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The Cloning *F* Gene of Pigeon Paramyxovirus TypeI (PPMV-1) PL Strain and the Study on DNA Vaccine

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Abstract: One pair of primers were designed based on *F* gene of Pigeon Paramyxovirus type I (PPMV-1) sequences reported in GenBank. *F* gene fragment about 1.66 kb was amplified by Reverse Transcription Polymerase Chain Reaction (RT-PCR) with the genome of Newcastle Disease Virus (NDV) PL strain as the template. Sequence analysis showed that *F* gene of PL strain shared 76.3-98.6% homology with the nucleotide sequence of *F* gene of domestic and foreign 17 strains PPMV-1 or NDV. The *F* gene of PL strain was inserted into eukaryotic expression vector PCDNA3.1V5HIS so eukaryotic expressing plasmid PCDNA3.1-PPMV-1-F was constructed. Researchers immunized some 1 month old young pigeons which were not immunized against NDV with the constructed eukaryotic expression recombinant plasmid. The dose was 100 μg/feather. After 2 weeks, researchers boosted immunization one time. Respectively, collected blood from vein under the wings and separated serum on days 0, 7, 14, 21 and 28 after booster immunization. The blood serum antibody titers of different groups were assayed by indirect ELISA. The results showed that the antibodies producing after immunizing young pigeons with eukaryotic expression plasmid specifically reacted with F protein of PPMV-1. The antibody began to produce on day 7 after immunization were maximum on day 14 and then the level began to decline. This indicated that F protein had good immunogenicity. All young pigeons were challenged with 100 times 50% Egg Infections Dose (EID50) of virus homologous to *F* gene on day 28 after immunization with eukaryotic expression recombinant plasmid. The results showed that the protective rate of immunized with recombinant plasmid group and immunized with pigeon NDV propolis inactivated vaccine group were respectively, 3.3 and 100% significantly higher than physiological saline group and PCDNA3.1V5HIS mock-vehicle group. The protective rate of pigeon NDV propolis inactivated vaccine group was also higher than the recombinant plasmid group. This indicated that the eukaryotic expressing plasmid of *F* gene constructed by us as candidated genetic vaccines could induce young pigeons to produce protective immune response but it also needed other methods to raise the efficiency of immunity.

Key words: PPMV-1, *F* gene, cloning, NDV, pigeon

INTRODUCTION

PPMV-1 disease also known as pigeon plague or pigeon Newcastle Disease (ND) is a highly contacting and acute infectious disease which was caused by Avian Paramyxovirus type I (APMV-1) and prevalent in pigeons. It is one of the main diseases which threaten to pigeon industry and characterized by enteritis, severe diarrhea and neurological symptoms (Alexander, 1990; Alexander *et al*., 1985). Written records showed that Hanson and Sinha first reported a pigeon disease similar to ND in Poultry Science in 1952. After that Ahmed and Sabri, Stewart, Hilbrich, Vindevogel, Maes and Canic successively also reported this disease (Alexander *et al*., 1984; Eisa and Omer, 1984). Then, the pathogen was diagnosed with PPMV-1 recognized by most researchers. The first report was the PPMV-1 epidemic which took place in Iraq in 1977. However, at that time some researchers believed that it was caused by the herpes virus which were called pigeon herpetic encephalomyelitis virus. Whereas Schrag considered it as A/PMV-3 (The Avian Study Group, 1984; GeIb *et al*., 1987). Outbreaks also occurred in Sultan and Egypt in 1981. From then on the outbreaks quickly spread and spread to

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Mediterranean countries and went into French and German (The Avian Study Group, 1984). Then, it quickly spread to Europe, America, Canada and so on (Alexander, 1990; Alexander *et al*., 1985, 1984) and it had spread to Asia by 1985. This disease was found in pigeons of the country (Bao *et al*., 1996) around 1986 and now is popular in various provinces and cities nationwide (ZiZhao, 1999; Ning *et al*., 1994).

Pigeons of different varieties and ages are all infected with PPMV-1 which spread quickly and the disease caused by which have high mortality. At present, the most effective way to prevent and treat PPMV-1 disease is vaccination but the vaccine against PPMV-1 is rare in the market and NDV vaccine was mainly used to prevent and treat PPMV-1 disease. Although, the cross-reaction between PPMV-1 and NDV is higher and many researchers also think NDV vaccine can prevent PPMV-1 infections but the immune effect is not enough real and effective in practice. At present, there are no specific drugs to treat this disease and many researchers are devoting themselves to studying an effective vaccine or drug to prevent and treat this disease. Increasing research and development efforts of PPMV-1 vaccine become the priority of preventing and treating pigeon ND.

PPMV-1 has characteristics similar to NDV. The Hemagglutinin and Neuraminidase (HN) on the surface of the virus can agglutinate red blood cells of chicken and some mammalian and the action of agglutination can be inhibited by specific serum. The viral genomes were a single-stranded negative-sense RNA which is about 15 kb long and includs 6 genes: 3'-NP-P-M-F-HN-L-5'. Among them, the fusion protein encoded by *F* gene plays a major role in virus penetrating on cells, hemolysis and fusion of cells to cells which is the mainly decisive factor in virulence (Liu *et al*., 2001). So, *F* gene can be used to develop subunit vaccine, live vector vaccine and DNA vaccine as candidate gene.

In the late 1980s, the development of ND recombinant *DNA* gene engineering vaccine which mainly includes subunit vaccine, live vector vaccine and nucleic acid vaccine was begun. The nucleic acid vaccine directly imports exogenous gene (DNA or RNA) encoding some antigenic proteins into animal somatic cell and antigenic proteins which induce the immune response of host to meet the goal of preventing and treating diseases were synthesized by the expression system in host cells. Compared with the traditional inactivated vaccine, subunit vaccine and genetically engineering vaccine, nucleic acid vaccine can induce the body to produce all-sided immune responses and has the advantages of safe and reliable, convenience for preparation, allogeneic strain cross protection, producing long-lasting immunity, etc. Nucleic acid vaccine is considered as the third generation vaccine after inactivated vaccine, live attenuated vaccine and subunit vaccine. *F* gene of PPMV-1 PL strain was cloned, eukaryotic expression plasmid PCDNA3.1-PPMV-1-F was constructed and its protective immunity was preliminarily studied in this study.

MATERIALS AND METHODS

Materials: PPMV-1 PL strain was isolated from infected pigeons of some pigeon farm in Kongtong region, Pingliang city, Gansu province and identified and preserved by The Animal Husbandry and Veterinary Research Institute of Gansu province. Experimental young pigeons were purchased from some pigeon farm in Pingliang city Gansu province.

PCDNA3.1V5HIS plasmid was a gift from by the Zoonosis Laboratory of Chinese Academy of Agricultural Sciences, Lanzhou Veterinary Research Institute. *E.coil* DH5α competent cells were purchased from Tiangen Biochemical Technology Co., Ltd. pMD-18T vector, DNA Marker DL-000, T4 DNA ligase, RNAiso Plus extracting reagent, one-step process reverse transcription kit (PrimeScript ®One Step RT-PCR kit Ver. 2), gel extraction kit, restriction enzymes and so on were purchased from TaKaRa Company. PPMV-1 F-ELISA antibody test kits were prepared by the laboratory. The other reagents were prepared domestic and analytical grade.

The cloning and sequence analysis of PPMV-1 PL strain: According to *F* gene of NDV published in GenBank, one pair of primers P1 and P2 the length of which was 1.66 kb were designed. Primers sequences are shown in Table 1. About 500 μL allantoic fluid of chick embryo containing PPMV-1 PL strain stored at -20°C were taken out. RNA was extracted from allantoic fluid in accordance with the instructions of TaKaRa Company and dried RNA precipitate were dissolved with appropriate amount of RNAase-free water and preserved at -80°C for future use. According to the instructions of PrimeScript One Step RT-PCR kit Ver. 2 kit, 2×1 Step Buffer, P1 and P2, PrimeScript 1 Step Enzyme Mix, RNA templates extracted and RNase Free dH_2O were added in sequence then gently flipped and instantly centrifugated the mixture. After uniformly mixing *F* gene were amplified by RT-PCR. The reaction was subjected to 30 cycles at 50°C for 30 min, 94°C for 2 min, 94°C for 30 sec, 55°C for 30 sec, 72°C for 2 min. The 5~10 μL RT-PCR reaction solution was prepared and performed agarose gel electrophoresis after the end of reaction. *F* gene through RT-PCR amplification and pMD-18T vector were connected and the products were transformed into DH5α competent cells. Positive bacterial colonies were screened by colony PCR and the plasmids were extracted. The recombinant plasmid was

Table 1: Primers and their sequences used in RT-PCR amplification

Primers	Primers sequences (5'-3')
P1	ATGGGCTCCAAACCTTCTAC
P2	TCATGCTCTTGTAGTGG

Table 2: Primers and their sequence used in PCR amplification of recombinant eukaryotic plasmid

Primers	Primer sequence
P3	CGCGGATCCGCCACCATGGGCTCCAAACCTTCT
P4	CCGCTCGAGCATGTA GTGGCTCTCATCTG

named pMD-18T-PPMV-1-F. The recombinant plasmid pMD-18T-PPMV-1-F containing *F* gene was delivered to Shanghai Yingjun Bio-Technique Co., Ltd. and sequenced. The homology of *F* gene nucleotide sequence of PPMV-1 PL strain and domestic and foreign 17 strains PPMV-1 or NDV was analyzed in BLAST and GenBank.

The construction and large scale preparation of the eukaryotic expression vector of *F* gene of PPMV-1 PL strain: According to the results of recombinant plasmid pMD-18T-PPMV-1-F sequencing, one pair of primers P3 and P4 were designed. Bam H and Xho endonuclease site were respectively, added in the 5' terminal of P3 and P4 and protective Base pair were also added. The length was about 1.66 kb and the primers sequences are shown in Table 2.

Using PMD-18T-PPMV-1-F as the template and P3 and P4 as the upstream and downstream amplification primers, PCR amplification was carried out and the reaction were performed at 94°C for 5 min and then subjected to 30 cycles at 94°C for 50 sec, 55°C for 50 sec and 72°C for 2 min followed by a final extension at 72°C for 10 min. After finishing amplification, 5 μL PCR products were separated on 1.0% agarose gel to identify whether the size of amplification products was correct. Amplification products were recovered and purified by DNA gel extraction kit after they were identified correct. After the purified PCR products of *F* gene and PCDNA3.1 V5HIS vector were doubly digested by Bam H and Xho, respectively, *F* gene fragment and linearized PCDNA3.1V5HIS vector fragment were connected and the products were transformed into DH5α competent cells. Positive colonies were screened by colony PCR and the plasmids were extracted. The recombinant plasmid was named PCDNA3.1-PPMV-1-F. The recombinant plasmid PCDNA3.1-PPMV-1-F containing *F* gene was delivered to Shanghai Yingjun Bio-Technique Co., Ltd. and sequenced. A lot of recombinant plasmids were prepared on large scale by the method of molecular cloning.

Used expression products of *F* gene of PPMV-1 PL strain to immune animals: About 60-30 days old young pigeons which weren't immunized NDV vaccine were randomly divided into four groups, 15 in each group. The young pigeons in physiological saline group, PCDNA3.1 mock-vehicle group, PCDNA3.1-PPMV-1-F group and pigeon NDV propolis inactivated vaccine group were intramuscularly injected at chest, respectively with physiological saline dosage, PCDNA3.1 mock-vehicle and PCDNA3.1-PPMV-1-F as the dose of 100 μg/feather, pigeon NDV propolis inactivated vaccine 0.5 mL/feather. The pigeons in experimental group were injected with PCDNA3.1-PPMV-1-F at different points of chest muscle and the pigeons in control group were injected with PCDNA3.1 mock-vehicle as the same method. The pigeons in recombinant plasmids group were boosted immunization once on 14th day. Respectively, collected blood from vein under the wings and separated serum on days 0, 7, 14, 21 and 28 after booster immunization. The blood serum antibody titers of different groups were assayed with PPMV-1- F-ELISA antibody test kits.

Toxicity attack and protect test of the expression products of *F* gene of PPMV-1 PL strain: All the experimental young pigeons were challenged with 100 times 50% Egg Infections Dose (EID50) of virus homologous to *F* gene on day 28 after first immunization. The pigeons were isolated breeding as grouped and minutely observed every day until on 7th day post challenge. Added up the number of cases and deaths and calculated the rate of immune protection.

RESULTS AND DISCUSSION

The RT-PCR amplification results of PPMV-1 PL strain: It can be shown from Fig. 1 that the length of *F* gene fragment through RT-PCR amplification is 1.66 kb which was consistent with the experimental design.

The PCR screening of the recombinant plasmid containing *F* gene of PPMV-1 PL strain: *F* gene through RT-PCR amplification and pMD-18T vector were connected and the products were transformed into DH5α competent cells. Recombinant-positive bacteria were screened by Colony PCR as shown in Fig. 2. Six positive recombinant bacterial colonies could be seen in the selected 8 bacterial colonies. Colonies were selected to extract plasmid and DNA-sequencing was done. The sequencing results were authentic which indicated that *F* gene of PPMV-1 PL strain was obtained. The positive-recombinant plasmid was named pMD-18T-PPMV-1-F.

The homology analysis of *F* gene of PPMV-1 PL strain and the reference strains: The sequencing results showed that *F* gene in pMD-18T-PPMV-1-F was a

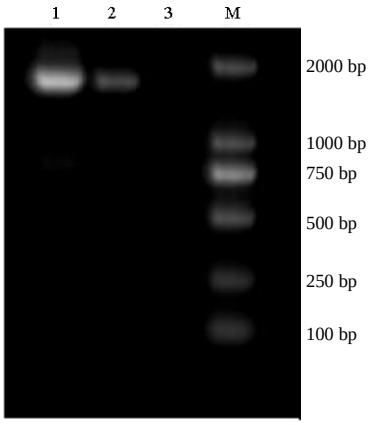

Fig. 1: The electrophotogram of RT-PCR products, 1: RT-PCR amplification 1, 2: RT-PCR amplification 2, 3: Blank control and M: DNA Mark

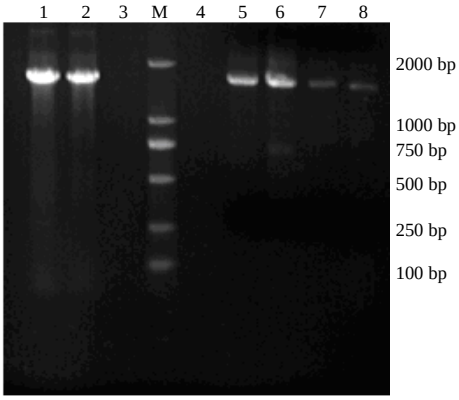

Fig. 2: The electrophotogram of colony PCR, 1, 2, 5, 6, 7 and 8 were positive colonies, 3, 4 were negative colonies and M: DNA Mark

fragment that was 1662 bp. The fragment entirely consistent with the expected results was complete *F* gene sequences. Through the comparison of homology domestic and foreign pigeon and chicken source partial NDV strains in BLAST and GenBank, the similarity between *F* gene of PL strains and 17 strains was between 76.3 and 98.6%. The results showed that the genetic relationship between pigeon NDV isolated by us and Chichen-Massachusette-344783 and Pigen-Minnesota-729 was the farthest and the similarity was only 76.3%. The genetic relationship between pigeon NDV and strain GZ strain was the closest and the similarity was up to 98.6%. The results were shown in Table 3.

The construction and identification of recombinant expression plasmid PCDNA3.1-PPMV-1-F: The purpose fragment and vector were doubly digested with BamH and

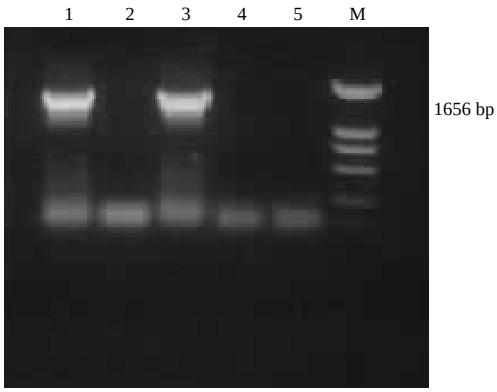

Fig. 3: The electrophoretogram for identification by colony PCR, 1 and 3 were positive recombinant plasmid, 2, 4, 5 were negative recombinant plasmid. M: The standard of DNA molecular weight

Xho, respectively and the two were connected after they were respectively recovered. The products were transformed into DH5α competent cells. Recombinant-positive bacteria were screened by PCR as shown in Fig. 3. Two positive recombinant bacterial colonies could be seen in the selected five bacterial colonies. Colonies were selected to extract plasmid and DNA-sequencing was done. The sequencing results were authentic which indicated that eukaryotic expression recombinant plasmid containing *F* gene of PPMV-1 PL isolates was obtained. The positive-recombinant plasmid was named PCDNA3.1-PPMV-1-F.

The results of using expression products of *F* gene of PPMV-1 PL isolates to immune animals: OD value data detected by indirect ELISA showed that the antibody levels of recombinant plasmid PCDNA3.1-PPMV-1-F and pigeon NDV propolis inactivated vaccine group began to rise on the 7th day after immunization and were significantly higher than physiological saline group and PCDNA3.1 mock-vehicle group on the 14th day reached the highest, pigeon NDV propolis inactivated vaccine group were also significantly higher than recombinant plasmid PCDNA3.1-PPMV-1-F group and physiological saline group and PCDNA3.1 mock-vehicle group had no change. The results were seen in Table 4.

Toxicity attack and protect test: The protective rate of recombinant plasmid PCDNA3.1-PPMV-1-F group and pigeon NDV propolis inactivated vaccine group were respectively 33.3 and 100% which were significantly higher than physiological saline group and PCDNA3.1 mock-vehicle group. The protective rate of pigeon NDV

Table 3: The comparison of nucleotide similarity of PPMV-1-PL and other paramyxo virus

Divergence	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
PPMV-1-PL	-	76.3	96.8	97.7	97.5	96.9	76.7	76.3	76.8	76.4	76.7	95.6	97.5	97.6	98.6	97.5	97.5	97.7
Chicken_Massachusetts _344783-3_2004	10.5	-	64.5	76.4	76.4	76.5	98.1	99.9	98.3	99.5	98.0	86.3	76.4	76.4	83.7	76.3	76.3	76.4
Chicken USA (CA)_1083(Fontana)_72	7.4	8.8	-	96.7	96.5	96.1	64.8	64.5	64.8	64.5	64.8	77.0	96.6	96.6	81.3	96.6	96.6	96.7
Japan_Ibaraki_85	6.7	8.9	5.5	-	98.8	98.1	76.8	76.3	76.9	76.5	76.9	95.6	99.5	99.5	98.5	98.8	98.8	99.6
Kr-102_89	7.2	8.8	6.1	4.9	-	98.0	76.7	76.4	76.9	76.5	76.9	95.6	98.7	98.8	98.4	98.8	98.7	98.8
P-Anhui_1_09	10.1	8.2	8.2	7.8	8.3	-	77.3	76.5	77.4	76.7	77.0	95.7	98.0	98.0	97.7	98.0	98.0	98.0
Pigeon_Indiana_18002_1991	8.8	6.2	7.1	7.0	7.2	4.7	-	98.1	99.8	98.3	98.6	85.3	76.8	76.8	84.0	76.7	76.7	76.8
Pigeon_Minnesota_723_2009	10.5	0.4	8.8	9.0	8.9	8.1	6.3	-	98.2	99.4	98.0	86.3	76.3	76.4	83.7	76.3	76.3	76.3
Pigeon_Minnesota_2446_1989	8.3	5.8	6.9	6.6	6.6	4.4	0.6	6.0	-	98.5	98.7	85.4	76.8	76.9	84.0	76.9	76.9	76.9
Pigeon_Nevada_241851_2003	10.0	1.7	8.5	8.4	8.3	7.3	5.5	1.8	5.1	-	98.2	86.1	76.5	76.5	83.8	76.4	76.4	76.5
Pigeon_New York_44407_1984	8.6	6.9	6.8	6.4	6.4	6.0	4.6	6.9	4.3	6.2	-	85.2	76.9	76.9	84.0	76.8	76.8	76.9
Pigeon_Pennsylvania_2062_2008	10.0	0.8	8.4	8.8	8.5	8.0	6.7	0.6	6.3	1.8	7.0	-	95.6	95.6	96.2	95.6	95.6	95.6
Sh-1_97	7.1	8.8	5.8	1.7	5.0	8.2	7.0	8.9	6.6	8.3	6.6	8.6	-	99.9	98.5	98.7	98.7	99.7
Sh-2_98	6.8	8.7	5.7	1.6	4.7	8.1	6.9	8.9	6.6	8.3	6.5	8.5	0.5	-	98.6	98.7	98.7	99.7
Strain GZ	2.4	9.4	6.6	5.4	5.8	8.9	7.6	9.4	7.3	8.9	7.5	8.9	5.9	5.7	-	98.6	98.6	98.7
XJ-1_91	7.1	9.0	6.0	4.7	4.9	8.0	7.1	9.2	6.4	8.6	6.7	8.7	5.1	5.1	5.5	-	100.0	98.9
XJ-3_97	7.1	9.0	6.0	4.7	4.9	8.0	7.1	9.1	6.4	8.6	6.7	8.7	5.1	5.1	5.6	0.2	-	98.8
ZhJ-2_86	6.5	8.8	5.3	1.3	4.8	8.0	6.9	8.9	6.5	8.3	6.3	8.6	1.3	1.3	5.3	4.6	4.7	-

Table 4: The results of serum antibody detected by ELISA

	Days				
Groups	0	7	14	21	28
PCDNA3.1-PPMV-1-F	0.029±0.013	0.253±0.021	0.642±0.034	0.598±0.029	0.524±0.033
Pigeon NDV propolis inactivated vaccine	0.024±0.005	0.786±0.032	1.457±0.035	1.243±0.031	1.112±0.019
PCDNA3.1 mock-vehicle	0.041±0.024	0.025±0.013	0.027±0.005	0.018±0.003	0.030±0.005
Physiological saline	0.031±0.008	0.028±0.006	0.031±0.007	0.021±0.005	0.027±0.004

Table 5: Results of toxicity attack and protect test in each group

Groups	Number of pigeons attacked by toxicity	Death	Rate of death (%)
PCDNA3.1-PPMV-1-F	15	10	66.67
Pigeon NDV propolis inactivated vaccine	15	0	0.00
PCDNA3.1 mock-vehicle	15	15	100.00
Physiological saline	15	15	100.00

propolis inactivated vaccine group was also significantly higher than recombinant plasmid PCDNA3.1-PPMV-1-F group. The results were shown in Table 5.

PMV-1 PL strain was separated from infected pigeons of some pigeon farm in Pingliang city, Gansu province by the research group which was confirmed virulent strain by analyzing its biological activity and amino acid sequence of the F0 cleavage site of *F* gene. In this study, the whole Open Reading Frame (ORF) of *F* gene of PPMV-1 PL strain the total length of which was 1662 bp was cloned. This encoded 553 amino acids (from the start codon AGT to the stop codon TAG) which was the same as the number of amino acids encoded by *F* gene of other NDV strains reported at home and abroad. Through the comparison of homology of 17 domestic and foreign pigeon and chicken source partial NDV strains in BLAST and GenBank and according to the results analysis of phylogenetic tree researchers could draw the conclusion that the similarity between the isolates and 17 strains was between 76.3 and 98.6%. The genetic relationship between pigeon NDV isolated by us and Chichen-Massachusette-344783 and Pigen-Minnesota-729 was the farthest and the similarity was only 76.3%. The genetic relationship between pigeon NDV and strain GZ strain was the closest and the similarity reached up to 98.6%.

Now the research on genetic engineering vaccine whose target gene was *F* gene of NDV is a hot spot. Taylor J made F protein of NDV express in FowlPox Virus (FPV) to recombine vFP-29 vaccine. The chickens were first immunized when they were 21 days old, second immunized when 28 days old and conteracted toxic substances when 49 days old. The protective rate of oral group was 50% droping eyes group was 90% scratching wings and webs group was 100% and intramuscular injection group was 100% (Taylor *et al*., 1990). Wei-dong Ding developed recombinant Fowlpox Virus vaccine rFPV-F and rFPV-HN with *F* and *HN* gene of NDV. SPF chicken was immunized as the dose of 103 PFU/feather (PFU was Plaque Unit) and the protective rate was 100% (ELD50 was embryo median lethal dose). The recombinant vaccines could induce body to produce HI antibody about 6.0log2 (Ding *et al*., 2005). In 2005, Loke used PEGFP-HN and pEGFP-F with Freund's complete adjuvant to immunize chickens. About 33.3% (3/9) of chickens immunized with pEGFP-HN were protected, 50.0% (5/10) with pEGFP-F and the protective rate reached up to 90% when the chickens were together immunized with pEGFP-HN and pEGFP-F. The results showed that

combined immunization was better than single-gene immunization (Loke *et al*., 2005). Between 2001 and 2004, Yong-Hou Jang, Sheng-Wang Liu, Xue-Ya Liang and Hui-Ming Gu from Chinese constructed *F* gene of NDV DNA vaccine applying PCDNA3.1 eukaryotic expression vector with *F* gene of NDV different isolates as target gene. Animals were immunized in intramuscular way and toxicity attack test was done. The protective rate reached 30-66.67% (Jiang *et al*., 2001; Liu *et al*., 2002; Liang *et al*., 2003; Gu *et al*., 2004).

In this study, *F* gene was inserted into eukaryotic expression vector PCDNA3.1V5HIS and eukaryotic expression plasmid PCDNA3.1-PPMV-1-F was successfully constructed. The young pigeons which weren't immunized with NDV vaccine were immunized with the eukaryotic expression recombinant plasmid inducing the body to produce corresponding and specific antibodies. Antibodies appeared on the 7th after immunization, the level of antibodies reached the highest on the 14th day and then the levels of antibodies began to decline. But the level of antibodies was overall not high. It indicated that *F* gene of pigeon NDV genetic vaccine could induce young pigeons to produce humoral immunity.

The results of toxicity attack test showed that immunization with *F* gene of pigeon NDV genetic vaccine could protect 33.33% of pigeons from the challenge of virulent NDV with a certain role in immune protection. However, the antibody titers and immune protection force were both significantly lower than the level of antibodies of pigeon NDV Propolis inactivated vaccine. The reason may be that the recombinant plasmids were degraded during the process from staying to totranscribing in inintercellular substance and cytoplasm after they were transferred into the host or the dose of vaccination wasn't enough or the inoculation site affected antigen presenting capacities and appropriate immunoadjuvant needed to be added. These need further study.

Pigeon NDV eukaryotic expression plasmid constructed in this study could induce the production of specific antibodies. The results of toxicity attack test showed that this had some immune protective effect which laid the foundation for next better developing and applying pigeon NDV DNA vaccine.

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REFERENCES

Alexander, D.J., 1990. Avian paramyxoviride recent developments. Vet. Microbiol., 23: 103-114.

Alexander, D.J., P. Russel and G. Parsons, 1985. Antigenic and biological characterization of avian paramyxovirus type-I isolates from pigeons an international collaborative study. Avian Pathol., 14: 365-376.

Alexander, D.J., P.H. Russell and M.S. Collins, 1984. Paramyxoviru stype 1 infections of racing pigeon: Characterization of isolated viruses. Vet. Record, 114: 444-446.

Ding, Z.D., L.P. Cao, T.Y. Zhang, X.Z. Liu, R.K. Zhang and Y.T. Wu, 2005. Genetic stability of the protective efficacy of recombinant fowlpox virus vaccines expressing F and HN genes of Newcastle disease virus. Chinese J. Vet., 41: 10-14 (In Chinese).

Eisa, M. and A. Omer, 1984. Avian paramyxovirus type 1infections of racing pigeons natural outbreak of Newcastle disease in pigeons in the Sudan. The Vet. Record, 114: 297-297.

GeIb, J. Jr., P.A. Fries and F.S. Peterson, 1987. Pathogenicity and cross-protection of pigeon paramyxovirus-I and newcastle disease virus in young chickens. Avian Dis., 31: 601-606.

Gu, H.M., S.H. Hou and X.L. Qiu, 2004. Construction and vaccination test of nucleotide vaccine of F glycoprotein gene from natural attenuated strain of Newcastle disease virus. Chinese J. Vet. Sci. Technol., 34: 39-44.

Jiang, Y.H., J.L. Chen, X.L. Song, T. Guangzhi, K. Xiangang and L. Zhonggui, 2001. Construction of the recombinant plasmid coexpressing NDV F gene and chicken IL2. Chinese J. Preventive Vet. Med., 23: 81-84.

Liang, X.F., W.H. Fang and L.L. Jiang, 2003. Safety, stability and immunogenicity of an oral DNA vaccine against newcastle disease. Chinese J. Biotechnol., 19: 24-29.

Liu, H.L., Y.K. Wang, W.W. Yan, G.Q. Zhu and J.H. Zhou, 2001. The progess of study on molecular biology of avian paramyxovirus type I. Progress Vet. Med., 22: 16-20.

Liu, S.W., X.G. Kong, G.Z. Tong, M.A. Yunyan and J.L. Lu, 2002. Protection of chickens from newcastle disease by vaccination with gene vaccine expressing the HN protein of newcastle disease virus. Scientia Agric. Sinica, 35: 1565-1569.

Loke, C.F., A.R. Omar, A.R. Raha and K. Yusoff, 2005. Improved protection from velogenic Newcastle disease virus challenge following multiple immunizations with plasmid DNA encoding for *F* and *HN* genes. Vet. Immunol. Immunopathol., 106: 259-267.

Ning, L., H. Yinxian, L. Naijin and Z. Guilan, 1994. Comparative studies on the biological characteristics of avian Paramyxovirus type 1 (PMV-1) in pigeon. Chinese J. Anim. Poult. Infect. Dis., 2: 1-5 (In Chinese).

Taylor, J., C. Edbauer, A. Rey-Senelonge, J.F. Bouquet and E. Norton *et al*., 1990. Newcastle disease virus fusion protein expressed in a fowlpox virus recombinant confers protection in chickens. J. Virol., 64: 1441-1450.

The Avian Study Group, 1984. Paramyxovirus infection of pigeons. Vet. Record, 114: 232-232.

ZiZhao, C., 1999. Comparison on clinical symptoms and pathologic changes in pigeons after experimental infection with paramyxovirus type I of pigeon origin and newcastle disease virus from chickens. Chinese J. Vet. Med., 25: 7-9.