Slov Vet Res 2013; 50 (1): 5-12
UDC 604.4:615.371:577.27:601.4:636.5:616.9:579.8

Original Scientific Article

STUDY ON IMMUNE EFFICACY OF GENOMIC EXPRESSION LIBRARY VACCINES AGAINST AVIAN *PASTEURELLA MULTOCIDA*

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Summary: Random DNA fragments (500–3000 bp) were obtained from the genome of avian *Pasteurella multocida* CVCC474 using the restriction enzyme *Sau*3AI and cloned into the *Bam*HI site of the eukaryotic expression vector pcDNA3.1(+) to construct a genomic expression library. The library was subdivided into five clone pools and recombinant plasmids were extracted from each pool. Balb/c mice were allocated to groups 1–5 and vaccinated with these recombinant plasmids, whereas mice injected with pcDNA3.1(+) and PBS were used as negative controls. The immune responses were evaluated based on the serum antibody level, a lymphocyte proliferation assay, and the IFN - γ level. The protective efficacy after challenge with the virulent avian *Pasteurella multocida* strain CVCC474 was evaluated based on the relative protection rates. A significant increase in the serum antibody levels was observed in mice in group 1. The lymphocyte proliferation (SI value) and IFN - γ levels were also higher in mice immunized with the group 1 vaccine compared with those vaccinated with group 2–5 vaccines ($p < 0.05$) and control groups ($p < 0.01$). Furthermore, group 1 vaccine provided better protection than other vaccines after challenge with avian *Pasteurella multocida* strain CVCC474. The results indicated that construction of a genomic expression vaccine library is a promising approach for the prevention of avian pasteurellosis.

Key words: avian *Pasteurella multocida*; expression library; immunization; vaccine

Introduction

Avian *Pasteurella multocida* is the etiological agent of fowl cholera (avian pasteurellosis), which is a widely distributed disease that affects poultry, particularly chickens, turkeys, ducks, and geese, in many countries (1-3). The high morbidity and mortality associated with fowl cholera results in significant economic losses in the poultry industry (4). The main control measure used against this disease is drug treatment, particularly antibiotics such as streptomycin and olaquindox. However, the disease frequently recurs after drug withdrawal and the pathogen develops drug resistance after long-term medication, while it may also have significant toxic effects in avian species. The laying rate may be decreased significantly in layers while drug residues may persist in broilers. Therefore, it is necessary to develop novel vaccines for the control of fowl cholera.

Current vaccines for the prevention of fowl cholera include attenuated vaccines and inactivated vaccines. However, the immune efficacy with commercial live vaccines is not optimal. The attenuated vaccine has several shortcomings, such as a short period of immunity and poor protection. Residual virulence may lead

Received: 1 April 2012
Accepted for publication: 15 December 2012

to a reduction in the laying rate and there may be a disease outbreak if it is used incorrectly (5). The efficacy of the inactivated vaccine is not as high as the live vaccine, so the inactivated vaccine is used only rarely.

The lack of an effective vaccine that prevents this disease has stimulated the search for new candidate immunogens for novel vaccines. Expression library immunization (ELI) is an alternative approach with the potential to identify vaccine antigens (6-8). ELI was first reported by Barry (9). Piedrafita demonstrated that DNA vaccination with genomic libraries of *Mycoplasma pulmonis* could induce protection against challenge-exposure in parental strains of mice (10). Hernández showed that experimental animals immunized with a genomic expression library of *Mycobacterium tuberculosis* had a significant reduction in the number of viable bacteria in their lungs and less pulmonary tissue damage (11).

In this study, we constructed a genomic expression library of avian *Pasteurella multocida*. Recombinant plasmids were extracted and mice were vaccinated with these plasmids. The immune response and protective efficacy were tested. The aim of this study was to provide a foundation for the screening of antigens, and for further research and development into vaccines to combat avian *Pasteurella multocida* infection.

Materials and methods

Animals, bacterial strains, plasmids, and cells

Female Balb/c mice (6 weeks old) were purchased from the Experimental Animal Center in Henan Province. The avian *Pasteurella multocida* CVCC474 strain was purchased from the Chinese Institute of Veterinary Drug Control (IVDC). Competent cells (TG1) and the eukaryotic expression vector pcDNA3.1(+) were maintained in the laboratory at He Nan University of Science and Technology in China.

Construction of the genomic expression library

Genomic DNA was extracted from avian *Pasteurella multocida* CVCC474 strain as described previously (12) and digested with the restriction enzyme *Sau*3AI to produce median sizes of 500–3000 bp. These fragments were purified using a gel extraction kit. The eukaryotic expression vector pcDNA3.1(+) was digested with *Bam*HI and treated with alkaline phosphatase. It was ligated with the genomic DNA fragments and transformed into competent *E. coli* TG1 via electroporation (13). The *E. coli* cells were plated onto Luria-Bertani (LB) agar plates and incubated overnight at 37℃ to generate a genomic expression library containing about 10^6 clones.

Identification of the library and extraction of recombinant plasmids

Eight clones were selected randomly from the library and inoculated into 5 ml LB with ampicillin (50 μg/ml), before overnight culture at 37℃. The recombinant plasmids were extracted and identified using the restriction enzymes *Eco*RI and *Hind*III. The library was assigned randomly to five clone pools. The plasmids in each pool were prepared on a large scale using the alkaline lysis method and adjusted to 1 μg/μl using phosphate-buffered saline (PBS, 0.01 M, pH 7.2) for further experiments. These plasmids were referred to as DNA vaccines 1–5.

Vaccination protocol

Six-week-old female Balb/c mice (n = 168) were assigned randomly to seven groups, i.e., groups 1–5, the pcDNA3.1(+) group, and the PBS group. The mice in groups 1–5 and the pcDNA3.1(+) group were immunized with plasmids from clone pool groups 1–5 and the empty vector, pcDNA3.1(+), respectively. The vaccine dose was 100 μg/100 μl per injection for the plasmids from each clone pool and the pcDNA3.1(+). Mice in the PBS group were injected with 100 μl PBS (0.01 M, pH 7.2). The animals in each group were injected in both tibialis anterior muscles three times at 2 week intervals using 1 ml aseptic disposable syringes.

Detection of serum antibody levels

Blood samples measuring about 150 μl were drawn from mice by tail-cutting each week after immunization and the serum specimens were isolated. The serum antibody titers were detected

using an indirect enzyme-linked immunosorbent assay (ELISA). The ELISA was performed as follows. Flat-bottomed 96-well plates were coated with 100 μl ultrasound lysates (20 μg/ml) of avian *Pasteurella multocida* CVCC474. The plates were washed with 0.01 M PBS containing 0.05% tween-20 (PBS-T, pH 7.2) and blocked with 3% sodium caseinate for 2 h at 37 ℃. The plates were incubated for 1.5 h at 37 ℃ with 100 μl of the serum samples (diluted 1/100 with PBS-T). The plates were washed three times with PBST and goat anti-mouse IgG-horseradish peroxidase (HRP) was added and the plates were incubaed at 37 ℃ for 1.5 h. Then the plates were washed three times with PBST and 50 μl ortho-phenylene diamine (OPD) was added and the plates were incubated for a further 20 min. The enzyme activity was stopped by adding an equal volume of 2 M H_2SO_4 and the absorption was measured at 492 nm. The antibody titers were determined for up to 6 weeks before the challenge. All of the serum samples from each weekly collection were tested on the same ELISA plate, using two replicates.

Spleen lymphocyte proliferation assay of the immunized mice

Two weeks after each immunization, three mice were sacrificed from each group by cervical dislocation after administration of euthanizing drugs. The mice were soaked in 75% ethanol for 5 min. The spleen was removed via aseptic manipulation, before removing the fat and connective tissue, and washing with 1 ml Hanks' solution. The spleens were cut into 1–2-mm pieces and the tissues were blown fully with a pipette so that the cells were suspended in the Hanks' solution. The solution was then filtered through a three-tier gauze. Finally, the Hanks' solution containing the cells was collected and carefully layered onto the surface of lymphocyte separation medium. After centrifugation at 1000 x *g* for 20 min, the lymphocytes were collected and washed twice with RPMI-1640 without fetal bovine serum. The spleen lymphocyte suspension was resuspended and diluted to 1 x 10^7 cells/ml. An 50-μl aliquot of cell suspension was seeded into a 96-well plate (Greiner Bio-One, Longwood, Germany). Each experiment was repeated three times. Each well received 50 μl of 10 μg/ml ConA (experimental well) or 50 μl of RPMI 1640 medium (Gibco, Grand Island, USA) (negative control) and the plates were incubated at 37 ℃ in a 5% CO_2 environment. After 36 h, 10 μl of 5 mg/ml 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT; Sigma–Aldrich) was added to each culture well, followed by incubation for 3 h. After centrifugation for 10 min, the supernatant was discarded and 150 μl of dimethyl sulfoxide (DMSO) was added to the pellet, followed by incubation for 10 min until the crystals dissolved. The optical density (OD) of each well was measured using a microplate reader (Bio-Rad Laboratories, model 680) at a wavelength of 450 nm. The stimulation index (SI) was estimated using the following equation: SI = OD experimental well/OD negative control well.

IFN - γ assays

Two weeks after each immunization, the spleen lymphocytes activated by ConA were prepared as described above. The cells were incubated at 37 ℃ in 5% CO_2 for 60 h and the supernatants were harvested. The IFN-γ levels in the supernatants were detected using a commercial ELISA kit (Lianshuo, Shanghai, China), according to the manufacturer's instructions. The ELISA microtiter plate was coated with mouse IFN-γ monoclonal antibody which was combined with the IFN-γ in spleen lymphocyte supernatants. After the addition of anti-mouse IFN-γ antibody-horseradish peroxidase (HRP) and tetramet hylbenzidine (TMB), TMB became from colorless to blue under the catalysis of HRP and finally generated yellow, followed by the addition of H_2SO_4. The depth of color was directly proportional to the content of IFN-γ in samples. After the absorption was measured at 450 nm, the IFN-γ levels could be calculated according the standard curve. The measurement range of this mouse IFN-γ commercial ELISA kit was 0-1000pg/ml.

Challenge study

Fifteen days after the final vaccination, all of the mice were challenged subcutaneously with 3 x10^2 (5 LD_{50}) of virulent strain CVCC474. The mice were reared for 15 days and the survival number and relative protection rates were determined.

Statistical analysis

Data from all experiments were analyzed using the origin75 program. Analysis of variance was used to determine the significance of differences between the means in the experimental groups. Differences where $p<0.05$ were considered significant differences, while $p<0.01$ indicated highly significant differences.

Results

Identification of the library

Clones were randomly selected from the library and recombinant plasmids were extracted, before digesting with two restriction endonucleases, *Eco*RI and *Hind*III. The enzyme-digested products were analyzed by agarose gel electrophoresis. Figure 1 shows that DNA fragments between 500 and 3000 bp were obtained, indicating that the genomic expression library of avian *Pasteurella multocida* CVCC474 was constructed successfully.

Serum antibody levels

Conserved plates containing library clones were combined and assigned to five clone pools. The plasmids were extracted from each pool on a large scale and injected into mice. Serum specimens were isolated from vaccinated mice 1–6 weeks after the first immunization and assayed to determine the presence of specific antibodies using indirect ELISA. As shown in Figure 2, antibody levels of serum from all the experimental groups (groups 1–5) exhibited an increasing trend after immunization. The serum antibody levels in group 1 were significantly higher than those in groups 2–5 ($p < 0.05$) and the two negative control groups, i.e., the pcDNA3.1(+) group and the PBS group ($p < 0.01$). There were no differences in the antibody levels among the other experimental groups ($p > 0.05$), although group 2 was slightly higher than the others. In addition, the serum antibody levels in groups 1–5 were significantly higher than those in the pcDNA3.1(+) group ($p < 0.01$).

Spleen lymphocyte proliferation assay

To investigate the cellular immune response induced by the genomic expression library, we assessed the proliferation of spleen lymphocytes at three different time points after each vaccination experiment and the results are shown in Figure 3. In each experiment, the SI values of groups 1 and 4 were higher than that of other groups. After the first immunization, the SI values of groups 2–5 were similar to that of group 1 ($p > 0.05$). After the second and the third immunizations, however, the SI value of group 1 was significantly higher than that of groups 2–5 ($p < 0.05$) and the negative control groups ($p < 0.01$). There were also no differences among groups 2–5 ($p > 0.05$), although group 4 was slightly higher than the others.

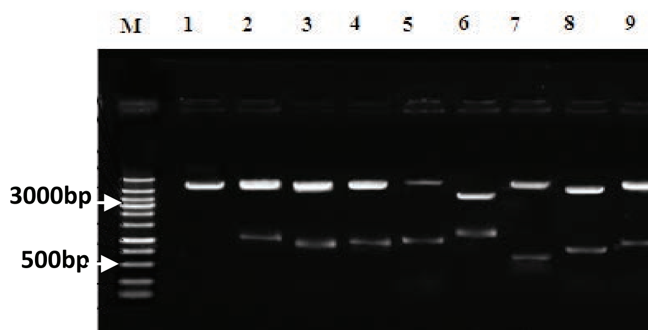

Figure 1: Electrophoresis of recombinant plasmids digested with *EcoR*I and *Hind*III. M: wide range DNA marker (100–6000); 1: pcDNA3.1(+) empty plasmid; 2–9: recombinant plasmids digested using *EcoR*I and *Hind*III

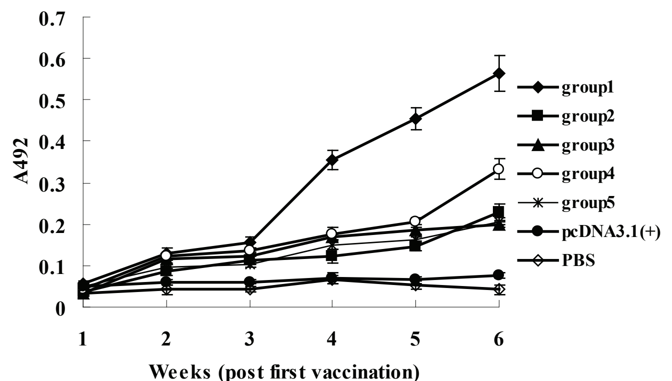

Figure 2: Dynamic changes in the serum antibody levels of immunized mice. The serum antibody levels were measured by indirect ELISA weekly, until 6 weeks. Mice were immunized with group 1 vaccine (■); group 2 vaccine (●); group 3 vaccine (▲); group 4 vaccine (▼); group 5 vaccine (◆); pcDNA3.1(+) vector (○); and PBS (△)

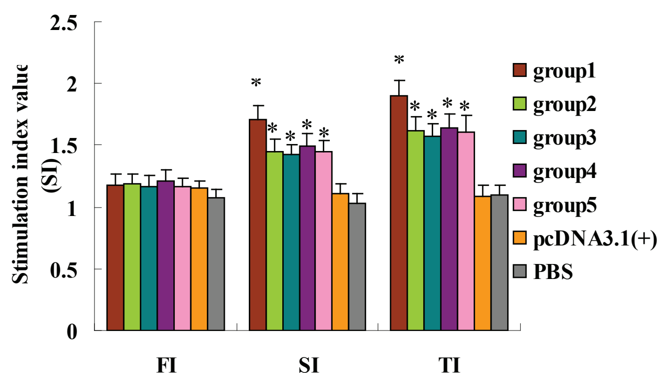

Figure 3: MTT detection of splenic lymphocyte proliferation in immunized mice. ConA was administered to stimulate mouse splenic lymphocytes 2 weeks after each immunization. FI: 1 week after the first immunization; SI: 1 week after the second immunization; TI: 1 week after the third immunization. *$p < 0.05$, **$p < 0.01$

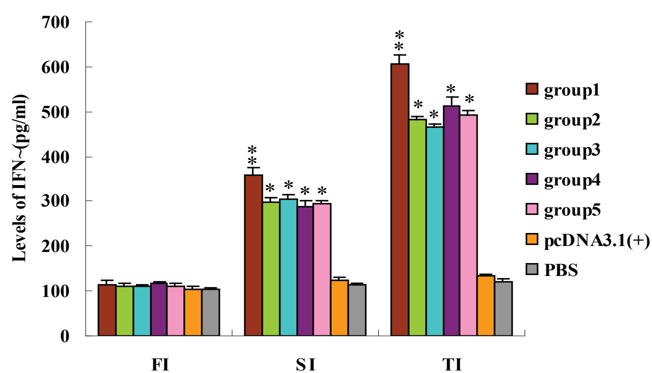

Figure 4: Analysis of mouse splenic lymphocyte-secreted IFN~γ levels. The results are expressed as the mean ± S.D. FI: first immunization; SI: second immunization; TI: third immunization. *$p < 0.05$, **$p < 0.01$

IFN~γ secretion

After ConA stimulation, the IFN~γ levels secreted by mouse splenic lymphocytes were higher in mice vaccinated with all recombinant plasmids (groups 1–5). After the first immunization, there were no significant differences in the IFN~γ levels ($p > 0.05$). After the second and third immunization, the IFN~γ level in group 1 was significantly higher than that in other groups ($p < 0.05$). The levels were similar among groups 2–5, but higher than in the two control groups ($p < 0.05$) (Figure 4).

Protection against challenge with avian Pasteurella multocida CVCC474

Groups of mice were challenged with the live virulent avian *Pasteurella multocida* CVCC474 strain 15 days after the last immunization. The survival number and relative protection rates were calculated up to 15 days (Table 1). The mortality of mice injected with PBS was 93.33% after challenge whereas that of the pcDNA3.1(+) group was 86.67%. The relative protection rates in groups 1 and 4 were higher than in the other groups ($p < 0.05$). The protective efficiency in group 1 was the highest (64.29%).

Table 1: Protective efficacy against lethal challenge with *avian Pasteurella multocida*

Groups	Survival number/total	Mortality(%)	Relative protection rate (%)
group 1	10/15	33.33	64.29**
group 2	5/15	66.67*	28.57*
group 3	3/15	80.00**	14.29
group 4	7/15	46.67*	50.00*
group 5	5/15	66.67*	28.57*
pcDNA3.1(+)	2/15	86.67**	0
PBS	1/15	93.33**	0

Relative protection rate (%) = (1 - mortality of experimental groups/mortality of control group) x 100%. *$p < 0.05$, **$p < 0.01$.

Discussion

Current candidate antigens for avian *Pasteurella multocida* DNA vaccines include outer membrane proteins (Omps), capsules, and Type 4 fimbriae (14-16). However, there are large differences in the immune efficacy of DNA vaccines constructed using these antigen genes. In general, the protective efficacy of these DNA vaccines rarely exceeds that of attenuated live vaccines (17). Thus, the screening of novel protective antigen genes is necessary to develop effective vaccines for controlling avian pasteurellosis. Immunization with genomic expression libraries has emerged as a novel technology (18, 19) for identifying candidate vaccine genes that provide protection against pathogens. Some studies have identified individual protective genes via the sequential fractionation of cDNA or genomic expression libraries (20-23). The identification of vaccine candidates to combat avian *Pasteurella multocida* using this technology is promising because another study demonstrated that fusion and combined DNA vaccines against avian pasteurellosis could induce a higher immune response than monovalent DNA vaccines (24).

In the present study, we constructed a genomic expression library of avian *Pasteurella multocida* and evaluated its immune efficacy. The humoral immune response is an important factor in the resistance to avian *Pasteurella multocida* infections. In this study, we detected the antibody levels induced by DNA vaccines extracted from a library of clone pools in mice (group 1–5) and we found that the antibody response induced by DNA vaccines extracted from clone pool group 1 was higher compared to other vaccines.

In addition to the antibody response, the cellular immunological response also has an influential role in the anti-infection process (25, 26). It is well-known that DNA vaccines can induce effective immune responses (27, 28). Lymphocyte proliferation assays and the detection of cytokine secretions are commonly used methods for evaluating the cellular immune function. The lymphocyte transformation rate after stimulation by ConA is frequently used to evaluate the T-cell immune response (29). In this study, the DNA vaccine prepared from clone pool group 1 induced higher lymphocyte proliferation levels and higher IFN~γ secretion from immunized mice compared with the other groups.

Challenge experiments are one of the important indices used to evaluate the protective efficacy of vaccines. In the present study, mice immunized with various recombinant plasmids were challenged with virulent avian *Pasteurella multocida*. Of these DNA vaccines, the plasmids from clone pool group 1 showed the most promise.

Future directions for our studies could include investigating Th2 cytokines, such as IL-4 and IL-10, which have been shown to modulate the immune responses to DNA vaccines (30, 31). The next task is to identify specific antigens in the library, which can be prepared as subunit and DNA vaccines to induce a protective immune response against fowl cholera. In conclusion, we demonstrated that immunization with an expression library enabled the screening of the avian *Pasteurella multocida* genome for potential vaccine candidates, which provided a valuable reference for the design of future DNA vaccines against fowl cholera.

Acknowledgements

This work was supported by a grant from the Natural scientific research Project of Henan province, China (No. 2011B180019)

References

1. Davies RL, MacCorquodale R, Caffrey B. Diversity of *avian Pasteurella multocida* strains based on capsular PCR typing and variation of the OmpA and OmpH outer membrane proteins. Vet Microbiol 2003; 91: 169–82.

2. Hussam AA, Takuo S, Hitoshi H, Yoshiya K, Noriyuki O, Osamu I. Invasion of chicken embryo fibroblast cells by *avian Pasteurella multocida*. Vet Microbiol 2004; 104: 55–62.

3. Shivachandra SB, Kumar AA, Gautam R, Singh VP, Saxena MK, Srivastava SK. Identification of avian strains of *Pasteurella multocida* in India by conventional and PCR assays. Vet J 2006; 172: 561–4.

4. Subaaharan S, Blackall LL, Blackall PJ. Development of a mμlti-locus sequence typing scheme for avian isolates of *Pasteurella multocida*. Vet Microbiol 2010; 141: 354–61.

5. Mariana S, Hirst R. The immunogenicity and pathogenicity of *Pasteurella multocida* isolated from poultry in Indonesia. Vet Microbiol 2000; 72: 27–36.

6. Kamath AT, Feng CG, Macdonald M, Briscoe H, Britton WJ. Differential protective efficacy of DNA vaccines expressing secreted proteins of *Mycobacterium tuberculosis*. Infect Immun 1999; 67:1702–7.

7. Liu SG, Gong Q, Wang C, et al. A novel DNA vaccine for protective immunity against virulent *Mycobacterium bovis* in mice. Immunol Lett 2008; 117: 136–45.

8. Geza E, Sameera S, Patricia A, et al. Development and characterization of a pooled *haemophilus influnzae* genomic library for the evaluation of gene expression changes associated with mucosal biofilm formation in otitis media. Int J Pedia Otorhinolar 2003; 67: 749–55.

9. Barry MA, Laiand WC, Johnston S. Protection against *mycoplasma* infection using expression library immunization. Nature 2002; 377: 632–35.

10. Piedrafita D, Xu D, Hunter D, Harrison RA, Liew FY. Protective immune responses Induced by vaccination with an expression genomic library of *Leishmania major*. J Immunol 1999; 163: 1467–72.

11. Hernández YL, Corona DY, Rodríguez SS, et al. Immunization of mice with a *Mycobacterium tuberculosis* genomic expression library results in lower bacterial load in lungs after challenge with BCG. Tuberculosis 2006; 86: 247–54.

12. Moore RJ, Lenghaus C, Sheedy SA, Doran TJ. Improved vectors for expression library immunization-application to *Mycoplasma hyopneumoniae* infection in pigs. Vaccine 2001; 20: 115–20.

13. Tollefsen S, Vordermeier M, Olsen I, Storset AK, Reitan LJ, Clifford D. DNA injection in combination with electroporation: a novel method for vaccination of farmed ruminants. Scand J Immunol 2003; 57: 229–38.

14. Entomack B, Sawada T, Kataoka Y, Ohtsu N, Akagi M, Nakamura SA. 39KDa protein mediates adhesion of avian *Pasteurella multocida* to chicken embryo fibroblast cells. Vet Microbiol 2003; 97: 229–43.

15. Prado ME, Dabo SM, Confer AW. Immunogenicity of iron-regulated outer membrane proteins of *Pasteurella multocida* A:3 in cattle: molecular characterization of the immunodominant heme acquisition system receptor (HasR) protein. Vet Microbiol 2005; 105: 269–80.

16. Hatfaludi T, Al-Hasani K, Boyce JD. Outer membrane proteins of *Pasteurella multocida*. Vet Microbiol 2010; 144: 1–17.

17. Yamile LH, Daniel YC, Sergio SR, et al. Immunization of mice with a *Mycobacterium tuberculosis* genomic expression library results in lower bacterial load in lungs after challenge with BCG. Tuberculosis 2006; 86: 247–54.

18. Alberti E, Acosta A, Sarmiento ME, et al. Specific cellular and humoral immune response in Balb/c mice immunised with an expression genomic library of *Trypanosoma cruzi*. Vaccine 1998; 16: 608–12.

19. Yero CD, Pajón FR, Caballero ME, et al. Immunization of mice with *Neisseria meningitidis* serogroup B genomic expression libraries elicits functional antibodies and reduces the level of bacteremia in an infant rat infection model. Vaccine 2005; 23: 932–9.

20. Melby P, Ogden G, Flores H, et al. Identification of vaccine candidates for experimental visceral leishmaniasis by immunization with sequential fractions of a cDNA expression library. Infect Immun 2000; 68: 5595–602.

21. Lvey FD, Magge DM, Woitaske MD, Johnston SA, Cox RA. Identification of a protective antigen of *coccidioides immitis* by expression library immunization. Vaccine 2003; 21: 4359–67.

22. Halea S, Kaltenboeckb B, DeGravesb FJ, et al. Screening the whole genome of a pathogen in vivo for individual protective antigens. Vaccine 2005; 23: 3016–25.

23. Beltrán PK, Gutiérrez OA, Puebla PA, et al. Identification of immunodominant antigens of *Mycobacterium bovis* by expression library immunization. Vet J 2010; 1: 181–3.

24. Gong Q, Cheng M, Niu MF. Out membrane protein DNA vaccines for protective immunity against virulent avian *Pasteurella multocida* in chickens. Procedia Environ Sci 2011; 8: 723–9.

25. Liao CM, Huang CJ, Hsuan SL, et al. Immunogenicity and efficacy of three recombinant subunit *Pasteurella multocida* toxin vaccines against progressive atrophic rhinitis in pigs. Vaccine 2006; 24: 27–35.

26. Register KB, Sacco RE, Brockmeier SL. Immune response in mice and swine to DNA vaccines derived from the *Pasteurella multocida* toxin gene. Vaccine 2007; 25: 6118-6128.

27. Lee CG, Choi SY, Park SH, Park KS, Ryu SH, Sung YC. The synthetic peptide Trp-Lys-Tyr-Met-Val-d-Met as a novel adjuvant for DNA vaccine. Vaccine 2005; 23: 4703–10.

28. Herrmann JE. DNA vaccines against enteric infections. Vaccine 2006; 24: 3705–8.

29. Stone KD, Feldman HA, Huisman C,

Howlett C, Jabara HH, Bonilla FA. Analysis of in *vitro* lymphocyte proliferation as a screening tool for cellular immunodeficiency. Clin Immunol 2009; 131: 41–9.

30. Huang Y, Babiuk LA, Hurk SD. The cell-mediated immune response induced by plasmid encoding *bovine herpesvirus* 1 glycoprotein B is enhanced by plasmid encoding IL-12 when delivered intramuscularly or by gene gun, but not after intradermal injection. Vaccine 2006; 24: 5349–59.

31. Williman J, Lockhart E, Slobbe L, Buchan G, Baird. The use of Th1 cytokines, IL-12 and IL-23, to modulate the immune response raised to a DNA vaccine delivered by gene gun. Vaccine 2006; 24: 4471–4.

ŠTUDIJA IMUNSKE UČINKOVITOSTI CEPIV PROTI AVIARNI *PASTEURELLA MULTOCIDA* NA OSNOVI GENOMSKE KNJIŽNICE

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Povzetek: Genomsko ekspresijsko knjižnico smo naredili z naključnimi odseki DNK (500-3000 bp), ki so bili pridobljeni iz genomske DNK ptičje bakterije *Pasteurella multocida* CVCC474 z restrikcijsko razgradnjo z encimom Sau3AI. Odseki so bili klonirani v mesta BamHI vektorja za izražanje v evkariontskih celicah pcDNA3.1(+). Knjižnica je bila razdeljena na pet delov in iz vsakega smo izolirali rekombinantne plazmide. Miši seva Balb/c smo razdelili v pet skupin in jih cepili s 5 seti rekombinantnih plazmidov, kontrolni skupini pa smo vbrizgali prazen vektor pcDNA3.1 (+) v slanem fosfatnem pufru. Imunski odgovor smo ocenili na osnovi serumske ravni protiteles, s testom proliferacije limfocitov in ravni IFN-γ.
Učinkovitost zaščite po okužbi z virulentnim sevom CVCC474 ptičje bakterije *Pasteurella multocida* smo ocenili na osnovi relativnih zaščitnih meril. Pri miših v skupini 1 smo opazili značilen dvig serumske ravni protiteles. Prav tako smo pri skupini 1 opazili statistično značilno povečanje proliferacije limfocitov in IFN-γ, tako v primerjavi s kontrolno skupino ($p < 0{,}01$) kot s testnimi skupinami 2 do 5 ($p < 0{,}05$). Rezultati so pokazali, da je cepivo v obliki genomske ekspresijske knjižnice obetaven pristop za preprečevanje ptičje pastereloze.

Ključne besede: ptičja *Pasteurella multocida*; ekspresijska knjižnica; cepljenje; cepivo