conjunctivitis in chickens and other birds (MEULEMANS et al., 2001; KUMAR et al., 2003a; 2003b; 2010). Fowl adenoviruses are easily transmitted both horizontally and vertically (McFERRAN and ADAIR, 2003; GRGIC et al., 2006). At present, the known FAdV strains are divided into five distinct species (*Fowl aviadenovirus* (FAdV) A-E) and 12 types (FAdV 1-11, 8a and 8b) (BENCO et al., 2005; SCHACHNER et al., 2018). FAdV 2 and 11 share a close serological and molecular relationship, hence, isolates belonging to either FAdV 2 or 11 are designated as FAdV-2/11 (STEER et al., 2011; DeHERDT et al., 2013).

In recent years, FAdV-2/11 has emerged as the most frequently isolated FAdV type from IBH cases worldwide (NICZYPORUK, 2018; KAJAN et al., 2019). Like other adenoviruses, FAdV virions are non-enveloped with a dsDNA genome with a diameter of 70-90 nm. The FAdV virion is an icosahedron containing 252 capsomers of the major structural proteins such as hexon and penton. Penton is further divided into fibre, which anchor the penton base. Some FAdV types (FAdV-1, 4, 10) have two fibres per penton base, while the rest possess only one (KAJAN et al., 2019). The knob and distal head domains of the fibre harbour the receptor-binding sites and thus play a critical role in virus attachment to the host cell (LOUIS et al., 1994; HENRY et al., 1994).

Pentons and hexons, which are major surface-exposed capsid proteins of adenoviruses, are key mediators of virus antigenicity, and harbour type-specific epitopes (NORRBY, 1969). The antibodies to hexons and fibres account for most of the neutralising activity in the mammalian humoral response against adenoviruses (GAHERY-SEGARD et al., 1998; BRADLEY et al., 2012; YU et al., 2013). *In vitro* trials have demonstrated varying degrees of neutralising capacity in antibodies raised against the recombinant hexon and fibre proteins of the egg drop syndrome virus (DAdV-1) (FINGERUT et al., 2003). The immune protection induced by the *E. coli*-produced recombinant proteins of fibre-1, fibre-2, hexon loop-1 and penton base against FAdV-4 was compared and fiber-2 offered excellent protection (SHAH et

al., 2012; SCHACHNER et al., 2014; WANG et al., 2018). All these studies indicate the potential of these proteins to induce immune protection. However, there is no study comparing the immune potency of penton base, fibre, or a combination of them for fowl adenoviruses other than FAdV-4. A comparison of these proteins individually and in combination in inducing immune protection would be useful for selecting vaccine candidates against FAdV-2/11.

## Materials and methods

**Viral DNA extraction.** A FAdV-2/11 isolate, Pantnagar/HA-14/R-21 (GenBank accession no. MH379245) from a natural outbreak, was propagated in chicken embryo liver (CEL) cells and DNA was extracted from the infected cell culture showing 70-80% CPE, using a DNAeasy Kit (Qiagen, USA).

**Cloning of penton base and fibre genes.** Oligonucleotide primers used in the study for the amplification of penton base (16223-17935) and fibre gene (30189-31907) region were designed using Gene Tools software (V 4.3.7.0, Syngene) and sequences having GenBank accession no. KU746335.1. The primer pairs: forward 5'-GGA TCC ATG CGT AGA AAC GGA AGA C-3', reverse 5'-AAG CTT TTA TTG TAA AGT GGA GCT GCT-3' and forward 5'-GGT ACC ATG GCG AAA TCG ACT CTT TT-3', reverse 5'-CTG CAG TTA GGG TTG TGT TAA TTT GTT GG-3' were used to amplify the penton base and fibre coding sequences, respectively. Cloning of the penton base and fibre gene PCR products at *Bam*HI, *Hind*III (penton base) and *AS*PI, *Pst*I (fibre) restriction sites of pJET vector was carried out using a CloneJET PCR Cloning Kit (Thermo Fisher Scientific, USA), and recombinant plasmids were constructed. Positive clones were selected by the antibiotic selection method. These positive clones were confirmed by colony PCR. The presence of inserts in the clones was further confirmed by double digestion with respective restriction enzymes. The nucleotide sequences of both inserts were determined, analysed, and submitted to GenBank with accession numbers MH371144 and MH371143 for the penton base and fibre, respectively.

*Expression of recombinant penton base and fibre.* After analysing the sequence of both genes and Open Reading Frame (ORF), sub-cloning of both genes was done at the respective restriction enzyme sites of the pQE30Xa expression vector (Qiagen, USA), and recombinant plasmids were constructed. The recombinant plasmids were then transformed into the M15 strain of *E. Coli* cells for expression of recombinant proteins. Selection of positive clones was done using the antibiotic selection method. Positive clones were confirmed by colony PCR and plasmid restriction enzyme double digestion.

The transformed strain was cultured in LB broth (100 ml) containing 100 µg/ml Ampicillin and 50µg/ml Kanamycin, incubated in an incubator shaker and induced by 1mM IPTG (isopropyl β-D-thiogalacto-pyranoside) at 37°C for 5 h with 150 rpm shaking until OD<sub>600</sub> reached 0.5-0.6. The cells were disrupted by cycles of sonication and freeze thaw. Supernatant and pellet (inclusion bodies) were separated by centrifugation at 24000 x g for 15 min at 4°C. The recombinant protein was detected by SDS-PAGE (Atto, Japan). *E. coli* cells harbouring wild type pQE30Xa plasmid were processed in the same way to serve as a negative control.

*Purification and characterization of recombinant proteins.* Cell lysates were loaded into chromatographic columns with Ni NTA resin (Invitrogen, USA), and allowed to pass through slowly to ensure maximum binding. The column was washed with 5.25 ml of the 1× Ni-NTA wash

buffer. A bounded fraction of the proteins was eluted by 1ml of 1× Ni-NTA elute buffer and elute fractions were collected in clean microfuge tubes. 10 µl each of washing and elute fractions were subjected to SDS-PAGE analysis.

The proteins from the SDS-PAGE gel were transferred onto a nitrocellulose membrane for western blot analysis (TOWBIN et al., 1979). The nitrocellulose membrane was incubated with the primary chicken antiserum against FAdV-2/11 (1:50). Goat anti-chicken HRP-conjugated secondary antibody (1:2000) was used to detect recombinant proteins using Di-amino-benzidine (DAB) containing 30% H<sub>2</sub>O<sub>2</sub> as the substrate (KUMAR et al., 2004).

*Evaluation of immune potential of recombinant proteins.* Day-old SPF chickens were randomly divided into 5 groups of 25 each. The birds were kept on litter up to day 7 and after that transferred into battery cages with ad-libitum food and water supplies. Recombinant penton base, fibre, and penton base + fibre (1:1) were administered with an equal volume of adjuvant, resulting in a total volume of 400 µl as detailed in Table 1. All groups except group 5 were challenged with 3×10<sup>7.5</sup> TCID<sub>50</sub> virulent virus (FAdV-2/11) on day 35 intramuscularly. Birds in group 5 (negative control) were administered the same amount of sterile PBS. Serum was collected from each group every week up to the termination of the experiment for antibody titre analysis.

Table 1. Details of immunization of SPF chicks with recombinant penton base and fibre proteins

S. No.	Group name (recombinant protein/s)	Quantity	Adjuvant/ route of immunization	Age of chicks at immunization	Age of chicks at challenge/ route
1.	Group-1 (penton base)	100µg	FCA/SC	14 <sup>th</sup> day	35 <sup>th</sup> day/IM
2.	Group-2 (fibre)	100µg	FCA/SC	14 <sup>th</sup> day	35 <sup>th</sup> day/IM
3.	Group-3 (penton+fibre)	50 µg+50 µg	FCA/SC	14 <sup>th</sup> day	35 <sup>th</sup> day/IM
4.	Group-4 (control challenged)	PBS	nil/SC	14 <sup>th</sup> day	35 <sup>th</sup> day/IM
5.	Group-5 (control unchallenged)	PBS	nil/SC	14 <sup>th</sup> day	-

FCA= Freund's complete adjuvant; PBS= phosphate buffered saline; SC= subcutaneously; IM= intramuscularly

The test and all the procedures performed on the experimental birds were discussed and approved by the institutional ethics committee and licensed by the Indian government.

*PCR for detection of virus in faeces.* To check shedding of the virus, cloacal swabs were collected after every 24 hour interval and kept in PBS at 4°C for further analysis. A lysate was made by heating the faecal material with PBS at 80°C for 30 minutes. PCR was performed in the same way as for the hexon loop1 gene (MEULEMANS et al., 2001).

*Enzyme-linked immunosorbent assay (ELISA).* To measure the antibody titre of all the groups at 0, 7, 14, 21, 28, 35, 42 days post immunization, serum was collected and analysed by single dilution ELISA (KUMAR et al., 2003b). Concentrations of coating antigen and conjugate were determined by checkerboard titration. Hyperimmune serum against FAdV-2/11 and serum of the SPF chickens collected prior to immunization were used as positive and negative controls, respectively. ELISA plates (Nunc, USA) were coated with each purified recombinant protein (5µg/well) diluted in carbonate bicarbonate buffer. The plate was incubated overnight at 4°C followed by washing and blocking using 2% bovine serum albumin. The plates were incubated for 1 h at 37°C followed by washing.

100 µl of the negative control serum (1:100) was added to the first three wells (A1-A3) and 100 µl of positive serum (1:100) was added to the first three wells of the last six wells of the last row (H7-H9) of the ELISA plate, and the last three wells of the last row (H10-H12) were kept as blank. To the remaining wells of the antigen-coated plate, 100µl of test serum (1:100) was added. The plate was incubated at 37°C for 1 h and washed three times with PBS-T. Then 100 µl of rabbit anti-chicken horse radish peroxidase conjugate (1:5000) (Sigma) was added to each well and the plates were incubated at 37°C for 1 h. To each well of the plate 100 µl of freshly prepared substrate solution 3,3',5,5'-Tetramethylbenzidine (TMB) in citrate buffer containing H<sub>2</sub>O<sub>2</sub> was added and then incubated at 37°C for 30 min in the dark, and the reaction was stopped by adding 100 µl of 1N H<sub>2</sub>SO<sub>4</sub>.

Absorbance (OD<sub>450</sub>) was recorded with an ELISA plate reader (Biotek, USA). The average absorbance of the positive and negative controls was calculated from the absorbance value of the ELISA plate, and the corrected positive control (CPC) value was determined by subtracting the average negative absorbance from the average positive absorbance.

The specific value (Sp. Value) was calculated using the following formula:

$$\text{Sp. Value} = \frac{\text{Average absorbance of test sample} - \text{Average absorbance of negative control}}{\text{Corrected positive control (CPC)}}$$

The titre was then calculated by the following formula:

$$\text{Log}_{10} \text{ titre} = [1.464 \times \text{Log}_{10} \text{ Sp. Value}] + 3.197$$

$$\text{Titre} = \text{Antilog of Log}_{10} \text{ titre.}$$

*Statistical Analysis.* Statistical analysis of antibody titres in the different experimental groups at different time intervals, to test significance by analysis of variance (ANOVA) was done following SNEDECOR and COCHRAN (1994), based on the computer program OPSTAT (SHEORAN, 2010). The means were separated using the Duncan multiple range test (DMRT) (DUNCAN, 1955) based on the SPSS16 (statistical product and service solutions) computer program.

## Results

*Cloning of the penton base and fibre genes and their expression.* The penton base and fibre genes were successfully cloned into the pJET cloning vector and subsequently expression vector pQE30 Xa, and the presence of inserts in the recombinant plasmid was confirmed by restriction endonuclease digestion. The nucleotide sequence of the penton base (1713 bp) and fibre (1668 bp) were determined, and their amino acid sequence (570 and 555 amino acid residues) were deduced.

The expressions and purifications of the recombinant proteins, penton base and fibre were identified using SDS-PAGE. The sizes of these

recombinant proteins fusing with tags from vector pQE30 Xa were 72 kDa (penton base) and 70 kDa (fibre), and the fibre protein contained two bands of similar size. Western blotting was done to further ascertain the identity and immunogenicity of the recombinant proteins. Both the proteins reacted strongly against the known FAdV antiserum. One single band was observed in the case of the penton base protein, in the induced culture lysate blot as well as in the purified protein lane, which was 72 kDa. In the fibre protein two distinct bands of similar size of 70 kDa were visualized in the induced culture lysate lane as well as in the purified protein lane.

*Evaluation of the immune potential of the recombinant proteins*

*Clinical signs, gross lesions, and mortality.* Dullness, ruffled feathers and slight diarrhoea were observed in the penton base and control challenged groups after the 3<sup>rd</sup> day post challenge (DPC). On the 4<sup>th</sup> DPC diarrhoea was observed in all groups except the unchallenged control and fibre groups. On the 5<sup>th</sup> DPC birds of the penton base, penton + fibre and control groups challenged showed dullness, hanging neck with closed eyes, and diarrhoea was observed in all groups except the unchallenged control and fibre group. On 6<sup>th</sup> DPC some birds from the penton base and challenged control group showed green colour diarrhoea. On the 7<sup>th</sup> and 8<sup>th</sup> DPC diarrhoea and dullness was observed in all groups except the unchallenged control. On the 9<sup>th</sup> DPC diarrhoea was only observed in the fibre group and one bird showed dullness and low activity. On the 10<sup>th</sup> DPC birds from all groups seemed healthy but slight diarrhoea was observed in the fibre groups. After the 10<sup>th</sup> DPC no apparent clinical signs were observed in any group.

Mortality was first observed on the 3<sup>rd</sup> DPC in the penton base group, in which one bird died showing typical PM lesions such as an enlarged liver with yellow necrotic foci, the kidney was pale, swollen, and anaemic, and petechial haemorrhage was also visible. Mortality continued until the 8<sup>th</sup> DPC and in total 9 birds died. On the 6<sup>th</sup> DPC 06 birds died in control challenged group, mortality continued till 9<sup>th</sup> DPC and total 17 birds died. In

penton+fibre group mortality started on 6<sup>th</sup> DPC and continued till 8<sup>th</sup> DPC and total 6 birds died. The fibre group exhibited mortality from the 7<sup>th</sup> DPC and it continued until the 9<sup>th</sup> DPC, and in total 5 birds died. From the 10<sup>th</sup> DPC no mortality was observed in any group. The PM lesions were a pale and enlarged liver and enlarged and haemorrhagic kidneys. Congested lungs were observed in some birds. In some birds the mottled appearance of the proventriculus, yellowish discoloration and petechial haemorrhage of the pericardial fat were common gross lesions. A pale, enlarged, and friable liver, congested and oedematous lungs, and atrophy of the bursa and thymus were also observed in affected birds. One bird from the unchallenged control group was also found dead, but on PM examination the typical lesions of IBH were absent. Overall, the highest, 68%, mortality was observed in the challenged control, while in the penton base group it was 44%, in the penton + fibre group 34%, and in the fibre group 20% mortality. The highest survivability rate of 80% was recorded in the fibre group. The fibre protein was found to be the best of all three combinations of recombinant proteins in terms of protection efficiency.

*Immune response and virus shedding.* Statistical analysis with two-way ANOVA at 95% confidence level revealed significant differences in antibody titres in the fibre group at every point of time. The antibody titre in the fibre group increased and peaked at seven days post immunization, and after that it decreased to the basal protective level and was maintained up to 21 days post immunization. After the challenge with the virulent virus it again increased drastically, which may be due to the anamnestic response. In other groups the same antibody pattern was also recorded but with a lower titre, so there was more mortality in these groups. The penton+fibre group showed the highest antibody titre at days 14 and 21 post immunization, however, the differences were statistically non-significant (Table 2). So, it can be inferred that recombinant fibre protein induced the highest antibody titre in comparison to the other groups and thus provided the best protection against the virulent IBH-HHS virus (FAdV-2/11).

Table 2. Antibody titres in experimental chicks at 7-day intervals post immunization

Groups / Days	0	7	14	21	28	35	42
Fibre	77.03 <sup>ab</sup> ±3.27	1960.09 <sup>ab</sup> ±87.06	243.32 <sup>ab</sup> ±7.26	230.54 <sup>ab</sup> ±7.10	6536.23 <sup>aA</sup> ±227.06	5810.16 <sup>ab</sup> ±365.92	4441.19 <sup>bc</sup> ±20.62
Penton base	9.26 <sup>cd</sup> ±1.37	573.37 <sup>b</sup> ±41.10	131.96 <sup>cd</sup> ±26.02	67.40 <sup>bc</sup> ±23.72	2489.45 <sup>cA</sup> ±127.09	2197.15 <sup>cA</sup> ±229.34	2484.59 <sup>cA</sup> ±417.91
Penton + fibre	18.91 <sup>ac</sup> ±1.41	362.72 <sup>bcB</sup> ±81.22	302.19 <sup>abC</sup> ±18.62	356.23 <sup>ab</sup> ±20.97	3145.07 <sup>bA</sup> ±509.23	2935.78 <sup>bA</sup> ±623.58	3018.80 <sup>bA</sup> ±482.53
Control challenged	125.39 <sup>bc</sup> ±6.88	146.76 <sup>bc</sup> ±8.52	169.68 <sup>bc</sup> ±27.24	98.51 <sup>ac</sup> ±62.66	1744.39 <sup>dA</sup> ±48.59	2008.80 <sup>cA</sup> ±33.68	1357.79 <sup>BB</sup> ±64.65
Control unchallenged	153.78 <sup>aA</sup> ±8.59	64.25 <sup>cA</sup> ±6.50	96.12 <sup>aA</sup> ±25.06	116.33 <sup>aA</sup> ±49.88	67.26 <sup>cA</sup> ±36.61	264.55 <sup>dA</sup> ±36.51	151.335 <sup>eA</sup> ±50.38

Similar small letters show group-wise non-significant data and similar large letters show non-significant data by day. Rest values found significant difference with others

The virus was detected in faeces up to the 10<sup>th</sup> day post challenge in the penton base group, as well as in the penton base + fibre group. In the fibre group the virus was present in faeces up to the 8<sup>th</sup> day post challenge. In the challenged control group the virus was present up to the 11<sup>th</sup> day post challenge, but the virus was not detected in the unchallenged control group on any day post challenge. The findings suggest that birds inoculated with fibre protein shed the virus in their faeces for less time than all the other groups.

### Discussion

Different types of vaccines are being used against IBH-HHS in different parts of the world. Numerous attempts have been made to develop inactivated vaccines of embryo and cell culture origin (GUPTA et al., 2005 and MAHMOOD et al., 2014). Prophylactic vaccination of birds using a formalin-inactivated vaccine prepared from the liver homogenate of infected birds, protected broilers under field conditions in India and Pakistan (CHISHTI et al., 1989; KUMAR et al., 1997; AHMAD and HASAN, 2004; KATARIA et al., 2013). Although this type of autogenous vaccine has been used with considerable success, these vaccines have inherent disadvantages and might compromise biosecurity (SHAH et al., 2012; KATARIA et al., 2013). In the present study, the penton base and fibre proteins of the virulent FAdV-2/11 were cloned, expressed in *E. coli* and used as a subunit vaccine in chickens. The protective efficacy of the recombinant and combinations thereof was assessed, and the results showed that the recombinant fibre protein is a potential candidate for subunit vaccines against FAdV-2/11 infection in domestic fowl.

In the past, most FAdV vaccines were formulated as inactivated vaccines. The protection rate varied from 80 to 100% with inactivated FAdV-4 against HHS (SCHACHNER et al., 2018), 90-100% against IBH (FAdV-2) (JUNNU et al., 2015), 98% against FAdV-8, and 92% against FAdV-11 (ALVARADO et al., 2007). An anti-IBH-HHS inactivated vaccine (oil emulsified) prepared from fowl adenovirus, propagated in a cell culture, provided protection within the first week of vaccination (KATARIA

et al., 1997). Similarly, GUPTA et al. (2005) developed a chicken embryo kidney (CEK) cell culture inactivated vaccine, which afforded 100% protection in broiler chickens when challenged with virulent FAdV-4. SHAH et al. (2012) reported 90% protection against HHS in broilers vaccinated with FAdV-4 recombinant penton base protein produced in *E. coli*. Approximately 62% and 96% protection against HHS was reported in SPF chickens vaccinated with recombinant fiber-1 and fiber-2 proteins (SCHACHNER et al., 2014). Other studies have also reported 90-100% protection against HHS in SPF chicks that were vaccinated with recombinant fiber-2 protein produced in *E. coli* (WANG et al., 2018; CHEN et al., 2018). In contrast to FAdV-4, the virus (FAdV-2/11) in our study has only one fibre attached to the penton base. Recombinant fibre, penton base and fibre + penton base proteins provided 80%, 56% and 66% protection, respectively. Previous studies have shown that Fibre-2 is better in providing protection against FAdV-4 (SCHACHNER et al., 2014; WANG et al., 2018; CHEN et al., 2018). Comparable protection was provided by fibre protein against FAdV-2/11, and the slightly lower value may be due to the increased number of immunizations and different adjuvant used. However, the penton base provided variable protection, i.e., 90% (SHAH et al., 2012) and 35% (WANG et al., 2018). No study has been done to evaluate the immune potential of recombinant penton base + fibre administered in combination; hence our results cannot be compared, but it provided better protection than penton base alone in the present study. FAdV-2/11 causes mortality ranging from 10 to 75% (SCHACHNER et al., 2018; BROWN et al., 2019). The high mortality observed in the challenged control group in the present study may be due to SPF chicks and the high challenge dose of the virus.

Humoral immunity plays the main role in protection against the IBH-HHS virus and its elimination from the host (COICO and SUNSHINE, 2015). The present study showed that fibre produced the strongest humoral immune response in comparison to penton base + fibre and penton base, as shown by the lowest mortality in birds in the fibre group. After the challenge with the

virulent virus, it again increased drastically, which may be due to the anamnestic response (SOLOFF, 2009). Fibre protein helps to activate Th2 helper cells, and increases the humoral immune response in comparison to other proteins (CHEN et al., 2018).

Virus shedding prevention is of utmost importance in the prevention of the spread of virus to susceptible birds, and to ensure the biosecurity of poultry farms (ONO et al., 2007; HAFEZ, 2010; KIM et al., 2014). The virus was detected in the faeces of the vaccinated and challenged control groups for different durations post challenge, and fibre protein was able to stop virus excretion early, in comparison to other proteins. These findings are partially not in agreement with SCHACHNER et al. (2014), who reported the presence of challenge virus DNA in faeces up to 21 DPC by real time-PCR, which may be due to the different virus type, infectious dose and method of detection. However, further studies are required to verify the effect of vaccination with fibre protein in preventing FAdV infection and excretion of the virus.

In conclusion, the detailed differences between the FAdV-2/11 capsid protein fibre, penton base and penton base + fibre in inducing immune protection were studied. Fibre induced the best protection against the FAdV-2/11 challenge followed by penton base + fibre and penton base. However, the present study demonstrates good humoral immune response, but weak cell-mediated immune response (TRIVEDI, 2018). Administration of fibre protein also shortened the duration of virus excretion in challenged birds. The present findings suggest that recombinant fibre is a potential candidate for the development of an effective and safe recombinant subunit vaccine. Further studies to elucidate the protective mechanisms supporting this finding might help to provide a more detailed understanding of FAdV infection pathways, and thus to devise improved control strategies for IBH-HHS caused by FAdV-2/11.

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**TRIVEDI, R. N., R. KUMAR, M. METWAL, A. MISHRA, P. BHATT: Usporedba imunološke učinkovitosti rekombinantne pentonske baze i vlaknastih proteina u pilića inficiranih adenovirusom peradi 2/11. *Vet. arhiv* 94, 119-128, 2024.**

### SAŽETAK

Adenovirus peradi (FAdV) ima 12 serotipova. Nedavno su, međutim, prijavljeni i hibridni tipovi. FAdV-2/11 najčešće je izoliran tip FAdV-a u pilića s hepatitisom s inkluzijskim tjelešcima (engl. inclusion body hepatitis, IBH) i hidroperikard-hepatitis sindromom (engl. hepatitis–hydropericardium syndrome, HHS), koji uzrokuje znatne gospodarske gubitke diljem svijeta. U nastojanju da se razvije podjedinična vakcina za infekciju FAdV-2/11, u bakteriji *E. coli* eksprimirani su virusni kapsidni proteini, pentonska baza i vlakna te je procijenjen njihov imunološki potencijal u domaćih pilića. Pročišćeni rekombinantni proteini primijenjeni su 14. dan u pilića bez specifičnih patogena (engl. specific pathogen-free, SPF) nakon čega im je 35. dan života apliciran virulentni virus. Vlakna su imala najbolji imunski odgovor na homolognu rekombinaciju, sa zaštitom od 80%. Umjeren zaštitni učinak, koji je rezultirao 66%-tnom zaštitom, imala je primjena pentonske baze s vlaknima, dok je 56%-tnu zaštitu pokazala skupina cijepljena samo pentonskom bazom. Analizirano je i postojanje virusa u fekalnim izlučevinama imuniziranih ptica. Zaključeno je da bi rekombinantni vlaknasti protein mogao biti potencijalan kandidat za podjedinično cjepivo protiv infekcije FAdV-2/11 u pilića.

**Ključne riječi:** adenovirus peradi; rekombinantni protein; pentonska baza; vlakna; podjedinična vakcina

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