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Effect of Different Routes of Inoculation on Plant-Derived VP2 Immunogenicity and Ability to Confer Protection Against Infectious Bursal Disease

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Abstract

Infectious Bursal Disease Virus (IBDV) is the etiological agent of an immunosuppressive and highly contagious disease that affects young birds causing important economic losses in the poultry industry. The structural protein VP2 has been used for the development of subunit vaccines in a variety of heterologous platforms. We have previously demonstrated that plant-derived VP2 (pVP2) is able to elicit a neutralizing antibody response in chickens when administered intramuscularly (i.m.) in a prime/boost scheme. However, administration via injection is impractical and carries the risk of needle stick injury or pain. Mucosal vaccination is noninvasive and has several advantages over traditional systemic vaccines. Taking this into account and the fact that natural infections with IBDV occur by the oral route, we decided to investigate whether pVP2 was also immunogenic when given intranasally (i.n.) or orally to chickens. In addition, we evaluated if intramuscular vaccination with VP2 plant extract in a more welfare-friendly scheme with less injections and without adjuvant was able to elicit a protective immune response against IBDV as previously seen. We determined that animals inoculated i.m., but not i.n., with the experimental vaccine developed high titres of specific antibodies, with virus neutralizing activity. Also, bursae of animals vaccinated i.m. with pVP2 presented few infiltrating T cells, low viral charge and normal morphology. However, chickens that received the immunogen via nasal or oral route were not protected after challenge. Considering the disadvantages of conventional live-attenuated and inactivated vaccines, a plant-based subunit vaccine represents a viable alternative in the veterinary field. Once again pVP2 has proven to be immunogenic when parentally inoculated. However, further investigations need to be done in order to find an alternative route of administration which is more practical than the intramuscular injection and capable of eliciting a mucosal immune response

Keywords

Infectious Bursal Disease; VP2; Plant based vaccine

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Introduction

Infectious Bursal Disease is an acute, highly contagious, immunosuppressive disease that affects young birds causing important economic losses in the poultry industry worldwide. Its etiological agent is the Infectious Bursal Disease Virus (IBDV), a non-enveloped icosahedral bisegmented double-stranded RNA virus, member of the Birnaviridae Family [1]. IBDV infects and destroys IgMbearing B-lymphocytes in the bursa of Fabricius (BF), which results in immunosuppression [2,3] and T cells infiltration into this organ [4].

Currently, vaccination with inactivated and live-attenuated vaccines induces immunity in the flock against virulent viruses. However, conventional vaccines have a number of disadvantages because of their viral nature. For instance, live-attenuated vaccines can revert to virulence by recombination of RNA segments [5], they usually produce a temporary state of immunosuppression in young chickens, and they can be inefficient in protecting birds from very virulent and variant IBDV strains [6,7]. Moreover, inactivated vaccines are costly and lack efficient immunogenicity unless they are adjuvated and administered in multiple inoculations, or delivered as a booster after priming with a replicating antigen. Thus, there is a genuine need to replace conventional virus-based vaccines by new ones with higher efficacy and fewer side-effects. The structural protein VP2, which contains the major neutralizing epitopes, has been used for the development of subunit vaccines in a variety of heterologous systems such as recombinant fowlpoxvirus [8], herpesvirus [9-11], adenovirus [12,13], baculovirus [14,15], *Escherichia coli* [16], *Pichia pastoris* [17] and plant virus [18]. In addition, DNA vaccines have been obtained [19, 20] and VP2 production and immunogenicity have been reported in transgenic *Arabidopsis thaliana* [21] and rice [22].

Since the past two decades plants have been considered a promising system to produce subunit vaccines given that they offer significant advantages over conventional expression systems, such as time and cost efficiency, lower risk of contamination from animal pathogens and nearly-unlimited scalability [23]. Furthermore, it is well documented that antigens expressed *in planta* are capable of inducing protective response when administered by oral or parenteral routes. For these reasons, the technology to produce recombinant vaccines in plant cells has evolved from modest proofs of concept to viable technologies adopted by some companies [24].

In a previous study, we investigated the expression, immunogenicity and protective efficacy of a plant-based VP2 (pVP2) vaccine against IBDV [25]. We determined that agroinfiltration of *N.benthamiana* leaves allowed the production of VP2 and that chickens intramuscularly immunized in a 3 doses scheme with adjuvated concentrated plant extract developed a specific humoral response with viral neutralizing capacity. We also demonstrated that pVP2 had the ability to prevent T-cell infiltration into the bursa as a parameter of protection against an infectious virus. However, administration via injection is impractical and carries the risk of needle stick injury or pain. Mucosal vaccination is non-invasive and does not involve the use of needles. Moreover, mucosal administration of vaccines is relatively easy and does not require specialized personnel.

Reduced adverse effects and the potential for frequent boosting may also represent further advantages over injectable vaccines [26].

Taking this into account and the fact that simultaneous stimulation of mucosal IgA and systemic IgG responses would be desirable for an anti IBDV vaccine since natural infections occur by the oral route [27], we decided to investigate whether pVP2 was also immunogenic when given intranasally or orally to chickens. Additionally, we evaluated if intramuscular vaccination with VP2 plant extract in a more welfare-friendly prime/boost scheme with less injections and without adjuvant was able to elicit a protective immune response against IBDV as previously seen.

Materials and Methods

Genetic engineering of the expression vector

Construction of the plant expression vector containing VP2 was described previously [25]. Briefly, the coding region of the mature VP2 (1323 bp) was amplified from the Argentinian field isolate LD-04 of IBDV kindly provided by Dr. Delamer (Empresa Delamer S.R.L., Argentina), cloned into the commercial 1.1tag vector and subcloned into the binary vector pBINPLUS (IMPACTVECTORTM, Wageningen UR, Netherlands). The resulting expression vector was introduced into *A. tumefaciens* strain GV3101 by electroporation.

Transient expression of VP2

Transient expression was performed by infiltrating *Nicotiana benthamiana* leaves with a suspension of recombinant bacteria as previously described [28]. A construction harboring the green fluorescent protein (GFP) was added as a negative control. The infiltrated leaves were harvested 4 days postinoculation and grounded in liquid nitrogen. Subsequently, 3 volumes of chilled extraction buffer (100 mM Sodium acetate pH 4, 1 mM EDTA, 5% glycerol, 0.5% NP40 and protease inhibitor cocktail (Roche, Mannheim, Germany)) were added. After 30 min incubation on ice, samples were centrifuged for 30 min at 20000×g and filtered through gauze. Samples pH were raised to 7 for animal inoculation and kept at −80°C until use.

Detection and quantification of the recombinant protein

VP2 expression was analyzed by Western blot assays. Briefly, extracted proteins were separated in 12% SDS-PAGE and blotted onto nitrocellulose membrane. Proteins were identified using an antiVP2 rabbit polyclonal antibody produced in our laboratory. For protein quantification, we performed a standard curve of bovine serum albumin (BSA). BSA and samples were subjected to SDS-PAGE and VP2 amounts were estimated after Coomassie Brilliant Blue staining.

Animals

Embryonated eggs laid by specific pathogen free White Leghorn hens were purchased from Instituto Rosenbusch S.A. (CABA, Argentina) and hatched in an automatic incubator (Yonar, CABA, Argentina). Chickens were kept in individual cages with food and water *ad libitum*. All procedures were approved by the Institutional Committee for the Care and Use of Experimental Animals (CICUAE–CICVyA–INTA).

Immunization scheme and challenge

Nine chickens of 21 days of age were randomly assigned to each group. Animals received 250 µl of a plant extract containing approximately 7.5 µg of VP2 via intramuscular (i.m.) (group 1), intranasal (i.n.) (group 2) or oral (group 3) administration. Control groups (4, 5 and 6) received plant extract containing GFP (pGFP) as a non-related antigen by i.m., i.n. and oral route respectively. Fourteen days post first immunization (dpi) chickens were boosted following the same scheme. All animals were bled by the wing vein every eleven days. Three weeks after boost (35 dpi), 6 out of 9 chickens per group were challenged by oral inoculation with 500 µl of the intermediate IBDV strain LZD (6934 $TCID_{50}$/ml) purchased from Laboratorios Inmuner (Entre Ríos, Argentina). 3 chickens of each group were left unchallenged. Five days later (40 dpi) animals were euthanized and bursae were removed.

Antibody response against IBDV

Sera were evaluated for the presence of specific antibodies against IBDV with a commercial kit (cat No. 99-09260, IDEXX Laboratories, Inc., USA). Titers were calculated following the manufacturer's instructions. Values above 396 were considered positive.

Seroneutralization assay

Seroneutralization assay was performed as previously described [25]. Briefly, sera were inactivated for 30 min at 56 °C, serially diluted twofold in culture medium and incubated with 100 $TCID_{50}$ of IBDV strain LZD for 1 h at 37 °C in 96-well plates. Subsequently, 100 µl of a cell suspension of 1.5×10^6 chicken embryo fibroblasts (CEFs)/ml were added to each well. Cells were cultured at 37°C, % CO_2 for 4 days, when cytopathic effect was observed. Neutralizing antibody titers were calculated as the inverse of the last dilution showing no cytopathic effect.

Lymphocyte isolation and flow cytometry analysis

Bursal samples were pooled (in pairs in the case of challenged animals or in threes for unchallenged animals) and used to study mononuclear cell populations by flow cytometry as described [29]. Briefly, bursae were mechanically disrupted in RPMI 1640 and cellular suspensions were passed through a 40 µm mesh (Cell Strainer, BD). Mononuclear cells were isolated by centrifugation over Histopaque density gradient. 1×10^6 cells per well were seeded on 96-well plates and stained with different combinations of antibodies. Monoclonal antibodies (mAbs) (CD3-SPRD, CD4-PE, CD8α-FITC, CD8β-PE) were purchased from Southern Biotech. (Birmingham, AL). Cell suspensions were analyzed with a FACS Calibur flow cytometer (BD Biosciences, San Jose, CA) and CellQuest software. The lymphocyte gate was defined by the forward/side scatter characteristics of the cells and 50,000 events were analyzed for each sample.

IBDV isolation from bursa

Pieces of bursae were mechanically disrupted in one volume of PBS and 3 frost/thaw cycles were performed. Homogenates were ten-fold serially diluted in culture medium (199 1X supplemented with triptose phosphate broth, HEPES 25 mM, pH 7) and used to infect CEFs monolayers seeded in 96-well plates. After adsorption for 1 hour at 37°C, virus was removed and fresh medium (199 1X supplemented with triptose phosphate broth, HEPES, 1.5% Fetal Bovine Serum and antibiotic/antimycotics) was added. After 4 days at 37°C, 5% CO_2, the presence of cytopathic effect was evaluated in each well. Viral titer was expressed as $TCID_{50}$/ml using the Reed and Muench method.

Histopathological observation of bursa

Bursal samples were placed in 10% neutral buffered formalin and paraffin embedded. Sections of the paraffin embedded BF were stained with haematoxylin and eosin following standard histological

procedures. The stained sections were microscopically examined for the presence of bursal lesions by light microscopy.

Results

Immunogenicity of plant-derived VP2 in SPF chickens

Before performing chicken experiments, expression of recombinant VP2 in plant extracts was confirmed by Western blot. As shown in Figure 1 a specific band corresponding to the mature VP2 was observed at the expected size. The estimated concentration of VP2 antigen in the plant extract was approximately 30 ng/µl.

To evaluate the immunogenicity of plant-derived VP2 by different routes of administration, chickens were inoculated intramuscularly, intranasally or orally with 250 µl of plant extract containing 7.5 µg of recombinant VP2 in a prime/boost scheme. Sera were analyzed for the presence of specific antibodies against IBDV using a commercial ELISA assay. Figure 2 shows that all animals i.m. injected with pVP2 (group 1, Figure 2B) mounted a humoral response detected at 21 dpi (one week after boost), reaching its highest titers by the end of the experiment. However, animals i.n. (group 2) or orally (group 3) vaccinated with the same immunogen failed to generate specific antibodies against IBDV. As expected, control groups that received plant extract containing GFP (groups 4, 5 and 6) had undetectable levels of antibodies independently of the inoculation route (Figure 2A).

Furthermore, antibodies against IBDV present in group 1 exhibited virus neutralizing ability. Figure 3 shows the neutralizing titers expressed as the $\log_{10}$ of the inverse of the last dilution without cytopathic effect of samples corresponding to 32 dpi (18 days after boost). Sera from other groups were not evaluated given that specific antibodies against IBDV were not detected.These results indicate that VP2 produced in plants is able to elicit an appropriate humoral response in chickens when administered intramuscularly but not intranasally or orally.

Evaluation of cellular populations by flow cytometry

After infection, IBDV replication in the bursa involves an infiltration of T cells into this organ [4]. Hence, we investigated the frequency of T cells in the bursa of vaccinated animals after challenge with a high dose of an intermediate IBDV strain to determine if the inoculation of pVP2 through different routes was able to elicit a protective immune response. Results are shown in Figure 4 and they are expressed as the fold increase of each sample normalized with the mean value of unchallenged chickens. Animals i.m. inoculated with pVP2 (group 1) showed less T lymphocyte infiltration (from a 1.3 to a 2.6 fold increase) than chickens from group 2 and 3 vaccinated with the same immunogen by the intranasal and oral route respectively, in which fold increase varied from 6.7 to 42.8 times. Within the infiltrating $CD3^+$ lymphocytes, most of them corresponded to $CD8\alpha\beta^+$ cells. As expected, pGFP groups showed elevated numbers of infiltrating T lymphocytes.

Morphological and histological observation of bursa

Bursae from animals immunized i.m. with pVP2 had no gross lesions and exhibited a normal morphology. However, bursae from chickens in groups 2 and 3 displayed a yellowish appearance and haemorrhages, typical signs of IBDV infection, as seen in pGFP groups (data not shown). Regarding microscopical observation, bursae from group 1 were, histopathologically comparable to those from animals unchallenged, although some of them contained few apoptotic lymphocytes. On the contrary, the rest of the animals had damaged bursae with different degrees of lymphoid atrophy, necrosis and apoptosis. Edema, congestion and small haemorrhages, together with lymphocyte and heterophile infiltration were also observed in these animals (Figure 5).

Viral isolation

Finally, to evaluate the protective effect of the recombinant vaccine by different routes of inoculation, viral isolation assays from bursae after challenge were performed. Results are shown in Table 1. As expected, IBDV was not detected in unchallenged chickens while high viral titers were observed in bursae from control animals inoculated with pGFP (groups 4, 5 and 6). Animals in group 1 which were i.m. immunized with pVP2 revealed a notable decrease in the viral titer recovered from bursa. In four out of six chickens, IBDV was not detected while the other two animals showed a reduction in the viral titer of approximately 10^5 times. However, chickens i.n or orally inoculated with pVP2 (groups 2 and 3 respectively) had viral titers undistinguishable from control groups.

Taken together, the results evidence that mucosal vaccination with pVP2, in the conditions tested, is unable to generate a protective response that prevents IBDV entrance or replication in bursa.

Discussion

In this study, we investigated if the inoculation of pVP2 through different routes was able to elicit a protective immune response against IBDV. We have previously demonstrated that chickens intramuscularly immunized with adjuvated concentrated plant extract at 0, 22 and 35 dpi developed a specific humoral response with viral neutralizing ability that prevented totally or partially the entrance of IBDV, reducing T-cell infiltration into the bursa [25]. Here we showed that two i.m. injections were enough to elicit a protective immune response with high titers of specific and neutralizing antibodies (Figures 2 and 3). In addition, we observed that this immunization scheme prevented the entrance or replication of IBDV in bursa as evidenced by the low viral titers in this organ (Table 1), as well as T-cell infiltration and bursal damage (Figures 4 and 5). Furthermore,

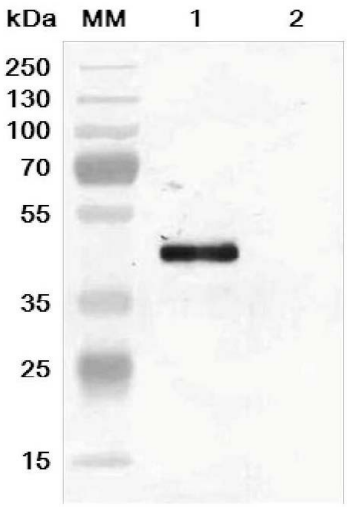

Figure 1: VP2 transient expression in *Nicotiana benthamiana* plants. Proteins were separated on a 12% SDS-PAGE and recombinant VP2 was identified using an anti-VP2 antiserum (lane 1).Control plant extract containing GFP was loaded in lane 2. MM: molecular marker (Page Ruler Plus Prestained Protein Ladder, Thermo Scientific).

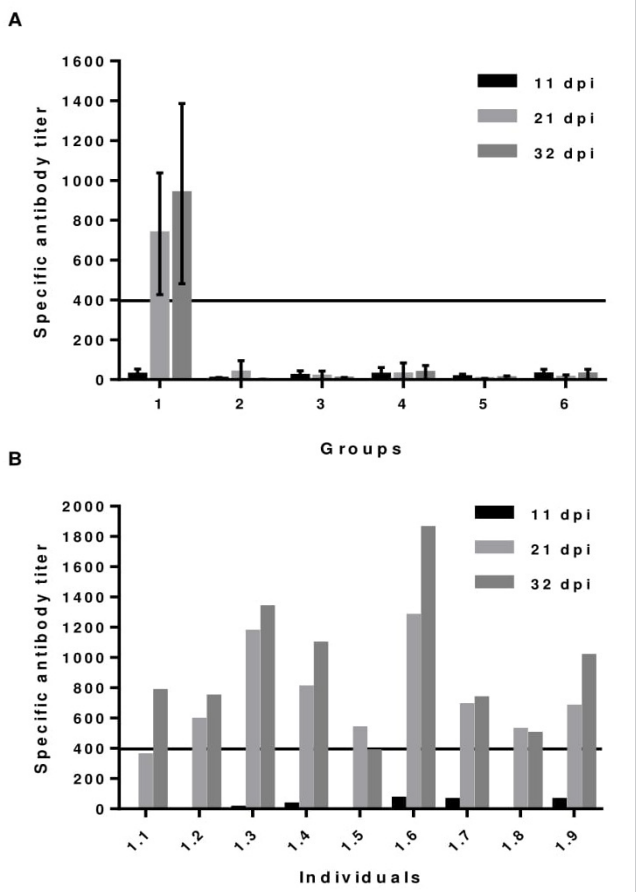

Figure 2: Evaluation of plasma samples by ELISA: Animals were vaccinated on 0 and 14 dpi with plant extracts containing VP2 by i.m. (group 1), i.n. (group 2) or oral (group 3) route. Control groups (4, 5 and 6) were inoculated with plant extract containing GFP using the same routes respectively, A. Anti-IBDV titers represented as the mean ± S.D. for each group and date of the time course. Titers above the cutoff point (396) were considered positive. B. Individual titers for chickens in group 1.

adjuvant was not needed in order to achieve immunoprotection against IBDV. Plant extracts, including *Nicothiana benthamiana's*, have been reported to modulate the monocyte differentiation in dendritic cells and their maturation, although the chemical nature of the compounds responsible for their immunomodulatory properties has yet to be determined [30-32]. Lack of adjuvants, which tend to cause inflammation and ulceration at the site of injection, as well as few inoculations, are desirable characteristics for a vaccine since they imply less distress in the animals. Therefore, we consider that our plant-based VP2 could be a promising alternative to traditional inactivated vaccines which are more expensive to produce and poorly immunogenic unless they are adjuvated and administered in multiple inoculations.

As regarding mucosal vaccination with pVP2, results were less encouraging. Neither intranasal nor oral vaccinations were able to produce an effective immune response in chickens. Specific antibodies were not detected and chickens were not protected from IBDV challenge. Their bursa had elevated viral titers and high T-lymphocyte infiltration which caused important bursal damage.

In all, these animals were undistinguishable from those inoculated with GFP.VP2 has been assessed as an immunogen by the oral route in previous investigations. Five oral doses at 3 days intervals of soluble VP2 expressed in *A. thaliana* (11.44 µg of VP2 in total) induced an antibody response and 80% of protection against challenge [21]. Furthermore, fasted chickens fed with rice seeds expressing VP2 (between 5 and 10 mg) produced neutralizing antibodies against IBDV and were protected (83.33%) against challenge [22]. Oral immunization using different strains of yeasts containing VP2 were also investigated with diverse outcomes. Only 10% of chickens administered *Kluyveromyces lactis* expressing VP2 (1-3 mg of recombinant protein in total) in a 2/2/2 scheme (two weeks feeding, two weeks break, two weeks feeding) were protected from depletion of B lymphocytes in the bursal follicles after challenge although no mortality or clinical signs were observed in none of these animals [33]. On the contrary, 4 doses of orally administered *Pichia pastoris* producing VP2, containing 400 µg or 4 mg of viral protein, induced a protective immune response against IBDV in chickens which increased survival rates to 60 % compared to 40% in the control groups [34]. These studies demonstrate that VP2 is resistant to gut degradation, based on the fact that it invoked an immune response. However, the amounts of antigen used in these reports were much higher than the concentration evaluated in our study, so we do not discard the possibility that larger mass of pVP2 or addition of an oral adjuvant [27,34-36] could be needed to elicit an immune response by the oral route. Protein concentration in our plant extract has always been a limiting factor; therefore we are looking into ways to improve VP2 expression and/or recovery.

Intranasal or oculonasal immunization in chickens is mostly used with live attenuated or vector vaccines [37-39], but there are few studies about protein subunit or inactivated vaccines delivered by this route. Intranasal administration of recombinant outer membrane protein H (rOmpH) expressed in bacteria has been evaluated as a potential fowl cholera vaccine candidate for chickens [40]. One inoculation of 50 µg of rOmpH adjuvated with *Escherichia coli* enterotoxin B or CpG oligodeoxynucleotides mounted IgY and IgA antibody responses and conferred 70% and 90% protection

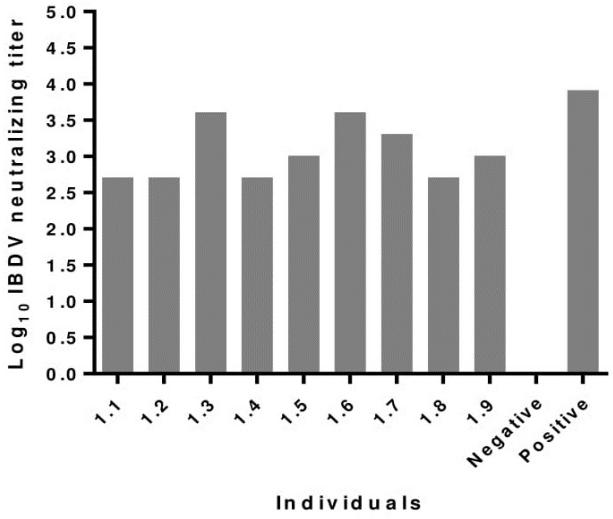

Figure 3: Neutralizing antibody response of animals vaccinated i.m. with VP2 at 32 dpi. Titers are expressed as the $\log_{10}$ of the inverse of the last dilution that prevented the appearance of cytopathic effect for individual samples. Negative control: serum from an unvaccinated animal, Positive control: serum from an animal inoculated with a commercial live attenuated vaccine.

Table 1: Viral isolation from bursa after challenge: Pieces of bursa from group 1 (i.m. pVP2), group 2 (i.n. pVP2), group 3 (oral pVP2), group 4 (i.m. pGFP), group 5 (i.n. pGFP) and group 6 (oral pGFP) were mechanically disrupted in PBS and 3 frost/thaw cycles were performed. Homogenates were ten-fold serially diluted and the dilutions were used to infect monolayers of chicken embryo fibroblasts seeded in 96-well plates. After 4 days at 37 °C, the presence of cytophatic effect was evaluated in each well. Viral titer was e×pressed as $TCID_{50}$/ml, using the Reed and Muench method. Neg: negative results correspond to a viral titer lower than 1.26×10^3 $TCID_{50}$/ml.

Sample	$TCID_{50}$ / ml
1.1	Neg
1.2	Neg
1.3	Neg
1.4	1.86×10^4
1.5	Neg
1.6	1.26×10^4
2.1	4.00×10^7
2.2	2.7×10^7
2.3	$> 1.26 \times 10^9$
2.4	8.6×10^8
2.5	$> 1.26 \times 10^9$
2.6	8.6×10^8
3.1	$> 1.26 \times 10^9$
3.2	1.26×10^8
3.3	6.80×10^8
3.4	$> 1.26 \times 10^9$
3.5	8.60×10^8
3.6	4.00×10^8
4.1	1.46×10^8
4.2	$> 1.26 \times 10^9$
4.3	$> 1.26 \times 10^9$
4.4	$> 1.26 \times 10^9$
4.5	4.00×10^6
4.6	7.10×10^8
5.1	$> 1.26 \times 10^9$
5.2	$> 1.26 \times 10^9$
5.3	$> 1.26 \times 10^9$
5.4	1.26×10^8
5.5	1.86×10^8
5.6	$> 1.26 \times 10^9$
6.1	8.60×10^8
6.2	4.00×10^8
6.3	1.26×10^7
6.4	$> 1.26 \times 10^9$
6.5	1.86×10^8
6.6	$> 1.26 \times 10^9$

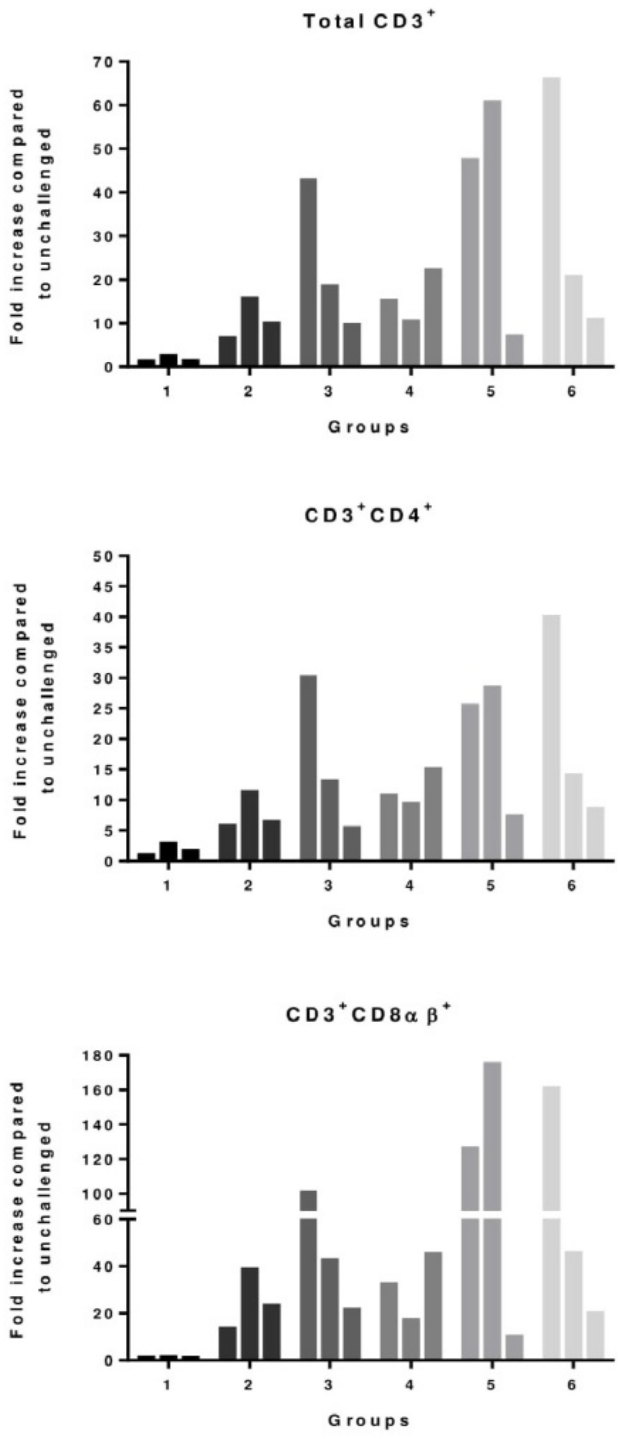

Figure 4: Evaluation of cellular populations in bursa by flow cytometry: Bursae from challenged animals in groups 1 (i.m. pVP2), 2 (i.n. pVP2), 3 (oral pVP2), 4 (i.m. pGFP), 5 (i.n. pGFP) and 6 (oral pGFP) were pooled in pairs and chicken leukocytes were isolated, stained with different combinations of antibodies and analyzed by flow cytometry. Lymphocyte population was gated according to their size and complexity. Results are expressed as the fold increase of each sample normalized with the mean values of unchallenged chickens.

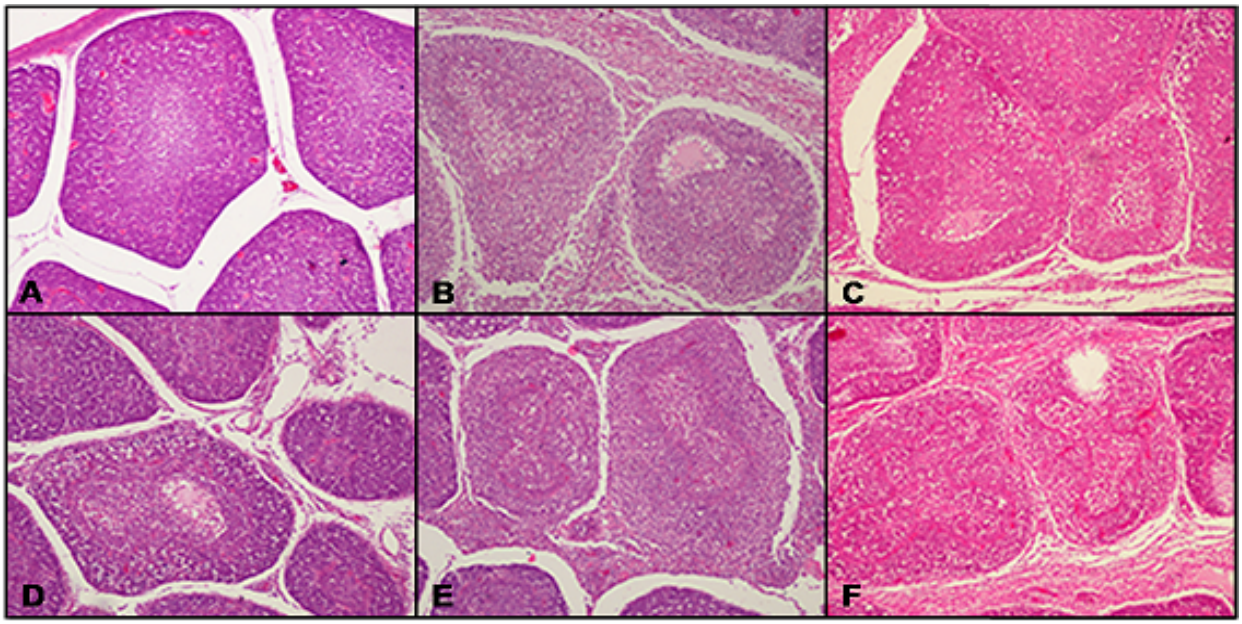

Figure 5: Histopathological observation of bursa. Bursal samples from group 1 (i.m. pVP2, A), group 2 (i.n. pVP2, B), group 3 (oral pVP2, C), group 4 (i.m. pGFP, D), group 5 (i.n. pGFP, E) andgroup 6 (oral pGFP, F) were paraffin embedded and stained with haematoxylin and eosin. Bursae from group 1 were histopathologically normal although some of them contained few apoptotic lymphocytes. The rest of the animals had damaged bursae with different degrees of lymphoid atrophy, necrosis, apoptosis, edema, small haemorrhages and lymphocyte and heterophile infiltration.

respectively. Another study demonstrated that i.n. administration of inactivated Newcastle disease virus (NDV) induced local humoral responses and protected birds from lethal challenge with virulent virus. Addition of cholera toxin B subunit to inactivated vaccine increased antibodies titers and protection rates [41]. We believe that supplementation of our recombinant vaccine formulation with an appropriate mucosal adjuvant could be necessary to achieve the desire protective state against IBDV since immunomodulators are often needed for strong mucosal immune responses [42].

Taken into account the disadvantages of the commercial live-attenuated and inactivated vaccines, a plant-based subunit vaccine represents a viable alternative in the veterinary field [43,44]. Our transiently expressed VP2 conserves antigenic determinants of the wild type protein and has proven to be immunogenic when parentally inoculated. However, further investigations need to be done to improve the pVP2 formulation in order to be able to use a more practical route of administration capable of eliciting a mucosal immune response.

In conclusion, our study reveals that an intramuscular vaccine based on plant material expressing the main immunodominant antigen of IBDV can be a welfare-friendly and desirable vaccine to apply in the poultry industry.

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References

1. Eterradossi N, Saif YM (2008) Infectious Bursal Disease. Dis Poult 54: 185-208.
2. Sharma JM, Dohms JE, Metz AL (1989) Comparative pathogenesis of serotype 1 and variant serotype 1 isolates of infectious bursal disease virus and their effect on humoral and cellular immune competence of specific-pathogen-free chickens. Avian Dis 33: 112-124.
3. Sharma JM, Kim IJ, Rautenschlein S, Yeh HY (2000) Infectious bursal disease virus of chickens: pathogenesis and immunosuppression. Dev Comp Immunol 24: 223-235.
4. Tanimura N, Sharma JM (1997) Appearance of T cells in the bursa of Fabricius and cecal tonsils during the acute phase of infectious bursal disease virus infection in chickens. Avian Dis 41: 638-645.
5. He CQ, Ma LY, Wang D, Li GR, Ding NZ (2009) Homologous recombination is apparent in infectious bursal disease virus. Virology 384: 51-58.
6. Berg TP, Gonze M, Morales D, Meulemans G (1996) Acute infectious bursal disease in poultry: Immunological and molecular basis of antigenicity of a highly virulent strain. Avian Pathol 25: 751-768.
7. Heine HG, Haritou M, Failla P, Fahey K, Azad A (1991) Sequence analysis and expression of the host-protective immunogen VP2 of a variant strain of infectious bursal disease virus which can circumvent vaccination with standard type I strains. J Gen Virol 72: 1835-1843.
8. Shaw I, Davison TF (2000) Protection from IBDV-induced bursal damage by a recombinant fowlpox vaccine, fpIBD1, is dependent on the titre of challenge virus and chicken genotype. Vaccine 18: 3230-3241.
9. Darteil R, Bublot M, Laplace E, Bouquet JF, Audonnet JC, Rivière M (1995) Herpesvirus of turkey recombinant viruses expressing infectious bursal disease virus (IBDV) VP2 immunogen induce protection against an IBDV virulent challenge in chickens. Virology 211: 490.
10. Tsukamoto K, Kojima C, Komori Y, Tanimura N, Mase M, et al. (1999) Protection of chickens against very virulent infectious bursal disease virus (IBDV) and Marek's disease virus (MDV) with a recombinant MDV expressing IBDV VP2. Virology 257: 352-362.
11. Perozo F, Villegas AP, Fernandez R, Cruz J, Pritchard N (2009) Efficacy of single dose recombinant herpesvirus of turkey infectious bursal disease virus (IBDV) vaccination against a variant IBDV strain. Avian Dis 53: 624-628.
12. Francois A, Chevalier C, Delmas B, Eterradossi N, Toquin D, et al. (2004) Avian adenovirus CELO recombinants expressing VP2 of infectious bursal disease virus induce protection against bursal disease in chickens. Vaccine 22: 2351-2360.

13. Perozo F, Villegas P, Estevez C, Alvarado IR, Purvis LB, et al. (2008) Protection against infectious bursal disease virulent challenge conferred by a recombinant avian adeno-associated virus vaccine. Avian Dis 52: 315-319.

14. Pitcovski J, Di-Castro D, Shaaltiel Y, Azriel A, Gutter B, et al. (1996) Insect cell-derived VP2 of infectious bursal disease virus confers protection against the disease in chickens. Avian Dis 40: 753-761.

15. Vakharia VN, Snyder DB, Lütticken D, Mengel-Whereat SA, Savage PK, et al. (1994) Active and passive protection against variant and classic infectious bursal disease virus strains induced by baculovirus-expressed structural proteins. Vaccine 12: 452-456.

16. Rong J, Jiang T, Cheng T, Shen M, Du Y, et al. (2007) Large-scale manufacture and use of recombinant VP2 vaccine against infectious bursal disease in chickens. Vaccine 25: 7900-7908.

17. Pitcovski J, Gutter B, Gallili G, Goldway M, Perelman B, et al. (2003) Development and large-scale use of recombinant VP2 vaccine for the prevention of infectious bursal disease of chickens. Vaccine 21: 4736-4743.

18. Chen TH, Hu CC, Liao JT, Lee CW, Liao JW, et al. (2012) Induction of protective immunity in chickens immunized with plant-made chimeric Bamboo mosaic virus particles expressing very virulent nfectious bursal disease virus antigen. Virus Res 166: 109-115.

19. Kumar S, Ahi YS, Salunkhe SS, Koul M, Tiwari AK, et al. (2009) Effective protection by high efficiency bicistronic DNA vaccine against infectious bursal disease virus expressing VP2 protein and chicken IL-2. Vaccine 27: 864–869.

20. Zhang H, Yang X, Xie Q, Ma J, Luo Y, et al. (2010) The potent adjuvant effects of chicken beta-defensin-1 when genetically fused with infectious bursal disease virus VP2 gene. Vet Immunol Immunopathol 136: 92-97.

21. Wu H, Singh NK, Locy RD, Scissum-Gunn K, Giambrone JJ (2004) Expression of immunogenic VP2 protein of infectious bursal disease virus in Arabidopsis thaliana. Biotechnol Lett 26: 787-792.

22. Wu J, Yu L, Li L, Hu J, Zhou J, et al. (2007) Oral immunization with transgenic rice seeds expressing VP2 protein of infectious bursal disease virus induces protective immune responses in chickens. Plant Biotechnol J 5: 570-578.

23. Kolotilin I, Topp E, Cox E, Devriendt B, Conrad U, et al. (2014) Plant-based solutions for veterinary immunotherapeutics and prophylactics. Vet Res 45: 117.

24. Salazar-González JA, Bañuelos-Hernández B, Rosales-Mendoza S (2015) Current status of viral expression systems in plants and perspectives for oral vaccines development. Plant Mol Biol 87: 203-217

25. Gómez E, Lucero MS, Chimeno Zoth S, Carballeda JM, Gravisaco MJ, et al. (2013) Transient expression of VP2 in Nicotiana benthamiana and its use as a plant-based vaccine against infectious bursal disease virus. Vaccine 31: 2623-2627.

26. De Magistris MT (2006) Mucosal delivery of vaccine antigens and its advantages in pediatrics. Adv Drug Deliv Rev 58: 52-67.

27. Zhai L, Wang Y, Yu J, Hu S (2014) Enhanced immune responses of chickens to oral vaccination against infectious bursal disease by ginseng stem-leaf saponins. Poult Sci 93: 2473-2481.

28. Gómez E, Zoth SC, Asurmendi S, Vázquez Rovere C, Berinstein A (2009) Expression of hemagglutinin-neuraminidase glycoprotein of Newcastle disease virus in agroinfiltrated Nicotiana benthamiana plants. J Biotechnol 144: 337-340.

29. Carballeda JM, Zoth SC, Gómez E, Gravisaco MJ, Berinstein A (2011) Activation of the immune response against Infectious Bursal Disease Virus after intramuscular inoculation of an intermediate strain. Immunobiology 216: 1028-1033.

30. Stein GM, Büssing A, Schietzel M (2002) Activation of dendritic cells by an aqueous mistletoe extract and mistletoe lectin-3 in vitro. Anticancer Res 22: 267-274.

31. Bradley WG, Widen RH, Weiser AM, Powers JJ, Fountain LB, et al. (2003) The novel differentiation of human blood mononuclear cells into CD1a-negative dendritic cells is stimulated in the absence of exogenous cytokines by an extract prepared from pinecones. Int Immunopharmacol 3: 209-223.

32. Di Bonito P, Grasso F, Mangino G, Massa S, Illiano E, et al. (2009) Immunomodulatory activity of a plant extract containing human papillomavirus 16-E7 protein in human monocyte-derived dendritic cells. Int J Immunopathol Pharmacol 22: 967-978.

33. Arnold M, Durairaj V, Mundt E, Schulze K, Breunig KD, et al. (2012) Protective vaccination against infectious bursal disease virus with whole recombinant Kluyveromyces lactis yeast expressing the viral VP2 subunit. PLoS One 7:e42870.

34. Taghavian O, Spiegel H, Hauck R, Hafez HM, Fischer R, et al. (2013) Protective oral vaccination against infectious bursal disease virus using the major viral antigenic protein VP2 produced in Pichia pastoris. PLoS One 8:e83210.

35. Zhang X, Yang Q (2007) Effect of compound mucosal immune adjuvant on mucosal and systemic immune responses in chicken orally vaccinated with attenuated Newcastle-disease vaccine. Vaccine 25: 3254-3262.

36. Lei H, Sheng Z, Ding Q, Chen J, Wei X, et al. (2011) Evaluation of oral immunization with recombinant avian influenza virus HA1 displayed on the Lactococcus lactis surface and combined with the mucosal adjuvant cholera toxin subunit B. Clin Vaccine Immunol 18: 1046-1051.

37. Svindland SC, Jul-Larsen Å, Pathirana R, Andersen S, Madhun A, et al. (2012) The mucosal and systemic immune responses elicited by a chitosan-adjuvanted intranasal influenza H5N1 vaccine. Influenza Other Respir Viruses 6: 90-100.

38. Zhao K, Chen G, Shi XM, Gao TT, Li W, et al. (2012) Preparation and efficacy of a live newcastle disease virus vaccine encapsulated in chitosan nanoparticles. PLoS One 7: e53314.

39. Nayak B, Rout SN, Kumar S, Khalil MS, Fouda MM, et al. (2009) Immunization of chickens with Newcastle disease virus expressing H5 hemagglutinin protects against highly pathogenic H5N1 avian influenza viruses. PLoS One 4: e6509.

40. Thanasarasakulpong A, Poolperm P, Tankaew P, Sawada T, Sthitmatee N (2015) Protectivity conferred by immunization with intranasal recombinant outer membrane protein H from Pasteurella multocida serovar A:1 in chickens. J Vet Med Sci 77: 321-326.

41. Takada A, Kida H (1996) Protective immune response of chickens against Newcastle disease, induced by the intranasal vaccination with inactivated virus. Vet Microbiol 50: 17-25.

42. Zaman M, Chandrudu S, Toth I (2013) Strategies for intranasal delivery of vaccines. Drug Deliv Transl Res 3: 100-109.

43. Ling HY, Pelosi A, Walmsley AM (2010) Current status of plant-made vaccines for veterinary purposes. Expert Rev Vaccines 9: 971-982.

44. Rybicki EP (2014) Plant-based vaccines against viruses. Virol J 11: 205.

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