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RESEARCH ARTICLE

RESULTS OF IMMUNOGENICITY ANALYSIS OF SUBUNIT VACCINE AGAINST BOVINE ADENOVIRUS TYPE 3 IN CATTLE

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ABSTRACT

Bovine adenovirus type 3 (BAdV-3) causes respiratory and gastrointestinal infections in feedlot cattle and the disease results in financial losses affected cattle industries. BAdV-3 is can cause conjunctivitis, pneumonia, diarrhea, and polyarthritis, among other symptoms. BAdV-3 is common in cattle worldwide and can infect wild animals through cross-species transmission. There lacks a commercial BAdV-3 vaccine on the market at current time. The aim of this research was to develop a BAdV-3 subunit vaccine to be used as a vaccine for BAdV-3 prevention. We expressed recombinant hexon protein (rhexon) of BAdV-3 in the Escherichia coli expression system to evaluate immune response in cattle. According to the results, immunized calves had an enhanced level of antibody response following primary inoculation than the control group, demonstrating that immunization with subunit using rhexon protein can stimulate an immune response. The recombinant hexon protein of BAdV-3 has immune-protective properties that could be helpful in creating subunit vaccine antigens against BAdV-3.

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INTRODUCTION

Bovine adenovirus type 3 is a member of the *Mastadenovirus* genus of the *Adenoviridae* family and is usually related with the bovine respiratory disease complex (1). Like other adenoviruses, it is an icosahedral, non-enveloped particle with a diameter of 75 nm that contains double-stranded linear genomic DNA (2). Several clinical symptoms, such as pneumonia, bipolar fever, dyspnea, irregular lung consolidation, necrotizing bronchitis, and alveolitis, can be caused by BAdV-3 (3). Among cattle worldwide, BAdV-3 is widely distributed geographically (1). The modified live vaccine and inactivated bovine adenovirus vaccination trials have been conducted for the cattle against BAdV-3. However, no commercial BAdV-3 vaccinations are currently available (4). The first type of vaccine was live attenuated vaccines, which have been used effectively for many years in economically significant animal diseases as well as in companion animals (5).

A live attenuated vaccine is made from microorganisms that have had their disease-producing ability weakened but still have immunogenic properties that can produce an immune response (6). Although modified live vaccinations are the most effective, they have some risks and disadvantages, including the risk of causing abortions, immunosuppression, and the establishment of latent infection (7). Inactivated viral vaccines are more stable and safer than live attenuated vaccines, but they generally require strong adjuvants and many doses to elicit an immune response (6). According to research on human adenovirus (HAdV) subunit vaccination, specific surface proteins can provide individuals with protective immunity (8). Due recombinant protein-based subunit vaccines are produced without the use of an infectious agent, they have been considered to be safer than conventional vaccines (9). The major antigens of the different serotypes of AdV are fibers, hexon, and penton bases, that comprise the components of AdV capsid proteins. Hexon protein could possibly be useful in the development of subunit vaccines (10,11).

AdV hexon protein is the major determinant of serotypes and the key mediator of the virus antigenicity to induce neutralizing antibodies (12,13). Hexons can play a role in the development of humoral and cellular immunity (14). The development of novel vaccinations has been made possible by recombinant technology using capsid proteins (15). In our previous studies, recombinant hexon protein-based subunit vaccination was able to induce antibody production, especially long-term antibody responses, and develop cellular immunity in mice and goats (16). In this study, we evaluate the immune response of subunit vaccine candidate using recombinant hexon protein in target animals.

MATERIALS AND METHODS

Virus isolation and confirmation by PCR: The BAdV-3 was isolated in subclinical cows in Tuv province, Mongolia in 2016. After the sample collection, 2mL PBS was added to swap and the mixture was filtered by 0.45 μm (Thermo Fisher, Waltham, USA). Next, the filtered mixture was infected to the MDBK cells in 6 well plates and cultured for 72 hours at 37°C, in an atmosphere of 5% CO_2. The culture medium was used to repeat the inoculation procedure once for viral amplification. Harvest the cell cultures when 80% of cytopathic effect (CPE) appeared in the monolayer of MDBK cells and stored at -80°C. Viral genome DNA was extracted using DNAzol Reagent (Invitrogen, CA, USA) according to the manufacturer's instructions. BAdV-3 were detected by PCR with hexon region-based primers (BALF: 5'- GRTGGTCIYT RGATRTR ATGGA-3'; BARF: 5'- AAGYCTRTCA TCYCCDGGCCA-3') with a predicted size of 641 bp (17).

Sequencing and identification of nucleotide: The PCR products were purified using the Gel/PCR DNA Isolation system (VIOGENE, New Taipei, Taiwan) according to the manufacturer's instruction, and sent to Tri-I Biotech, Inc (Taipei, Taiwan) for sequencing with Cycle Sequencing Applied Biosystems 3730 DNA Analyzer BigDyeR Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, USA). The results were cross referenced with DNAStar (DNASTAR, USA) and BLAST gene pool (NCBI, USA). Sequence were aligned using the Clustal W 1.8 program.

Production of recombinant hexon protein of BAdV-3: A fragment of 693bp of the hexon gene was amplified by PCR using the following primers: forward AATGAGCTCC TTCAGAGCACTCTG and reverse ACTGCGGCCGCA GTTTCTATGGTTCAC. PCR products were cut with *Not I/Sac I* respective restriction sites and ligated into the vector, pET-32a (Novagen, Darmstadt, Germany). The ligated plasmids were transformed into competent *E. coli* BL21 strain was grown in Luria Broth (LB broth) supplemented with ampicillin (100mg/mL) at 37°C. Protein expression was induced with 1 mM isopropyl-β-D thiogalactopyranoside (IPTG) (Amresco, Ohio, USA) for 6 hours. After induction, cells were harvested by centrifugation at 4°C and were stored at -20°C till further use.

Vaccine preparation: The subunit vaccine was prepared to contain 25 μg/ml of recombinant hexon protein with water in oil in water adjuvant (ISA 206 VG SEPPIC, Puteaux, France) as described previously. The sterility of the vaccines was confirmed by culturing of the vaccine in trypticase soy agar (TSA, 37°C), thioglycolate agar (TGA 37°C), and sabouraud dextrose agar (SDA, 25°C), (HACH, Colorado, USA) separately. During the experimental period, all test vessels or plates didn't show cloudiness or colonies, and vaccines were confirmed to be free of bacteria and fungi.

Animals: Ten five-six-month-old, BAdV-3 negative calves were obtained from herder Lhagva Bat-ochir in Khan-uul district, Ulaanbaatar, Mongolia. All experimental protocols for the animal vaccine trials were approved by the Animal Care and Uses Committee at the Institute of Veterinary Medicine, Mongolian University of Life Science (IVM-MULS-23/01/15). The experiments were conducted according to the Ethical Rules of the Mongolian University of Life Science.

Immunization of cattle: BAdV-3-negative, ten 5-6-month-old calves were randomly divided into a vaccinated group (5 calves) and a control group (5 mock-vaccinated calves). The group of experimental calf was immunized and boosted two weeks later intramuscularly with 25 μg/ml of 2 mL rhexon protein-based subunit vaccine. The levels of antibodies were measured by taking blood samples before injection, two, four, and eight weeks following the first immunization.

Antibody analysis by indirect enzyme-linked immune sorbent assay (ELISA): Serum samples were taken from immunized calves and a control group of calves for indirect ELISA analysis. Indirect ELISA plates were coated with 1.25 μg/mL of rhexon protein in coating buffer (15 mM Na_2CO_3, 35 mM $NaHCO_3$, 3 mM $NaNO_3$ pH = 9.6) at 4°C overnight. The plates were washed with PBST (0.5% tween-20) three times and blocked by 1% BSA (KPL, Gaithersburg, MD, USA) in PBST at 37°C for 1 hour. After blocking, serum samples were diluted at 1:250 with PBS and incubated at 37°C for 1.5 hour. Serum from the saline group acted as a negative control. The plates were washed six times with PBST and incubated for 1.5 hour at 37°C with 1:5000 dilution of secondary antibody (goat anti-bovine IgG) (KPL, Gaithersburg, MD, USA). Each well received 100 μL of TMB 2-Component Microwell Peroxidase Substrate (KPL, Gaithersburg, MD) after six times of PBST washing. After five minutes, 100 μL of TMB stop solution (KPL, Gaithersburg, MD) was added for stopping the reaction. A multiwall plate reader (Anthos 2020; Anthos, Cambridge, UK) was used to read the plates at 450 nm.

Statistical analysis: All data were analyzed by the statistical software, SAS (Version 9.0, Cary, NC, USA). For the animal trial, differences among the treatments at each time point were analyzed by analysis of variance (ANOVA) and Duncan's multiple comparisons ($^{a, b,}$ indicate a statistically significant difference between groups). A p-value of <0.05 was considered significant.

RESULTS

Detection of BAdV-3: The BAdV-3 was inoculated into the culture of MDBK cells and incubated until 72 hours. Compared with control MDBK cells, the inoculated MDBK cells formed with cytopathic effect (CPE) after an incubation period of 48 hours (Fig 1.) The culture of BAdV-3-infected supernatant was extracted from the viral genomic DNA, which was then identified by PCR using hexon region-based primers with an expected size of 641 bp (Fig. 2).

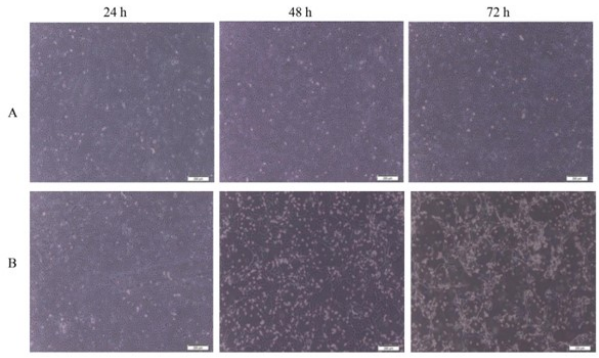

Figure 1. BAdV-3 culture in MDBK cells. A. Mock cell (original magnification × 200), B. BAdV-3 inoculated cell (original magnification × 200)

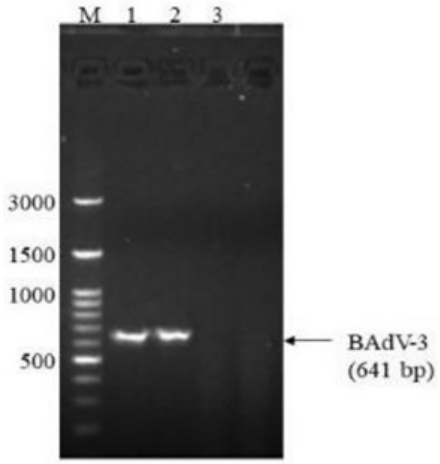

Figure 2. PCR detection of BAdV-3. Lane 1 -BAdV-3 supernatant, Lane 2- Positive control, Lane 3-Negative control, and Lane M- marker (100 bp - 3000 bp)

The amino acid of the BAdV-3 sequence was compared with other animals hexon region sequences (Fig. 3).

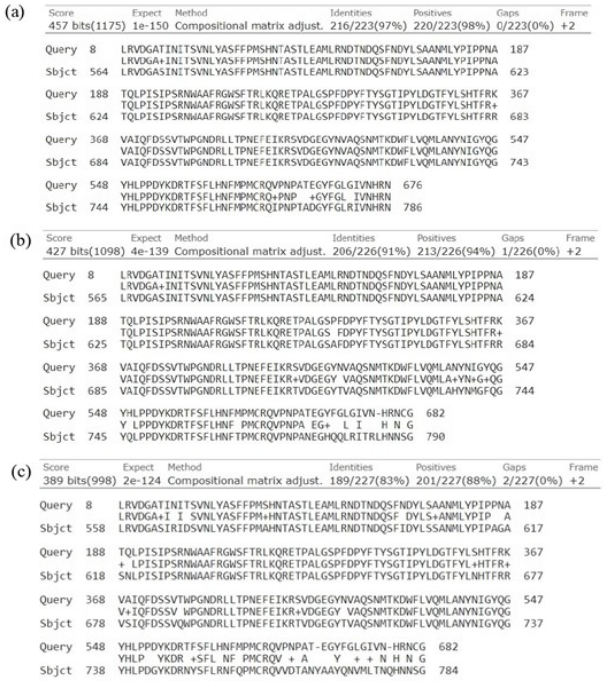

Figure 3. Amino acid sequence alignment of the hexon region of BAdV-3. BAdV-3 isolated from Mongolia, (a). Deer Mastadenovirus YP009373247.1, (b) Ovine adenovirus type 8 VP010087266.1, (c) Porcine adenovirus type 5 NP108667.1

Recombinant hexon protein purification and quantification analysis: The hexon protein's fragments of DNA were cloned into the pET32a (+) plasmid for protein expression in an E. coli expression system. The recombinant plasmids harboring the foreign genes were used to transform into *E. coli* BL 21. After induction of recombinant proteins were expressed as His-tagged fusion proteins with the expected size of 44 kDa, and it was confirmed by Western blotting using the Mice Anti-His Antibody. After purification, proteins were refolded by progressively reducing urea concentrations through dialysis in PBS. We examined SDS-PAGE analysis for recombinant hexon protein quantitation. The purified recombinant hexon proteins were quantified by comparison with different concentrations of standard bovine serum albumin (BSA conc. 1000–62.5 µg/mL). The concentration of recombinant hexon proteins was 248.3 µg/mL (Fig. 4).

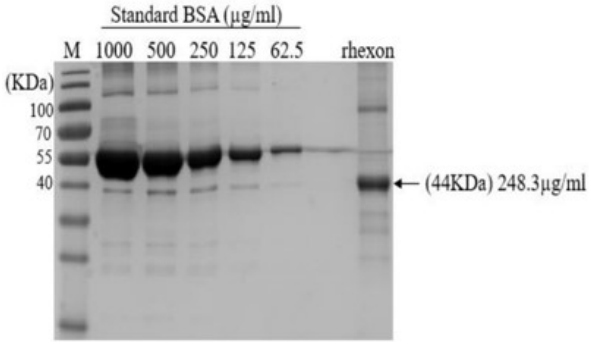

Figure 4. SDS-PAGE analysis for protein quantitation. Lane 1-5 – BSA standards for 1000-62.5 µg/mL, Lane 7- Purified recombinant hexon protein. The concentration of purified rhexon proteins quantitation indicates 248,3 µg/mL

The subunit vaccine induced antibody response in cattle

Antibody titers were measured in vaccinated target animal calves at 0, 2, 4, and 8 following weeks primary inoculation with subunit vaccine using rhexon protein. Recombinat hexon protein was used as the coating antigen to detect the antibody response by indirect ELISA. After two weeks, the calves that got the rhexon protein-based subunit vaccination produced significantly higher total levels of IgG than the control group (Fig. 5).

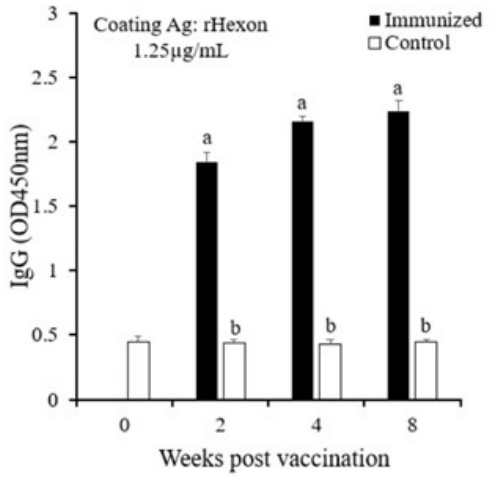

Figure 5. Antibody response of rhexon protein in cattle. Cattles were immunized twice (weeks 0 and 2) intramuscularly with subunit vaccine using rhexon protein. Indirect ELISA was performed with 1.25 µg/mL of rhexon protein. Data represent means ± SD. Duncan's significance test analyzed differences between groups. (Different superscript letters (a, b) indicate significantly different $P < 0.05$)

DISCUSSION

BAdV-3 is a cause of respiratory illness in cattle, and respiratory infections are the causative factors of animal production loss (18). One of the best strategies for controlling and decreasing infectious disease is vaccination (19). However, new vaccines against BAdV-3 are urgently needed to prevent BAdV-3 infections, as there are currently no BAdV-3 vaccines on the market. The Ad capsid proteins were investigated for their ability to elicit an effective immune response capable of neutralizing the virus (15). It has previously been demonstrated that human protective immunity can be induced by HAdV-2 and HAdV-5 subunit vaccines based on purified hexon and fiber proteins. As well, previous research have demonstrated the effectiveness of developing BAdV type 2, 3, and 7 hexon subunit vaccinations that induce viral neutralizing responses (8). The adenovirus capsid proteins fiber 1, fiber 2, hexon, and penton base were also expressed in the in the E. coli or baculovirus expression system in make to develop a FAdV-4 subunit vaccine, and it was shown that these proteins provided good protection in both in vivo and in vitro experiments (11). Our previous study, BAdV-3 subunit vaccine candidate induced higher level of antibody production in mice and produced long-term immunity in immunized in mice and goats. Additionally, mice (IL-2 and IFN-γ) and goats (IL-21 and IFN-γ) had their Th1- and Th2-type immune responses balanced, and both species produced more Th1-type cytokines (16). IL-21 and interleukin 2 are closely related cytokines with substantial structural similarities that may have resulted from gene duplication (19). Natural killer T cells and CD4+ T cells are the primary producers of interleukin 21 (20). The cytokines T helper 1, IL-2, INF-γ, and IL-21 mediate pro-inflammatory actions that are essential for the development of cell-mediated immune responses and are important for eliminating of viral infections (21). In this study, to have a more realistic evaluation of this vaccine, target animals were immunized with the same vaccination as before the study. Also, higher level of antibody production was observed in calves in our study. Our further study will experiment with the humoral or cellular immune responses in the target animals. The rhexon protein could be a subunit vaccine candidate against BAdV-3.

CONCLUSION

In the target animal test, a recombinant hexon protein-based BAdV-3 subunit vaccine candidate induced an immune response. The recombinant hexon protein could be a subunit vaccine candidate against BAdV-3.

Conflict of Interests: The authors declare no conflict of interests.

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REFERENCES

1. Shi, H.F., Zhu, Y.M., Yan, H., Ma, L., Wang, X.Z., Xue, F., 2014. Pathogenesis of a Chinese strain of bovine adenovirus type 3 infection in albino guinea pigs. Archives of Virology 159:3211–3218. https://doi.org/10.1007/s00705-014-2181-5
2. Ayalew L.E., Kumar, P., Gaba, A., Makadiya, N., Tikoo, S.K., 2015. Bovine adenovirus-3 as a vaccine delivery vehicle. Vaccine 33:493-499. https://doi.org/10.1016/j.vaccine.2014.11.055
3. Shen, Y., Liu, J., Zhang, Y., Ma, X., Yue, H., Tang, C., 2020. Prevalence and characteristics of a novel bovine adenovirus type 3 with a natural deletion fiber gene. Infection, Genetics and Evolution 83:104348. https://doi.org/10.1016/j.meegid.2020.104348
4. Lehmkuhl, H.D., 1978. A bovine adenovirus type 3: Isolation, characterization, serologic survey and experimental infection in calves. Iowa State University Capstones, Doctoral dissertation. 6503 https://lib.dr.iastate.edu/rtd/6503
5. Ndi, O.L., Barton, M.D., Vanniasinkam, T., 2013. Adenoviral Vectors in Veterinary Vaccine Development: Potential for Further Development. World Journal of Vaccines 03:111–121. https://doi.org/10.4236/wjv.2013.33016
6. Gadissa L, Yune N, n.d. 2018. Update on Veterinary Viral Vaccines: A Review. Journal of Veterinary Science Research 3(3): 000163.
7. Zakhartchouk, A.N., Pyne, C., Mutwiri, G.K., Papp, Z., Baca-Estrada, M.E., Griebel, P., Babiuk, L.A., Tikoo, S.K., 1999. Mucosal immunization of calves with recombinant bovine adenovirus-3: induction of protective immunity to bovine herpesvirus-1. Journal of General Virology 80:1263-1269 doi: 10.1016/j.vaccine.2014.03.073
8. Jane Kwun-Lai Kan Battles., 1985. Isolation and evaluation of the immunizing subunits of bovine adenovirus types 2, 3 and 7 (Program in immunobiology). Iowa state university, Ames, Iowa. Doctoral dissertation
9. Tan, M., Jiang, X., 2017. Recent advancements in combination subunit vaccine development. Human Vaccines and Immunotherapeutic. 13(1):180–185. https://doi.org/10.1080/21645515.2016.1229719
10. Thaci, B., Ulasov, I. v., Wainwright, D.A., Lesniak, M.S., 2011. The challenge for gene therapy: Innate immune response to adenoviruses. Oncotarget. 2(3):113-121 https://doi.org/10.18632/oncotarget.231
11. Wang, X., Tang, Q., Chu, Z., Wang, P., Luo, C., Zhang, Y., Fang, X., Qiu, L., Dang, R., Yang, Z., 2018. Immune protection efficacy of FAdV-4 surfaces proteins fiber-1, fiber-2, hexon and penton base. Virus Research 245:1–6. https://doi.org/10.1016/j.virusres.2017.12.003
12. Chen, L., Yin, L., Zhou, Q., Peng, P., Du, Y., Liu, L., Zhang, Y., Xue, C., Cao, Y., 2019. Epidemiological investigation of fowl adenovirus infections in poultry in China during 2015-2018. BMC Veterinary Research 15:271 https://doi.org/10.1186/s12917-019-1969-7
13. Liu, J., Mei, N., Wang, Y., Shi, X., Chen, H., 2021. Identification of a novel immunological epitope on Hexon of fowl adenovirus serotype 4. AMB Express 11:153 https://doi.org/10.1186/s13568-021-01309-2

14. Russell, W.C., 2009. Adenoviruses: Update on structure and function. Journal of General Virology 90:1-20 https://doi.org/ 10.1099/vir.0.003087-0
15. 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. https://doi.org/10.1016/S0264-410X(03)00117-8
16. Gundegmaa U, Raadan O, Wu HC, Wang HY, Wu MC, Chu CY. 2023. Recombinant hexon protein as a new bovine adenovirus type 3 subunit vaccine candidate. J Vet Res. 67(1):23–31. doi: 10.2478/jvetres-2023-0014
17. de Motes, C.M., Clemente-Casares, P., Hundesa, A., Martín, M., Girones, R., 2004. Detection of Bovine and Porcine Adenoviruses for Tracing the Source of Fecal Contamination. Applied and Environmental Microbiology 70(3):1448–1454. https://doi.org/10.1128/ AEM. 70.3.1448-1454.2004
18. Ali Y, Khalafalla AI, Rahman MEA, Intisar KS, Ali YH, Khalafalla. 2010. Adenovirus type 3 infections in camels in Sudan. Afr J Microbiol Res [Internet]. 4(13):1356–8. Available from: https://www.researchgate.net/ publication/ 228671265
19. Cid R, Bolívar J.2021. Platforms for production of protein- based vaccines: From classical to next-generation strategies. Vol. 11, Biomolecules. MDPI; doi: 10.3390/ biom11081072
20. Hinrichs C.S., Spolski R., Paulos C.M., Gattinoni L., Kerstann K.W., Palmer D.C., Klebanoff C.A., Rosenberg S.A., Leonard W.J., Restifo N.P.: IL-2, and IL-21 confer opposing differentiation programs to CD8+ T cells for adoptive immunotherapy. Blood 2008, 111, 5326–5333, doi: 10.1182/BLOOD-2007-09-113050
21. Leonard W.J., Wan C.-K.: IL-21 Signaling in Immunity. F1000Res 2016, 5, 224, doi: 10.12688/ f1000research. 7634.1

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