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A genotype VII Newcastle disease virus-like particles confer full protection with reduced virus load and decreased virus shedding

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ABSTRACT

Newcastle disease (ND) is one of the most severe avian infectious disease inflicting a great loss on poultry industry worldwide. The control of ND relies on proper vaccination strategies. The vaccine strains of Newcastle disease virus (NDV) mainly belong to genotype I, II or III, which cannot fully prohibit virus shedding against the prevalent genotype VII virulent strain attack. To develop a safe, genotype matched vaccine candidate, we employed a bac-to-bac expression system and constructed a genotype VII NDV strain based virus-like particles (NDV VLPs). It was constructed with NDV M protein as the skeleton, and protective antigen F and HN proteins displayed on the surface. The NDV VLPs exhibited a similar appearance to the live NDV particles, but with denser F and HN proteins displayed on the surface. The immunization assay indicated that NDV VLPs stimulated a longer protection period, less tissue virus loading and shorter virus shedding period than the commercialized LaSota-formulated vaccine when challenged with genotype VII NDV strain. These results proposed the potential role of NDV VLPs as an alternative to current live genotype unmatched vaccine for the control and eliminate NDV in the avian flocks.

1. Introduction

Newcastle disease (ND) is a highly contagious disease that may result in mortality as high as 100% in susceptible species, being the fourth most problematic disease to poultry industry [1,2]. The etiology of ND is Newcastle disease virus (NDV) of genus *Avulavirus*, family *Paramyxoviridae*. The virus genome is a single negative stranded RNA, composed of genes encoding six major structural proteins (3′-NP-P-M-F-HN-L-5′) [2]. Among them, F and HN proteins are the major protective antigens. Based on the amino acid sequences of these proteins, NDV is sorted into class I and class II. Class I isolates are lentogenic, possessing only 1 genotype (genotype 1), while class II isolates are composed of 18 genotypes (genotype I-XVIII) [3–7]. The prevalent virulent strains circulating in Asia mostly belong to genotype VII of Class II [8–10].

The control of ND requires both adequate biosecurity to protect flocks from contacting virulent strains and proper vaccination to resist virus attack. The most commonly used ND vaccines are formulated with live or inactivated NDV strains isolated in the last century, including LaSota, B1, Ulster/67, Mukteswar and VG/GA strains [1,11]. All these strains belong to genotype I, II and III, their genetic distance with prevalent strains (genotype VII) ranging from 18.3% to 26.6% [12]. The mismatched genotypes lead to incomplete protection, manifested by persistent existence of virus in the flock and atypical clinical symptoms [13–16]. Apart from causing economic loss in poultry industry, it also inflicts an evolutionary pressure on wild strains towards more virulent strains [17]. Additionally, these vaccines are normally used in the form of live vaccines, which also inherently bears the risk of recombination with wild strains and reversion to virulence [18]. Thus, it is in great need of a safe, genotype-matched and efficient vaccine.

Traditional vaccine development strategies mainly concentrate on recombinant protein vaccines, genetically modified live vaccines and inactivated vaccines. However, these vaccine candidates are all produced from egg-based systems, which have several drawbacks, such as low yields, time consuming, scattered risk and contamination of other avian pathogens [19–21]. Viruses like particles (VLPs) are a group of novel vaccine candidates constructed by viral structural proteins, preserving a similar spatial antigenicity to live viral particles and can fully avoid the risk of viral replication [22,23]. Several VLP-based commercialized vaccines have appeared on the market: Engerix 03 (hepatitis B virus),

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Cervarix 03 (human papillomavirus), Recombivax HB 03 (hepatitis B virus), Gardasil 03 (human papillomavirus) and Ingelvac CircoFLEX® (Porcine Circovirus Type 2) [23]. Early Newcastle disease virus like particles (NDV VLPs) were mostly generated through eukaryotic transfection of ELL-0 avian fibroblast cells [24]. These NDV VLPs were proved to be an efficient immunogen in a mouse model, demonstrating a proficient T cell response [24,25]. Shen et al. generated avian influenza virus based VLPs containing NDV proteins through a high efficient and economical Bac-to-Bac system [26,27]. We previously constructed NDV VLPs through the Bac-to-Bac system containing genotype VII NDV strain M and F protein, which demonstrated dendritic cell (DC) activating and immune stimulating ability in mouse [28,29]. However, the protective efficacy of ND VLPs against a lethal circulating strain (genotype VII) challenge in commercial chickens has not been explored.

In this study, we developed a type of genotype matched NDV VLPs, containing three structural proteins, namely M, F and HN of NDV NA-1 strain. In immunization examination, its satisfying immunogenicity and protection ability was proved in commercial layers. Additionally, compared to NDV genotype II strain based vaccine, the genotype VII NDV VLPs resulted in lower tissue viral load and reduced virus shedding period, suggesting its promising role in the control and elimination of ND.

2. Materials and methods

2.1. Virus and cloning of M, F and HN genes into recombinant baculoviruses

NDV NA-1 strain (GenBank: DQ659677) was isolated from diseased goose population by our laboratory and characterized to be a velogenic genotype VII virus, which was highly pathogenic to chickens and geese [15]. The full-length genes encoding the M, F and HN proteins of NDV NA-1 strain were codon-optimized and cloned into pFastBac1 transfer vector, respectively (Invitrogen, Carlsbad, CA, USA). The expression of these genes was under the control of polh promoter, generating the recombinant plasmids, pFastBac1-M, pFastBac1-F and pFastBac1-HN. These plasmids were separately transformed into *E. coli* DH10Bac competent cells that contained the AcMNPV baculovirus genome (Invitrogen, Carlsbad, CA, USA) to generate recombinant Bacmids. They were then transfected into Sf9 cells using Cellfectin II reagent (Invitrogen, Carlsbad, CA, USA) for the production of recombinant baculovirus (rBVs). Five days later, the supernatant was collected and subjected to the BacPAK Baculovirus Rapid Titer Kit (Clontech, Mountain View, CA, USA) to quantify the rBVs.

To confirm the expression of M, F and HN proteins in rBVs, indirect fluorescence assay (IFA) were performed. In detail, Sf9 cells were respectively infected with rBV at a multiplicity of infection (MOI) of 1 for 48 h at 27 °C, and then the cells were solved with 4% paraformaldehyde for 25 min. Subsequently, the fixed cells were gently washed in phosphate buffered saline (PBS, pH 7.2) and incubated with chicken anti-NDV polyclonal serum (Weike biotechnology Co. Ltd, Harbin, China) (1:200) containing 1% bovine serum albumin for 1 h. The cells were extensively washed with PBS containing 0.5% Tween-20 (v/v, PBST) and incubated with FITC-conjugated rabbit anti-chicken secondary antibody (Bioss, Beijing, China) (1:1000) and 0.2% Evans blue for 1 h before observed with a fluorescence microscopy.

2.2. Generation and characterization of NDV VLPs

For production of NDV VLPs, Sf9 cells were adjusted to 2×10^6 cells/ml and co-infected with rBV-M, rBV-F and rBV-HN at the MOI of 5, 4 and 3, respectively. The cells were maintained in serum-free SF900II medium (Life technologies corporation, Gaithersburg, MD, USA) at 28 °C in spinner flasks rotated at a speed of 150 × g. Cell supernatants were harvested five days post-infection and subjected to sucrose gradient ultracentrifugation. The VLPs were then confirmed by western blotting. Briefly, pelleted cell debris was removed by centrifugation at 5000× *g* for 30 min. VLPs in the supernatant were filtered with a 0.45 μm filter and pelleted by centrifugation at 140,000× *g* for 2 h at 4 °C. The fraction was suspended in 1 mL PBS overnight and subjected to a 20%, 40% and 60% interface discontinuous sucrose gradient centrifugation (100,000 *g* for 1 h at 4 °C) using SW41 rotor (Beckman Coulter, Miami, FL, USA). The product was then washed with PBS once to remove sucrose.

The resultant VLPs were examined by SDS-PAGE of 10% polyacrylamide gels. For western blot analysis, proteins were transferred to polyvinylidene difluoride (PVDF) membranes and then detected with 1:250 dilution of chicken anti-NDV polyclonal serum (Weike biotechnology Co. Ltd, Harbin, China) as primary antibody. It was then incubated with 1:5000 dilution of HRP-conjugated rabbit anti-chicken IgY (EarthOx, San Francisco, CA, USA) as the secondary antibody.

The titer of NDV VLPs was determined by haemagglutination assay (HA) as described before. Briefly, NDV VLP samples were serially diluted in 2-fold increments in 25 μL. To each VLP dilution, 25 μL 1% chicken red blood cell (RBC) (freshly produced by our lab) was added. The plates were incubated at room temperature for 30 min before observation [30].

For negative staining transmission electron microscopy (TEM), the VLP samples were negatively stained with 1% phosphotungstic acid and dried by aspiration on a copper gird. VLPs were visualized with a Hitachi H-7650 transmission electron microscope (Hitachi Ltd, Hitachi, Japan). For immune electron microscopy (IEM) observation, VLPs were pre-incubated with mouse anti-NDV F monoclonal antibody (provided by Dr. Guo from the Henan Academy of Agricultural Sciences) or mouse anti-NDV HN monoclonal antibody (generated and stored by our lab), and gold-labelled (10 nm) anti-mouse IgG antibody (Sigma, St Louis, MO, USA) before phosphotungstic acid staining.

2.3. Immunization and challenge in commercial chickens

NDV VLPs based vaccine (NDV VLPs+Alum) was formulated with the alum adjuvant. Briefly, the purified VLPs were solved 0.1% formaldehyde at 37 °C for 24 h to inactivate the recombinant baculoviruses, which were confirmed by Sf9 infection. Protocol to mix VLPs with the alum adjuvant was performed as the manufacturer's instructions (Thermo Fisher Scientific, Waltham, MA, USA). The commercial vaccine (formulated with inactivated NDV LaSota strain) was purchased from the Shandong HuaHong Biological Products Co., LTD (Veterinary drug approval number, 2013-150102008).

48 one-day-old commercial Hy-line Variety Brown chickens (purchased from the Jilin Academy of Agricultural Sciences, China) were randomly separated into four groups. The maternal antibody level against NDV was monitored through hemagglutination inhibition assay (HI), as described previously [30]. At day 21, when all the HI titers were lower than $2Log_2$, chickens were randomly divided into four groups (n = 12). Different groups were immunized with NDV VLPs (256 HAU/0.2 mL), NDV VLPs+Alum (256 HAU/0.2 mL), commercial vaccine (256 HAU/0.2 mL, equivalent to 2×10^8 EID_{50}/0.2 mL), or PBS through subcutaneous (s.c.) injection. Boost was administered seven weeks later with the antigens same to the primary immunization. Nine weeks after boost, all the

groups were intranasally (i.n.) challenged with 1×10^6 ELD_{50} of virulent NDV NA-1 strain (genotype VII) in 0.1 mL. During the experiment, the chickens were kept at a formal breeding condition which mimics normal poultry farms in China.

2.4. Serological tests and virus isolation

After the primary vaccination, the blood samples were collected weekly via the wing vein to monitor serum NDV antibody level by HI assay using 1% chicken red blood cell (RBC) with 4 hemagglutination units (HAU) of standard NDV antigen (Weike biotechnology Co. Ltd, Harbin, China) as described [30].

After challenge, the chickens were monitored for clinical signs, body weight change and mortality for 14 days. Oropharyngeal and cloacal swabs were collected at 2, 4, 7 and 10 days post-challenge (dpc) for the detection of virus shedding.

2.5. Examination of virus shedding

The swabs were incubated to nine-day-old embryonated chicken eggs to confirm the existence of virus through a real-time polymerase chain reaction (RT-PCR). In detail, swab samples were suspended in 1 mL of sterilized RNA-free PBS supplemented with 1000 units of penicillin and 1000 units of streptomycin. The samples were then centrifuged at 4000× *g* for 5 min. 250 μL supernatant was used for RNA extraction through a RNeasy Mini Kit (QIAGEN, Dusseldorf, Germany) and went through reverse transcription (Promega, Madison, WI, USA). For virus quantification, RT-PCR was performed under the following conditions: 42 °C 5 min; 95 °C 10 s, 56 °C 30 s for 40 cycles. The primers and probe were as follows: NDV F 5′-CAYTGACYACTTTGCTCA-3′, NDV R 5′-GCATTYTGRTTGGCTTGT-3′, probe 5′ FAM-CACCTATAAAGCGTTTYTGYCTCCT-BHQ-3′. A standard curve was established with quantified viral RNA extracted from pure NDV NA-1 virus. Ct values higher than 35 were counted as positive, the system was developed by our lab previously (data unpublished).

2.6. Detection of immunohistochemistry

Immunohistochemistry (IHC) detection of viral antigen in chicken tissues was also performed. At 5 dpc, three chickens of each group were sacrificed for the collection of lungs and brains. The IHC assay was performed with a SABC IHC staining kit (BOSTER, Wuhan, China), using 1:250 dilution of mouse anti-NDV nucleoprotein (NP) protein antibodies (stored by our lab) as primary antibody and goat anti-mouse IgG (EarthOx, Millbrae, CA, USA) as secondary antibody.

2.7. Statistical analysis

The frequencies of virus isolation among immunized groups were analyzed by Fisher's exact test. Virus titers of the swabs were analyzed by one-way ANOVA with Student-Newman-Keuls multiple comparison test. A *P*-value <0.05 was defined as statistically significant relationship.

2.8. Ethics statement

Animals were treated humanely and with regard for alleviation of suffering. All animal experiments were carried out following the experimental practices and standards approved by the Animal Welfare and Research Ethics Committee at Jilin University.

3. Results

3.1. Generation and identification of recombinant baculovirus

To obtain the rBVs, codon-optimized sequences of M, F and HN genes of NDV NA-1 strain were respectively cloned into the pFastBac1 vector surrounded by 5′ polyhedron promoter (PH) and 3′ transcription termination signal p(A) (Fig. 1a). After confirmed by gene sequencing, recombinant Bacmids were respectively transfected into Sf9 cells and generated recombinant baculovirus (rBVs), including rBV-M, rBV-F and rBV-HN. After passaged three times, the titers of rBV-M, rBV-F and rBV-HN reached 6.0×10^7, 1.0×10^8 and 8.8×10^7 infectious units (IFU)/mL. To confirm the expression of F, HN and M protein in Sf9, immunofluorescence assay was performed. The result showed that the three structural proteins were correctly expressed when Sf9 infected with rBVs at MOI = 1, indicated by strong fluorescence. However, the expression of M protein was somewhat weaker than those of F protein and HN protein, indicated by slightly lower green fluorescence density (Fig. 1b–e).

3.2. Production and identification of NDV VLPs assembled from insect cells

To produce NDV VLPs, Sf9 cells were co-infected with rBV-M, rBV-F and rBV-HN at a ratio of 5:4:3. The supernatant of co-infected Sf9 cells were processed and purified as described above. The white layer at the interface between 40% and 60% sucrose concentration was collected (Fig. 2a). TEM result showed that NDV VLPs exhibited typical paramyxovirus structure. The size of VLPs ranged from 100 to 300 nm and the surface peplomers and the spikes were readily observable (Fig. 2b). The presence of M, F and HN proteins in NDV VLPs was detected by western blotting with chicken anti-NDV serum, showing expected molecular weight of 38 kDa (M), 55 kDa (F) and 70 kDa (HN) (Fig. 2c). Further IEM observation showed a more denser gold labels on the surface of NDV VLP than live NDV particles, suggesting a higher F and HN insertion efficacy on NDV VLPs (Fig. 2d, e). Functional incorporation of HN protein was then confirmed by HA assay. The final HA titers of purified NDV VLPs were 12log2, proving the incorporated HN protein retained its hemagglutination activity. These results showed that NDV M, F and HN proteins could be assembled into VLPs.

3.3. Immunogenicity effect of NDV VLPs

The immunogenicity of NDV VLPs were tested in commercial chickens. The serum samples were collected weekly to monitor NDV antibody level through HI assay. No behavioral change was noticed in any groups and no local reactions were observed at the vaccine injection sites. The results showed that the NDV antibody level of NDV VLPs, NDV VLPs+Alum and commercial vaccine groups reached their peaks at two (HI = 5log2), three (HI = 6log2) and four (HI = 7.5log2) weeks post primary vaccination, respectively. The levels gradually declined afterwards (Fig. 3). As the serum antibody baseline for protection against NDV was 4 log_2, the effective protection period was also calculated [30]. It showed that the protection period after the primary immunization of NDV VLPs+Alum was the longest (4 weeks), while that of commercial vaccine and NDV VLPs groups were 3 weeks and 2 weeks, respectively. To compare the efficiency of a single immunization of different formulations, boost was administered when the HI value dropped below 4log2, namely week 7 after primary immunization. After boost, the antibody levels soon increased in one week, with NDV VLPs group being the fast but sustained the shortest

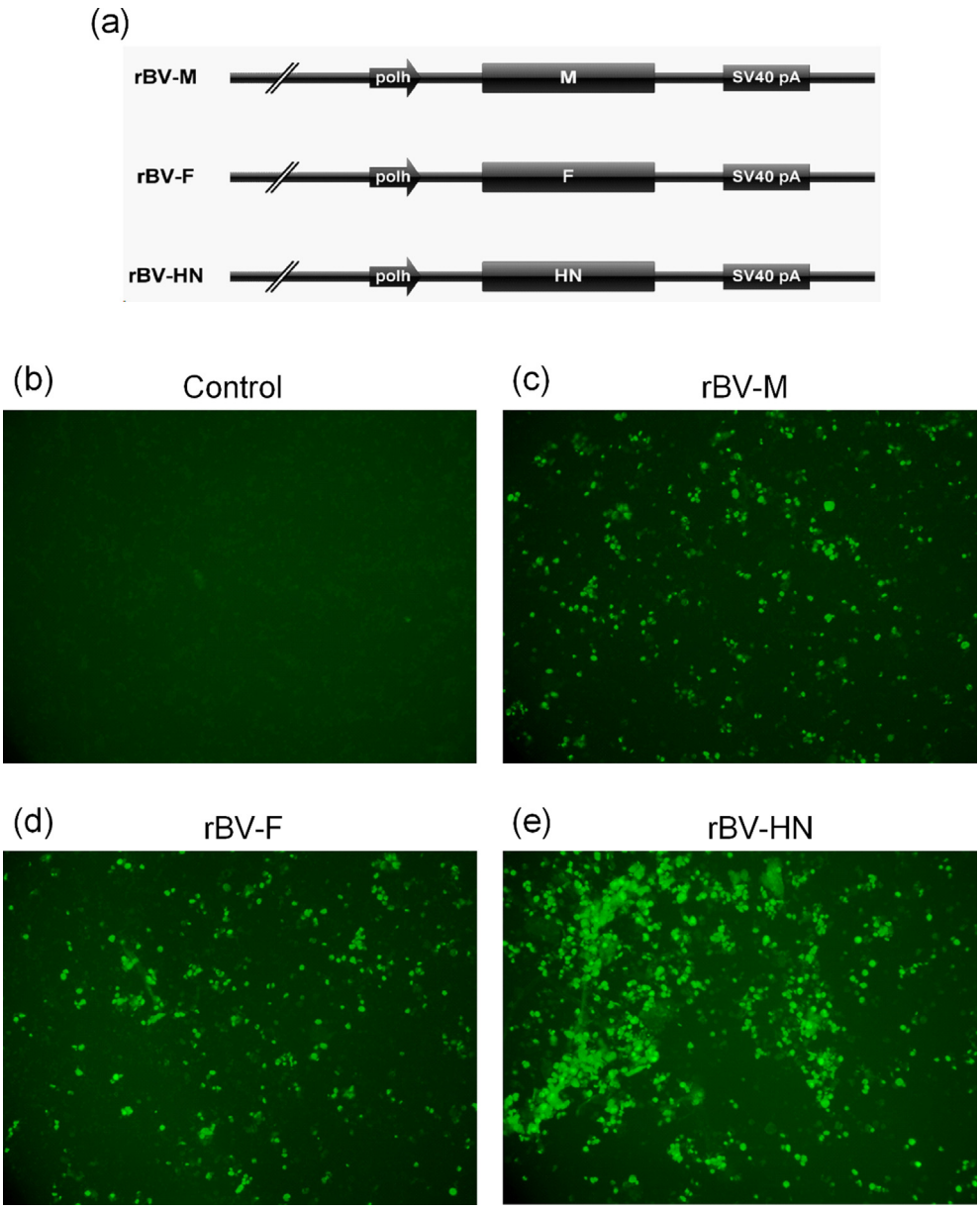

Fig. 1. (a) *Construction scheme of rBVs.* Condon optimized NDV NA-1 strain M, F and HN gene were respectively inserted between polh and SV40 pA. (b), (c), (d) and (e) *IFA observation of rBV infected Sf9 cells.* Sf9 cells were separately incubated with wild type BV (b), rBV-M (c), rBV-F (d) and rBV-HN (e) and incubated with anti-NDV polyclonal antibodies as primary antibody.

(4 weeks). NDV VLPs+Alum group came to be the second fast, while its protection period was 8.5 weeks, significantly longer than commercial vaccine group. In general, NDV VLPs group showed the fastest antibody increase with the shortest protection period while NDV VLPs+Alum group resulted with the second fast antibody response, highest HI level and the longest protection period both after the primary and second immunization. Additionally, PBS (Control) group kept the HI titer of 0–1log2 throughout the experimental period.

3.4. NDV VLPs conferred protection against NA-1 strain challenge

To assess the protective efficacy, all the groups were challenged nine weeks after boost with a lethal dose of NDV NA-1 strain intranasally. Clinical signs, mortality and body weight change were observed for 14 days. All the chickens immunized with NDV VLPs, NDV VLPs+Alum or commercial vaccines survived to the end of the experiment (Fig. 4a). As to body weight change, chickens of NDV VLPs+Alum and commercial vaccine groups experienced normal bodyweight increase, while chickens of NDV VLPs group showed a temporary weight loss 1–5 dpc but it soon bounced back (Fig. 4b). In the meanwhile, PBS group showed a significant loss in bodyweight and exhibited severe clinical signs from 1dpc, and soon succumbed to virus infection within 6 dpc (Fig. 4b).

Other aspects that can reflect the vaccine efficacy were virus shedding, lung tissue pathology and virus load. Lung tissue samples collected 5 dpc were examined through IHC for the existence of NDV nucleoprotein (NP). The result showed that PBS group was obviously positive for NP and commercial vaccine group were observed with low level of viral NP, while samples of VLPs+Alum group were negative (Fig. 4c–e).

Virus shedding from the oropharynx and cloaca is the main source for the spread of NDV among the flocks. Oropharyngeal and cloacal swaps were collected 3, 5, 7 and 10dpc for the detec-

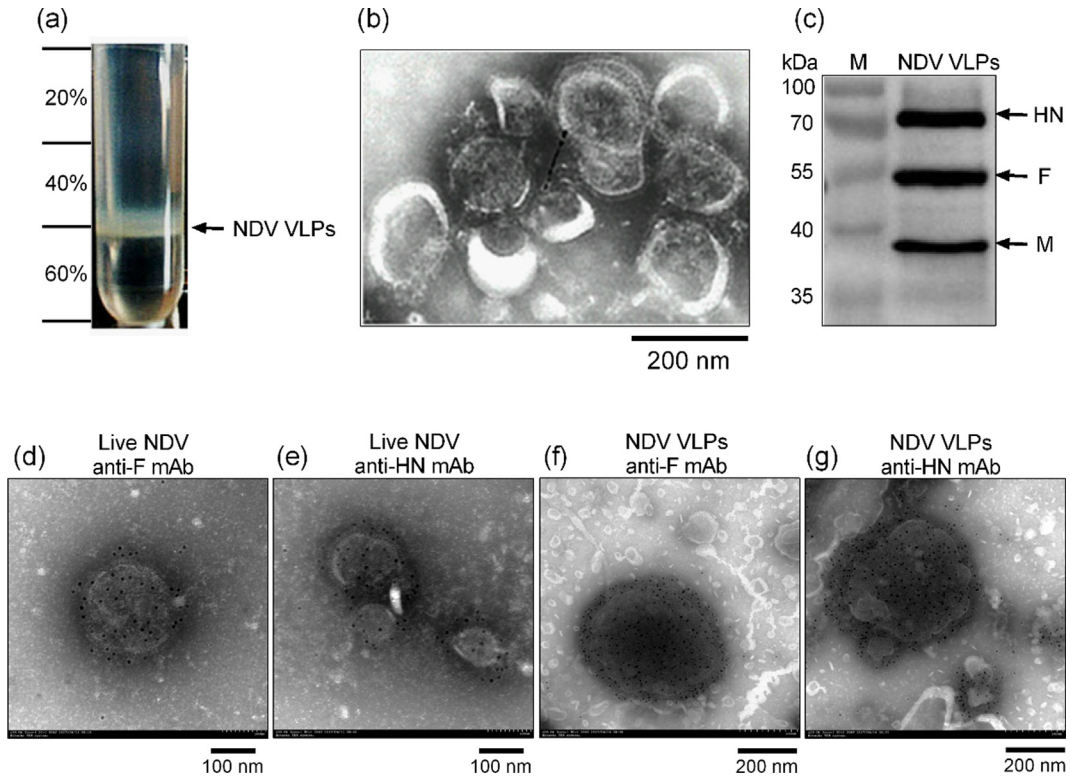

Fig. 2. (a) *Observation of NDV VLPs after sucrose density gradient separation.* The white layer between the 40% and 60% part was the expected condensed NDV VLPs. (b) TEM observation of NDV VLPs, showing a typical paramyxovirus spherical shape. (c) Western blotting detection of M, F and HN protein co-inserted on NDV VLPs with anti-NDV polyclonal antibody. (d) and (e) *IEM observation of live NDV particles.* NDV particles respectively incubated with anti-F antibody (d) and anti-HN antibody (e). (f) and (g) *IEM observation of NDV VLPs.* NDV VLPs particles respectively incubated with anti-F antibody (d) and anti-HN antibody (e).

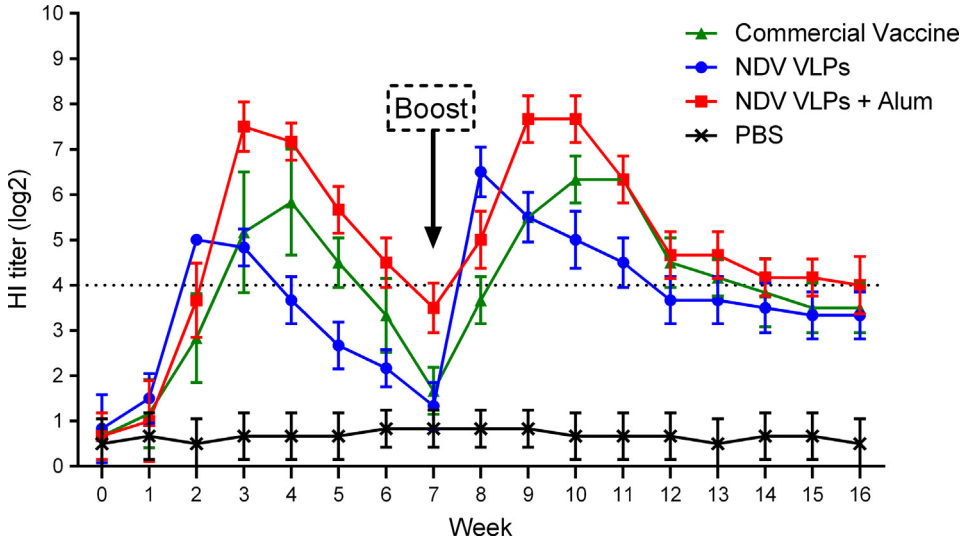

Fig. 3. *Serum antibody level change after immunization.* Serum HI antibody levels of different groups (PBS, NDV VLPs and NDV VLPs+Alum) were monitored for 16 weeks after primary immunization.

tion and quantification of NDV NA-1 strain. Results showed that all the samples collected from the control group were positive for NDV at 3 and 5dpc (Tables 1 and 2). Virus shedding of commercial vaccine group continued for at least 7 dpc, while that of NDV VLPs +Alum and NDV VLPs stopped at 5 dpc. In addition, the NDV VLPs +Alum group exhibited lower positive rate than NDV VLPs group (Tables 1 and 2). Therefore, NDV VLPs+Alum group produced to the shortest virus shedding both oral and cloacal swabs among the three groups while commercial vaccine group showed longest virus shedding.

4. Discussion

The worldwide circulation of NDV caused great economic loss in poultry industry. Vaccination is mandatorily performed in several countries and has successfully reduced epizootic outbreaks [2,13,14]. However, the most commonly used genotype I, II or III strain NDV vaccines (Ulster/67, LaSota, B1 or Mukteswar) cannot offer full protection against the virulent genotype VII strain attack, inducing atypical clinical signs, persistent virus existence and continuous virus shedding in the flock. It poses potential danger to

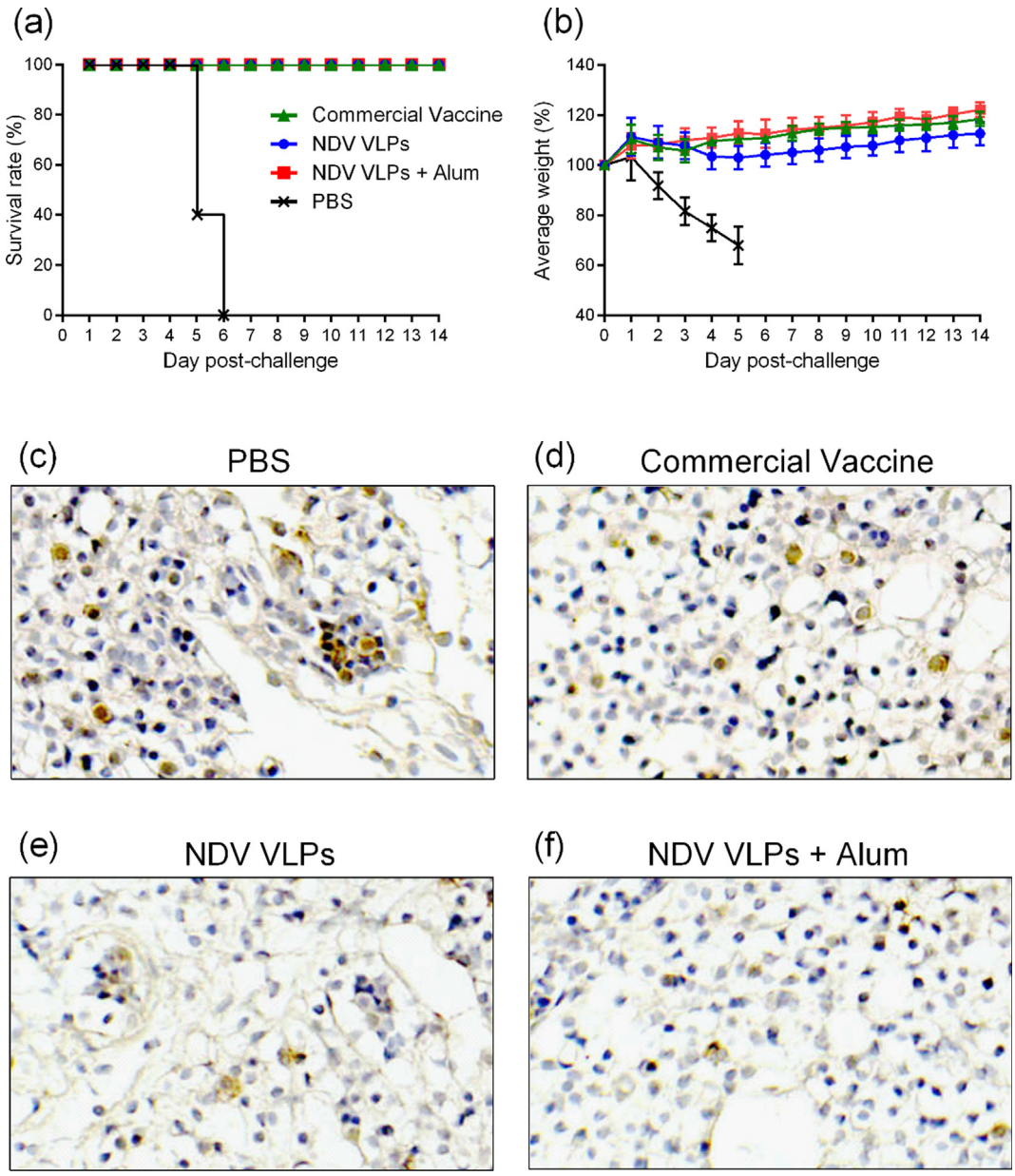

Fig. 4. (a) *Survival rate after NA-1 strain challenge.* (b) *BW change after NA-1 strain challenge.* (c), (d), (e) and (f) Lung tissue virus loading of different groups (PBS, NDV VLPs and NDV VLPs+Alum) detected with anti-NP antibodies.

Table 1
Isolation of virus from oropharyngeal and cloaca route after challenge.

Group	Oropharyngeal route				Cloaca route			
	3 dpc	5 dpc	7 dpc	10 dpc	3 dpc	5 dpc	7 dpc	10 dpc
PBS	12/12	12/12	NA	NA	12/12	12/12	NA	NA
Commercial vaccine	8/12	5/12	3/12	0/12	4/12	7/12	3/12	1/12
NDV VLPs	5/12	3/12	0/12	0/12	2/12	4/12	2/12	0/12
NDV VLPs+Alum	2/12	1/12	0/12	0/12	2/12	2/12	0/12	0/12

NA represents all death of experimental birds.

poultry industry [12–16]. There is one genotype VII matched attenuated vaccine developed by Prof. Liu's team, which can provide sufficient protection against the prevalent virulent NDV strain invasion [13]. However, all these vaccines mentioned above were produced through embryonated chicken eggs or avian source cell lines. The system can potentially be sabotaged by several vertical transmitted avian pathogens, like CIAV and FAdV as reported in LaSota vaccine production [20,21]. The Bac-to-Bac production system for NDV VLPs is totally insect origin, making it a proper solution as the alternative to avian production system. In addition to its guaranteed safety, the NA-1 strain used in our research was isolated from diseased geese flock. A previous study showed that

Table 2
RT-PCR detection of virus from oropharyngeal and cloaca route after challenge.

Group	Oropharyngeal route				Cloaca route			
	3 dpc	5 dpc	7 dpc	10 dpc	3 dpc	5 dpc	7 dpc	10 dpc
PBS	12/12	12/12	NA	NA	12/12	12/12	NA	NA
Commercial vaccine	9/12	6/12	3/12	0/12	5/12	8/12	4/12	2/12
NDV VLPs	6/12	2/12	0/12	0/12	3/12	5/12	0/12	0/12
NDV VLPs+Alum	3/12	1/12	0/12	0/12	2/12	3/12	0/12	0/12

NA represents all death of experimental birds.

the inactivated oil emulsion NA-1 vaccine can effectively protect chickens, as well as geese against homologous NDV strain challenge and significantly reduce virus shedding [15]. Therefore, the insect cell line produced, genotype VII NA-1 strain based NDV VLPs is a promising vaccine candidate for the control of NDV in both chicken and waterfowl flocks.

The most widely used *E. coli* VLP production system was not a proper option for NDV VLPs due to its deficiency in cell membrane and inadequate glycosylation to eukaryotic proteins [23,24,28]. To overcome such drawbacks, the Bac-to-Bac insect system was introduced. McGinnes et al. found M protein was the core for the assembly of VLPs and they also proved the biological activity of NDV VLPs [24,25]. Shen et al. generated an avian influenza virus M1 based VLP with the insertion of NDV HN protein through this system [26]. Park et al. also proposed AIV M1 as a core protein to produce VLPs containing NDV F protein, and assessed its protection efficacy in SPF chickens [27]. However, a total NDV based NDV VLPs may have the advantage for mass production for its high assembly rate [24]. As expected, NDV VLPs produced in our research possessed several biological similarities with live NDV particles and even denser surface antigen expression as observed through IEM, implying its effectivity in immune stimulating process.

To test its practical immunogenicity and protection length after a single administration, the second immunization was performed when the serum HI antibody level dropped below 4log2. It was found that non-adjuvant NDV VLPs initiated the fastest antibody secretion but sustained the shortest time (2 weeks) among the three groups, which may be attributed to its strong DC activating ability. The assistance of aluminum adjuvant slightly delayed its antibody response speed but efficiently prolonged its protection period to four weeks, while that of commercial vaccine was one week shorter. After boost, the protection length of NDV VLPs+Alum group was extended to 16 weeks, which time point coincided with the start of egg-laying maturity for hens. In terms of protection efficacy, both NDV VLPs and NDV VLPs+Alum groups exhibited a reduced virus shedding period (5 dpc), while that of commercial vaccine group continued to 10 dpc (Tables 1 and 2). The data obtained from two detection method showed a similar trend. Commercial vaccine group produced longer shedding period, while the results of RT-PCR detection were with higher NDV positive rates (Tables 1 and 2). The pattern of virus shedding also applied to virus loading in lung tissue. Namely, NDV VLPs+Alum group was with the least NDV NP protein detection, while commercial vaccine resulted with the most detectable NDV. Taking advantage of its prolonged protection period, sufficient protection rate and assured safety, NDV VLPs emphasized its potential as an appealing alternative to current live strain as primary immunization in practice to eliminate NDV.

5. Conclusions

In summary, the causative agent of recent ND outbreaks in vaccinated chickens from China was found to belong to velogenic genotype VII. Therefore, we developed a genotype-matched NDV VLPs vaccine candidate using the baculovirus vector expression system. Our results demonstrated that NDV VLPs alone or in combination with alum adjuvant was sufficient to protect commercial chickens from lethal challenge. In addition, the shedding and virus load in lung tissue were significantly reduced compared to that of inactivated LaSota vaccine vaccinated chickens, indicating that NDV VLPs are promising vaccine candidates for the control of genotype VII velogenic NDV in the field.

Conflict of interest

The authors have declared that no competing interests exist.

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