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The construction and immune efficacy of recombinant *Lactobacillus casei* strains expressing VP3 from goose parvovirus

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ABSTRACT

Goose parvovirus (GPV) is a pathogen that causes Derzsy's disease, which causes severe economic losses to China's waterfowl breeding industry. The mortality rate of Derzsy's disease reaches 90% in the 7 days. *Lactobacillus casei* (*L. casei*) is a common candidate vector in oral vaccines. It is widely used to prevent animal infectious diseases, parasites and tumors. *L. casei* is a promising vector for developing novel live bacterial vaccine vectors. In this study, *L. casei* was used as a presentation vector to construct a recombinant *Lactobacillus casei* expressing the VP3 gene of GPV, which was used as an oral vaccine to immunize goslings. and to evaluate its immunogenicity. In this study, we constructed recombinant *Lactobacillus casei* expressing the VP3 gene of goose parvovirus, and the immunogenicity and protective efficacy of Lc-pPG-612-VP3 were evaluated in SD rats and goslings. After oral immunization, Lc-pPG-612-VP3 colonized the intestine for approximately 34 days and had good immunogenicity. The results showed that Lc-pPG-612-VP3 can induce humoral and mucosal immune responses in goslings, and enhance the transcriptional levels of cytokines in various tissues. The challenge experiment showed that the recombinant *Lactobacillus casei* had a certain preventive effect against Derzsy's disease (30%), which was the same as the protection efficiency of the commercial vaccine.

Key words: goose parvovirus; Derzsy's disease; live recombinant bacteria vaccine; *Lactobacillus casei*

Introduction

Derzsy's disease has high mortality and morbidity. It mainly infects Muscovy ducks and goslings less than three weeks old (GOUGH et al., 1981; GLÁVITS et al., 2005; NING et al., 2018). Derzsy's disease is mainly divided into peracute, acute and subacute types (BROWN et al., 1995). Goslings infected with Derzsy's disease mainly have symptoms such as somnolence, dysphagia, slow growth, and frequent yellowish-white diarrhea (JANSSON et al., 2007; YU et al., 2016). The VP3 protein is the main capsid protein of GPV and accounts for 80% of the total amount of capsid

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protein. It is one of the main candidate antigens for the development of GPV vaccine research and serological diagnosis (YIN et al., 2012; LI et al., 2016).

At present, injecting these vaccines is the main method of preventing Derzsy's disease. However, injecting goslings at an early age causes stress that can result in death. Therefore, the development of a novel vaccine is urgent.

Bacterial DNA vaccines have good research prospects in the prevention of animal diseases (ZHI et al., 2021), parasites (WANG et al., 2014) and tumors (KITAGAWA et al., 2021). The advantages of bacterial DNA vaccines are as follows: (a) bacteria can protect foreign antigens from degradation by nucleases; (b) target cells can internalize bacteria carrying foreign antigens; (c) bacterial culture is simple and takes less time to manufacture than other vaccines; (d) the process of plasmid amplification and purification is not needed, which can greatly reduce the costs and labor; and (e) live recombinant bacterial vaccines can be administered to animals through oral immunization or nasal immunization to reduce their stress response (PEREIRA et al., 2014).

L. casei belongs to the genus *Lactobacillus* and tolerates acidic environments. In recent years, *L. casei* has been used as a carrier vector to prevent animal infection with *Aeromonas victorini* (ZHANG et al., 2018), porcine epidemic diarrhea virus (LI et al., 2021) and Newcastle disease (JU et al., 2021). However, there are no reports on the use of *L. casei* in DNA vaccines for the prevention of waterfowl parvovirus.

In this study, we tested the immunogenicity and intestinal tract colonization of a recombinant *L. casei* vaccine for GPV, and evaluated the vaccine in goslings, aiming to lay the foundation for the research and development of oral vaccines in waterfowl.

Materials and methods

Goslings. One-day-old goslings weighing 100±10 g were obtained from a farm in Changchun. Every experiment procedure was completed as per the Guidelines for Animal Experiments of our university (JLAU08201409).

Bacterial strains, carriers and growth conditions. The *L. casei* ATCC 393 and the plasmid pPG-612 used in this study were provided by the College of Animal Science and Technology, Jilin Agricultural University. *L. casei* ATCC 393 was cultivated in Man Rogosa and Sharpe (MRS) broth, or MRS agar plates at 37°C.

Virus isolation. A goose infected with Derzsy's disease was obtained from a farm in Changchun. The small intestine and liver of the infected goose were cut and ground into a homogenate. Sterile phosphate-buffered saline (PBS) containing penicillin and streptomycin was added to the homogenate. To ensure that the virus was successfully released into the cells, the prepared virus homogenate was repeatedly frozen and thawed. The homogenate was centrifuged, and the supernatant was collected. After the goose embryos had been cultured for 13 days, the supernatant was injected. We placed the goose embryos in the incubator and continued to hatch goose embryos for 5 days. The allantoic fluid in the remaining goose embryos was collected after 5 days. The extracted allantoic fluid was successively passaged 5 times, and the fifth-generation allantoic fluid was collected to extract DNA to design GPV-specific primers for PCR verification.

Vector construction and transformation. The viral DNA was extracted from the allantoic fluid of GPV-inoculated goose embryos. On the basis of the complete sequence of VP3 (U25749.1) in GenBank on NCBI, Primer 5.0 software was used to design a pair of primers for the *Not* □ and *Spe* □ restriction sites: forward: 5'-ATTT GCGGCCGC ATGGCAGAGGGAGGAGGC-3', reverse: 5'-GG ACTAGT TACAGATTTTGAGTTAG ATATCTGGTTCCAATCAATC-3'. The viral DNA was used as a template. The fragment of the VP3 gene (1605 bp) of the goose parvovirus was amplified by PCR. The PCR product was cut with *Not* □ and *Spe* □ restriction sites and inserted into the corresponding sites of pPG-612, then cloned into the relevant sites of pPG-612 to acquire recombination vector pPG-612-VP3. Then, recombinant pPG-612-VP3 was transformed into *L. casei* ATCC 393 by electroporation. Meanwhile, *L. casei* transformed with pPG-612 was utilized as the NC.

VP3 protein expression and Western blotting. Recombinant Lc-pPG-612 was induced in MRS medium (containing 2% lactose and 10 μg/mL chloramphenicol) at 37°C for 15 h, and pPG-612 was induced under the same conditions, as a control. The proteins were harvested from the culture lysates by lysozyme and added to the culture lysates at 37°C for 30 min. The protein was mixed with 25 μL of 5×SDS–PAGE Buffer (including DTT) and boiled for 10 min. The protein sample was stored at -20°C for Western blotting.

The protein was subjected to SDS-PAGE and transferred to a nitrocellulose membrane (NC membrane) (Bioss, Beijing, China). The membrane was blocked with PBS containing 5% BSA for 1 h, and then probed with the mouse antiVP3 serum (Solarbio, China) at 1:100 ratio under 4°C for 12 h. Horseradish peroxidase (HRP)-conjugated goat anti-mouse IgG was used as the secondary antibody and diluted at a 1:1000 ratio. Finally, the VP3 protein was visualized using a Western Blotting DAB Chromogenic Reagent Kit (Solarbio, China) in a chemiluminescent gel imaging system (Analytik Jena, Germany).

Immunofluorescence assay. We used an indirect immunofluorescence assay (IFA) to detect the expression of the GPV VP3 protein. Briefly, recombinant Lc-pPG-612-VP3 was grown in MRS broth at 37°C and washed with sterile PBS three times. The cells were resuspended in 1 mL of sterile PBS containing mouse anti-VP3 serum (diluted at 1:200) and then incubated at 37°C for 1 hour. The cells were washed with sterile PBS three times and then incubated with fluorescein isothiocyanate (FITC) -conjugated rabbit anti-mouse IgG antibody (diluted at 1:500) at 37°C for 30 min. The cells were washed in sterile PBS, air-dried and fixed on a glass slide. The immunofluorescence analysis of the cells was visualized by fluorescence microscope (Zeiss LSM710). In parallel, Lc-pPG was utilized as a negative control.

Hereditary stability of recombinant L. casei-pPG-612-VP3. To determine the stability of recombinant Lc-pPG-612-VP3, the recombinant Lc-pPG-612-VP3 was continuously transferred for 48 generations in MRS broth at intervals of 12 h with 10 μg/mL of chloramphenicol. Then, the plasmid of each generation was extracted, using PCR to detect the presence of the target fragment using specific primers for the target gene.

Vaccine preparation, oral immunization and sample collection. For oral vaccine preparation, recombinant Lc-pPG-612-VP3 strains were cultivated into MRS broth containing 2% lactose and 10 mg/mL chloramphenicol for 15 h. After centrifugation, the cells were washed three times with sterile PBS, and the concentration was adjusted to 1.0×10^{10} CFU. Recombinant Lc-pPG-612 and *L. casei* were used as controls.

The gosling individuals were randomly divided into four groups (n=42 per group). Regarding the immunization dose, three groups of goslings were orally fed 1 mL of Lc-pPG-612-VP3, *L. casei* and PBS. Goslings were immunized orally twice a day for three consecutive days and boosted twice at 2-week intervals. The inactivated vaccine group of goslings was injected with the inactivated vaccine according to the vaccine instructions.

To detect the content of IgM and sIgA antibodies in the goslings, serum was collected and stored at -80°C. The intestines of the goslings were cut longitudinally and rinsed with sterile PBS. The supernatant was collected and stored at -20°C after centrifugation. The heart, liver, spleen, kidney and intestines of the goslings were frozen in liquid nitrogen and stored at -80°C for RNA extraction.

ELISA. The levels of IgM and IgG antibodies induced by VP3 were evaluated with ELISA, where the specific steps were basically as described above (TARASIUK et al., 2019). Briefly, 96-well polystyrene microtiter dishes were coated with 10 μg/mL VP3 protein in overnight storage at 4°C. The plate was blocked with phosphate-buffered saline Tween (PBST) containing 2% BSA at 37°C for 2 h. The samples were added in triplicate and incubated for 1 h at 37°C. HRP-conjugated goat anti-goose IgM and HRP-conjugated goat anti-goose sIgA were added as secondary antibodies to the wells, and incubated at 37°C for 1 h. Further, the plate was washed 5 times with PBST, TMB substrate (3,30,5,50-tetramethylbenzidine) was added away from the light and allowed to react, and the absorbance was measured at $OD_{450\ nm}$.

Cytokine detection. Overall RNA was extracted from gosling tissues using the TRIzol method, and reverse transcription was performed according to the instructions of the RT EasyTM □ kit. The resulting cDNAs were used for RT–PCR detection of cytokines. On the basis of the GenBank sequence, specific primers were designed for target immune-associated genes (IL-10, IL-1β, IFN-γ and TNF-α.) and β-actin was utilized as the inner reference. The primer sequences are listed in Table 1. Each 20 μL RT reaction contained 1000 ng of RNA sample, a 1 μM final concentration of specific primers, 2 × SYBR Green Master Mix (GenStar, China) and ddH_2O. The data were analyzed by Stratagene MxPro software (Stratagene Mx3005p, USA). All the qPCR samples were completed in triplicate.

Table 1. Primer sequences of real-time PCR

Gene	Forward primers squences (5'-3')	Reverse primers squences (5'-3')
IL-10	AATGGCACAGGCTCGTTCACAC	GTTGACTCAAGGGAGAGGGAAACAAG
IL-1β	CCTCGCCTGGATTCTGAGCA	GCTGTCAGCAAAGTCCCTGC
IFN-γ	TGAGCCAGATTGTTTCCCTGTACTTG	CAGGTCCACGAGGTCTTTGAACTTC
TNF-α	TCCGTACACAGGACAGCCTATGC	GGAAGGGCAACACATCTGAACTGG
β-actin	GCATCCACGAGACCACCTTCAAC	GCTGTGATCTCCTTCTGCATCCTG

Challenge. Forty goslings at the age of 1 day were randomly divided into four groups: PBS, *L. casei*, recombinant Lc-pPG-612-VP3 and inactivated vaccine (n=10). Five days after immunization, the goslings were injected with GPV (1 mL/goslings). After the injection of GPV, the temperature and weight of the goslings were recorded daily. Statistics on the deaths of goslings were obtained, and the intestinal contents of dead goslings were collected to extract DNA according to the instructions of the viral DNA extraction kit. The DNA was used as a template for PCR identification, and two primers were synthesized by Shanghai Sangon Co. as follows: forward: 5'-CCAAGCTACAACAACCACATCTAC-3', reverse: 5'-CTGCGGCAGGGCATAGACATCCG AC-3'.

Statistical Analysis. Statistical comparisons were performed using Prism 6.0 software (GraphPad Software) and Tukey's analysis. Differences were considered statistically significant at $P<0.05$ (*), $P<0.01$ (**) or $P<0.001$ (***).

Results

Virus isolation and identification. The allantoic fluid of the dead goose embryos was collected and named GPV-WZ. GPV-WZ was detected by PCR, sterile water was used as a negative control, and GPV-SP was used as a positive control. The results are shown in Fig. 1. GPV-WZ had a specific band at 375 bp, which was consistent with the results of the positive control GPV-SP strain, and in line with the expected result. The GPV-WZ sequence, analyzed by Shanghai Sangon Co., showed that the isolated strains GPV-WZ and GPV-B had 99.6% identical nucleotide sequences. This means that GPV-WZ was further proven to belong to GPV.

Construction of recombinant L.casei and expression of VP3 protein in L.casei. Lc-pPG-612-VP3 was confirmed by enzyme digestion and PCR (Fig. 2A-B). The VP3 protein was detected by Western blotting. The result showed that pPG-612-VP3 was detected at 60 kDa (Fig. 2C), which was consistent with the expected effect. In the immunofluorescence assay, specific green

fluorescence was observed for pPG-612-VP3 in *L. casei* (Fig. 2D). In contrast, no fluorescence was detected in Lc-pPG-612. The results of the Western blotting and immunofluorescence assays showed that the VP3 protein was correctly expressed in *L. casei*.

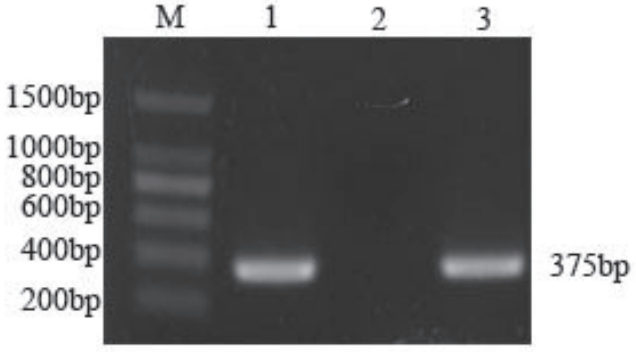

Fig. 1. PCR assay for virus identification
Lane M, DL 2000 DNA Maker; lane 1, positive control; lane 2, negative control; lane 3, isolation of GPV

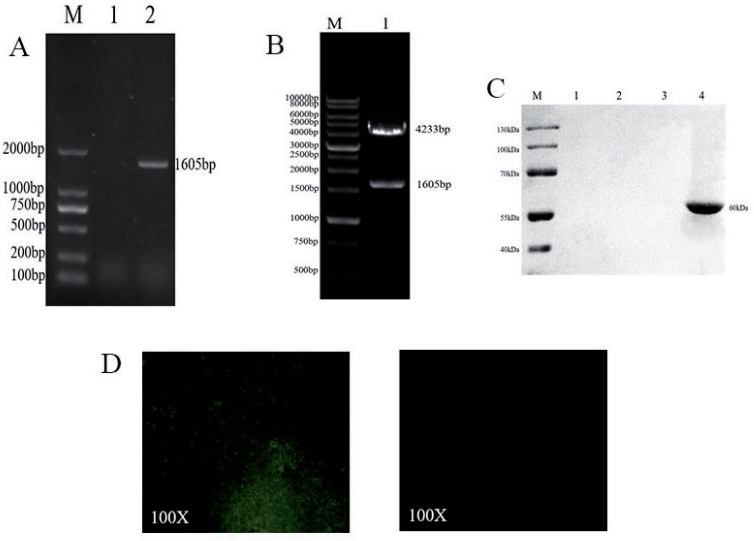

Fig. 2. Expression of GPV VP3 protein on *L.casei*

(A) Identification of recombinant pPG-612-VP3 by PCR. Lane M, DL 2000 DNA Maker; lane 1, negative control; lane 2, PCR amplification product of pPG-612-VP3. (B) Identification of recombinant pPG-612-VP3 digested by restriction enzymes. Lane M, DL 10000 DNA Marker; lane 1, identification of recombinant plasmid digested by restriction enzymes. (C) Western blot analysis. Lane M, the molecular mass markers; lane 1, pPG-612 before induction; lane 2, pPG-612 induction; lane 3, pPG-612-VP3 before induction; lane 4, pPG-612-VP3 before induction. (D) Immunofluorescence microscopy analysis. On the left Lc-pPG-612-VP3 and on the right Lc-pPG.

The hereditary stability of recombinant L. casei expressing VP3. The recombinant Lc-pPG-612-VP3 was detected by PCR and sequencing technology to analyze the hereditary stability of Lc-pPG-612-VP3. Fig. 3 shows that Lc-pPG-612-VP3 was stably inherited over 48 generations. The bands at 1605 bp were observed by sequencing techniques and matched to the target sequences. The results showed that the recombinant *L. casei* was stably inherited.

IgM and sIgA antibody content of the goslings. After immunization, the serum and intestinal cleansing solution were collected to detect the specific IgM and sIgA in the goslings. The results showed (Fig. 4A) that recombinant Lc-pPG-612-VP3 and inactivated vaccine induced goslings to secrete IgM antibodies. The inactivated vaccine induced the highest IgM content in goslings at 12 d after immunization, which was significantly higher than that induced by recombinant Lc-pPG-612-VP3 ($P<0.05$). At 15 d after immunization, the goslings immunized with recombinant Lc-pPG-612-VP3 reached the highest level of IgM antibodies compared with the control group. Both Lc-pPG-612-VP3 and inactivated vaccine induced sIgA secretion in the goslings (Fig. 4B) .

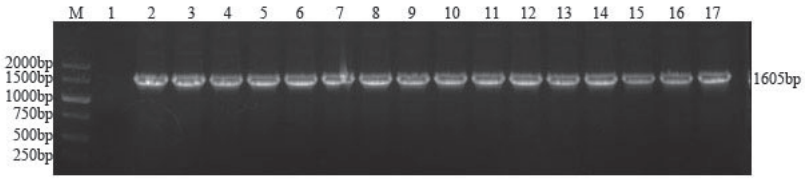

Fig. 3. Hereditary stability of recombinant Lc-pPG-612-VP3 detected by PCR with VP3-specific primer pairs
Lane M, DL 2000 DNA Maker; lane 1, negative control; lanes 2-17: PCR product of recombinant plasmid after 1/3/5 /7/10/14/18/22/26/30/33/36/39/42/45/48 generations

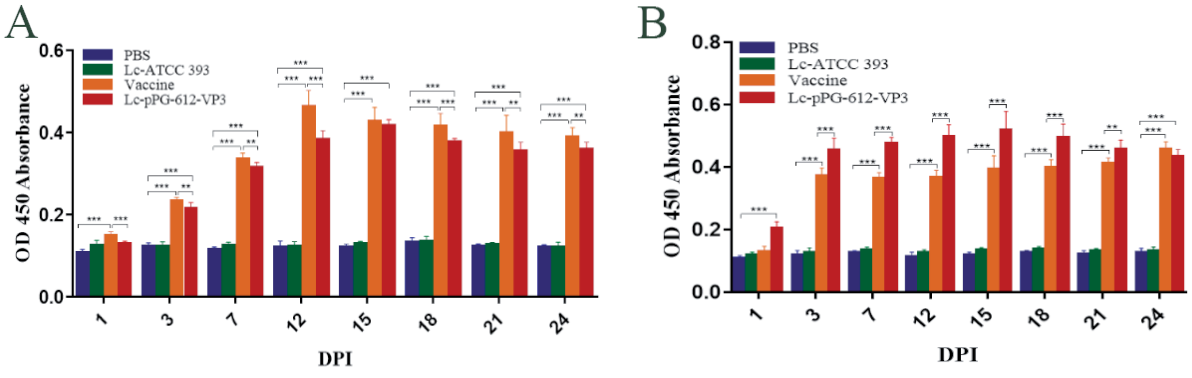

Fig. 4. The IgM and sIgA of recombinant Lc-pPG-612-VP3
(A) is the specific IgM level in the serum of goslings after immunization, (B) is sIgA levels in the serum of goslings after immunization

Cytokine expression. The effect of recombinant *L. casei* in the stimulation of immune responses was examined by measuring the relative expression of IL-10, IL-1β, IFN-γ and TNF-α by qRT-PCR analysis (Fig. 5; Fig. 6; Fig. 7; Fig. 8). The results showed that both Lc-pPG-612-VP3 and the inactivated vaccine upregulated the expression of four immunity-associated genes in the spleen and kidneys. However, Lc-pPG-612-VP3 and the inactivated vaccine showed differences in the four upregulated cytokine transcription levels in different organs, and the expression levels of the four cytokines in the same organ were also different. Compared with the inactivated vaccine, Lc-pPG-612-VP3 upregulated the expression of cytokines in a short period of time after the first immunization. However, the duration of Lc-pPG-612-VP3 immunization was temporary, and multiple immunizations were required to maintain high levels of cytokine transcription (Fig. 6E; Fig. 7A, B; Fig. 8B, D).

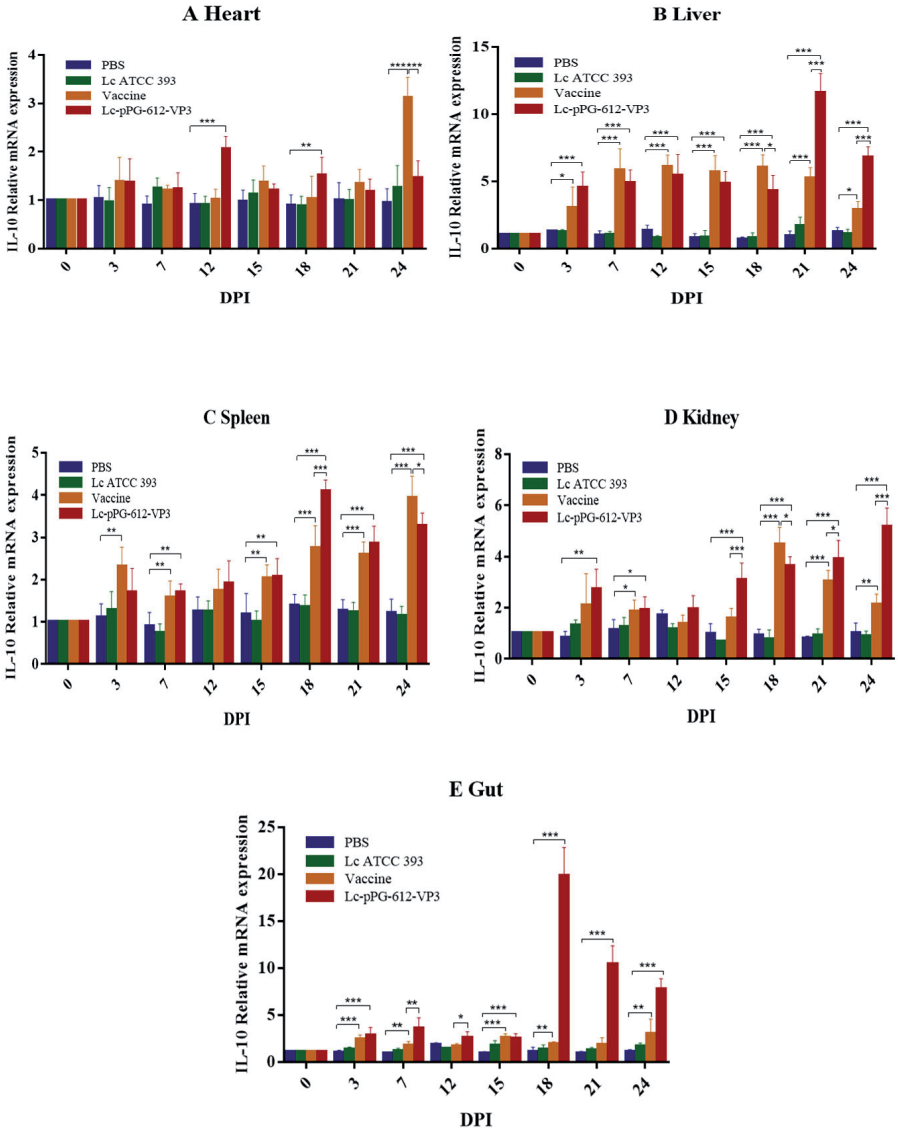

Fig. 5. IL-10 levels in the different organs of the goslings after immunization

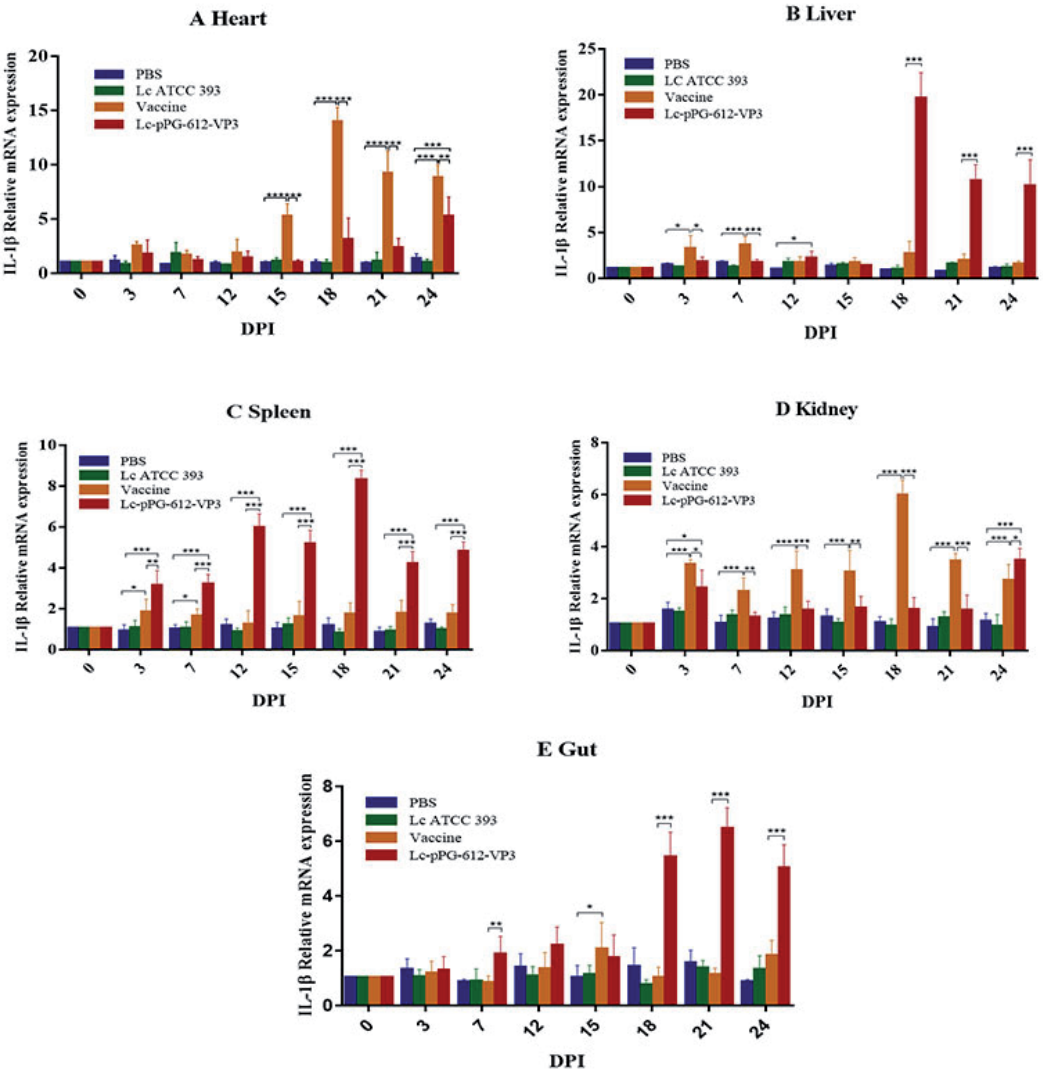

Fig. 6. IL-1β levels in the different organs of the goslings after immunization

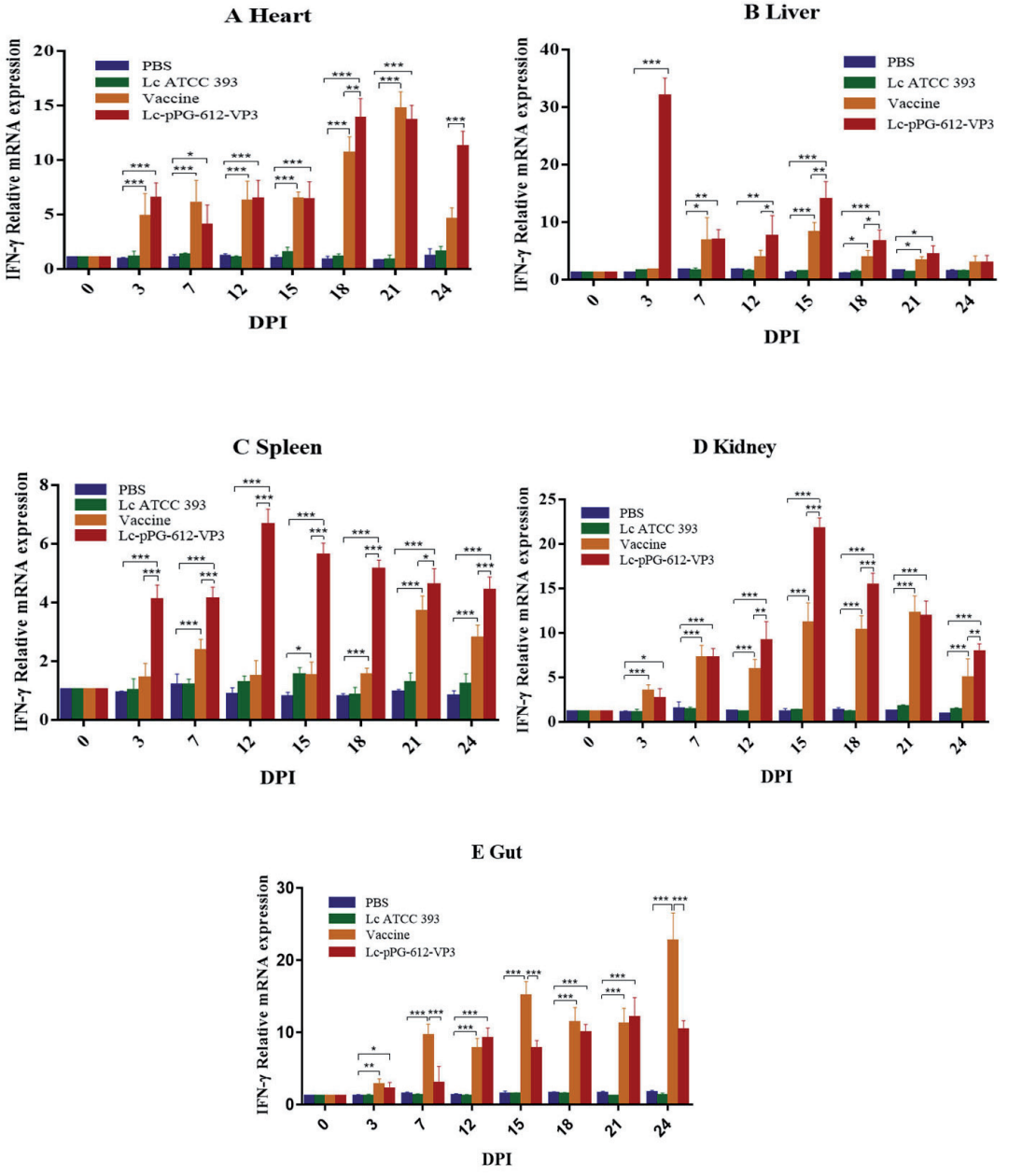

Fig. 7. IFN-γ levels in the different organs of the goslings after immunization

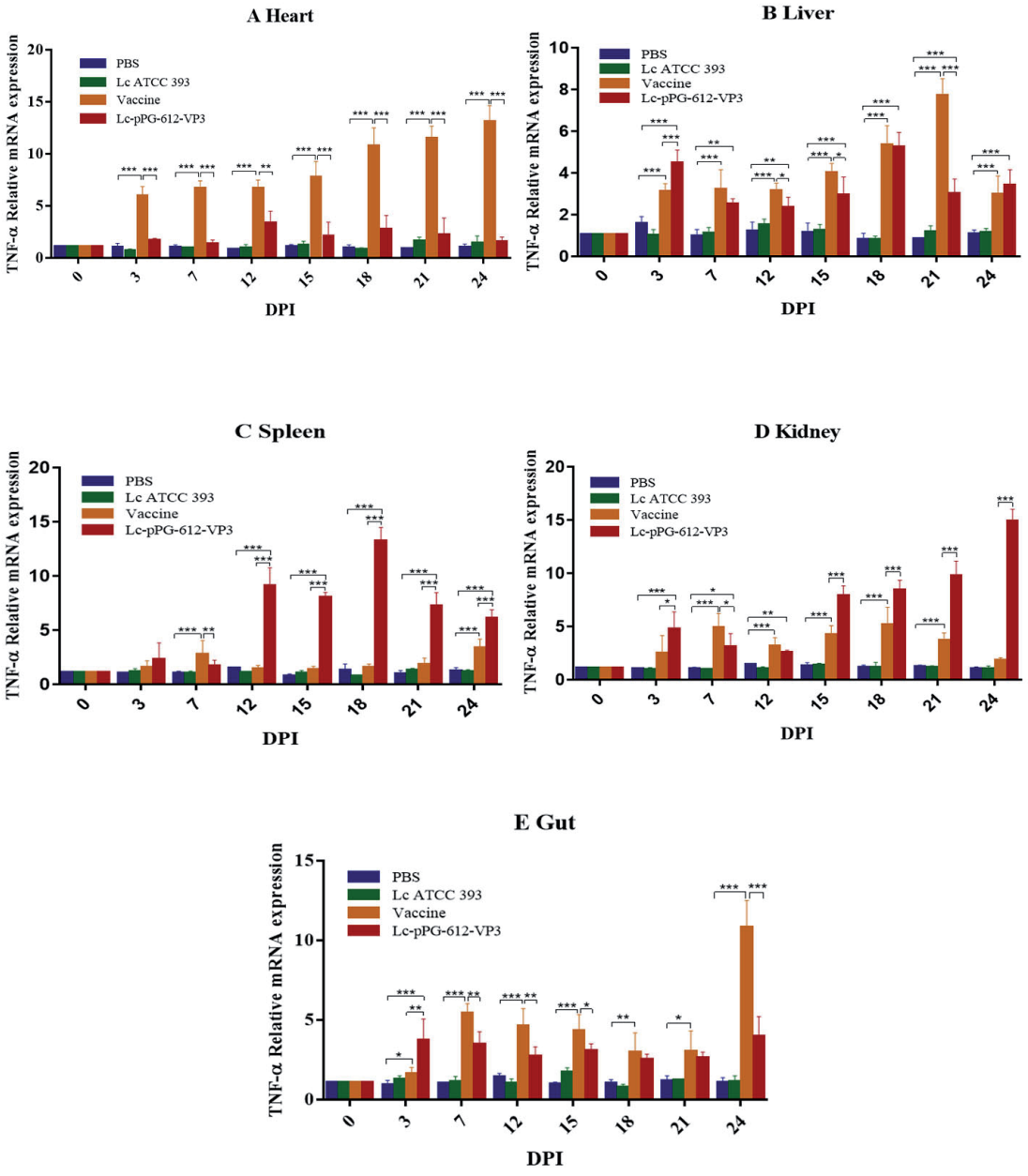

Fig. 8. TNF-α levels in the different organs of the gosling after immunization

Survival rate of goslings after the challenge. To evaluate the protective effects induced by vaccination with Lc-pPG-612-VP3, the immunized goslings were infected with the GPV-WZ strain 5 d after immunization. Temperature detection is shown in Fig. 9A. After the challenge, the goslings of the PBS group had the strongest temperature growth trend, followed by *L. casei* ATCC 393. There was no significant difference between the temperature growth trend of the inactivated vaccine group and the Lc-pPG-612-VP3 oral vaccine group. The weight results are shown in Fig. 9B. The weight

growth trend of goslings immunized with *L. casei* ATCC 393 and Lc-pPG-612-VP3 was higher than that of goslings treated with inactivated vaccine and PBS. It was speculated that *L. casei,* as a probiotic, can promote the appetite of goslings to effectively control their malnutrition and consequent weight loss. After the challenge, the death rate was documented for the successive 10-day period. According to Table 2, the goslings immunized with PBS had 50% death rates, while the goslings immunized with inactivated vaccine and Lc-pPG-612-VP3 had the lowest death rates of 30%. The DNA extracted from the intestinal cleansing fluid of the dead goslings was used as a template for PCR detection. The results shown in Fig. 9-C indicate that the goslings died because of GPV.

Table 2. Statistical data on 10-day-old gosling mortality

Group	PBS	Lc-ATCC 393	Vaccine	Lc-pPG-612-VP3
The number of deaths goslings	5	4	3	3
Mortality rate	50%	40%	30%	30%

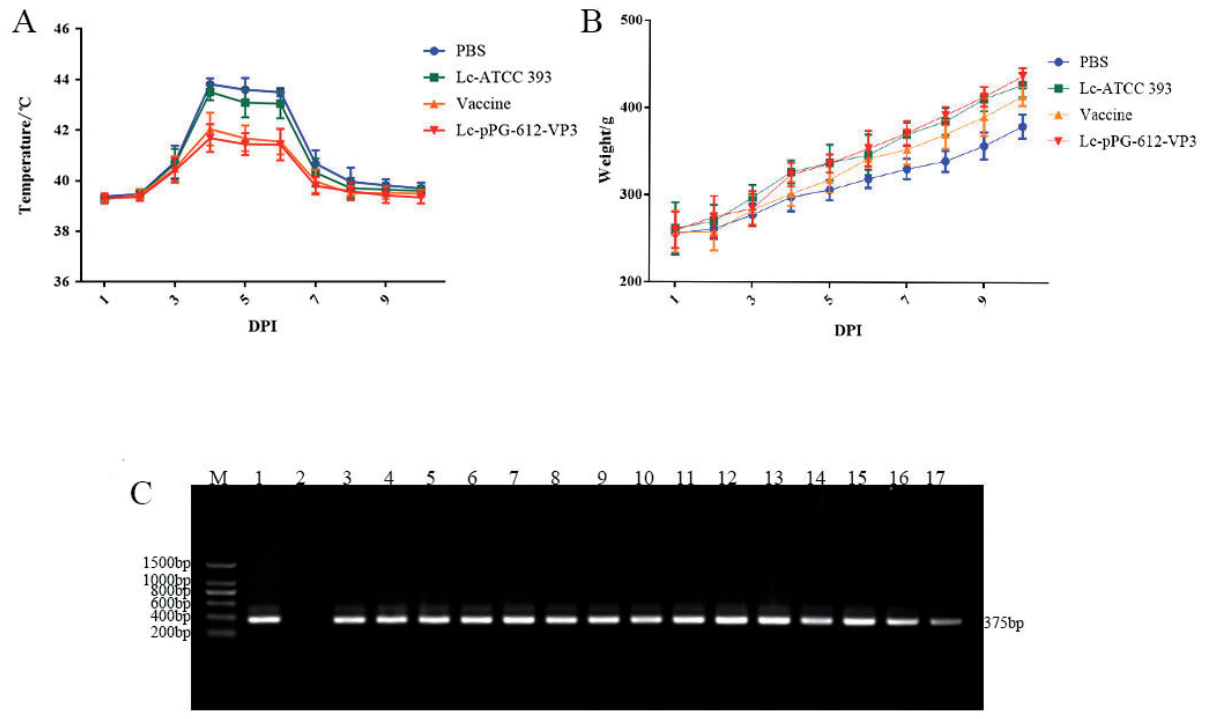

Fig. 9. After-challenge temperature and weight of goslings, and PCR verification of the intestines of goslings that died (A) is the temperature of 10-day-old goslings. (B) is the weight of 10-day-old goslings. (C) is the PCR result of DNA extracted from the intestinal contents of dead goslings. Line 1, positive control; line 2, negative control; line 3-17, PCR amplification product from dead goslings

Discussion

The most typical clinical symptoms of Derzsy's disease are loss of appetite, somnolence, and frequent yellowish-white diarrhea (LIU et al., 2014; NING et al., 2018). Since infected goslings cannot be isolated in good time this means the waterfowl poultry industry has experienced serious economic losses. Bacterial DNA vaccines are novel vaccines that have been developed rapidly in the past few decades. Research has shown that *L. casei* can effectively inhibit the growth of pathogenic microorganisms, and reduce diarrhea caused by bacteria, viruses and other microorganisms in the body (TOH et al., 2013; HILL et al., 2018). Adding *L. casei* into animal feed can promote animals' appetite, improving the animals' conversion efficiency of nutrients in their feed and improving animal immunity (DOWARAH et al., 2017; MARKOWIAK and ŚLIZEWSKA, 2018).

In this study, VP3 protein was successfully expressed in *L. casei*. The genetic stability results showed that the recombinant Lc-pPG-VP3 was passed on for 48 generations in *L. casei*. Due to the special acid-resistant nature of *L. casei*, it can protect the VP3 protein from degradation by gastric acid. Currently, *L.casei* is being widely used in veterinary research as a vehicle for DNA delivery and to induce both humoral and cellular immune responses (LIU et al., 2012; WANG et al., 2019).

Mucosal immune tissues mainly secrete IgM and sIgA antibodies to induce local mucosal responses, and participate in assisting cell responses and humoral responses (ALONSO et al., 2014). The sIgA is the main effector of the mucosal immune response (LI et al., 2020). The recombinant *Lactococcus lactis* effectively induced and maintained high levels of sIgA in chickens (LEI et al., 2015; SHA et al., 2020). Our results showed that recombinant *Lactobacillus casei* can induce goslings to secrete sIgA more quickly than the inactivated vaccine after immunization. This might be because recombinant *Lactobacillus casei* enters the body and finally colonizes the intestine. The intestines are one of the important sites for the mucosal immune response. The dendritic cells and M cells in the intestines can quickly process the VP3 protein, therefore, compared to traditional intramuscular vaccine injection, *L. casei* can cause plasma cells to secrete sIgA more directly and quickly.

In recent decades, cytokines have become a topic of intense interest in medical research (DINARELLO, 2007). IL-10 is a typical anti-inflammatory factor that reduces the excessive inflammatory response of antigen presentation (CASTRO-DOPICO and CLATWORTHY, 2019). IL-1β plays a major role in the early defense of the host against viral infection, and promotes the proliferation of CD8+ T cells (DINARELLO, 1996; BEN-SASSON et al., 2013a; BEN-SASSON et al., 2013b). IFN-γ can upregulate the efficiency of antigen processing and presentation, and promote immunoglobulin production and the proliferation and differentiation of B cells (SCHRODER et al., 2004). TNF-α plays a dual role in regulating the immune response (AKDIS et al., 2016). MAQSOOD et al. (2018) proved that recombinant *Lactobacillus plantarum* can significantly upregulate the expression of IFN-γ in chickens. Recombinant *Lactobacillus* upregulated the expression of cytokines in chickens (WANG et al., 2013). In this study, we detected the expression of IL-10, IL-1β, IFN-γ and TNF-α in five organs of the goslings. Recombinant *Lactobacillus casei* and inactivated vaccines upregulated cytokine expression, but the expression levels of the cytokines differed in different organs. In addition, recombinant *Lactobacillus casei* was not as effective as inactivated vaccines, so the immunization doses and cycles need to be further improved.

The immune protection efficiency of recombinant *Lactobacillus casei* in goslings was detected by the challenge protection experiment. The results showed that the protective efficiency of recombinant *Lactobacillus casei* and the inactivated vaccine in goslings reached 70%. Daily monitoring of the weight of goslings showed that the weight growth trend of goslings immunized with recombinant Lc-pPG-612-VP3 and *L. casei* was higher than that of the other groups. *L. casei*, as a probiotic, stimulates the appetite of animals, and stably regulates the intestinal flora of animals. The digestion and absorption capacity of animals were

improved and it increased the weight gain trend and reduced the impact of poor immunity caused by malnutrition. YANG et al. (2017) found that recombinant *Lactobacillus plantarum* alleviated the weight loss caused by the challenge.

In summary, an oral Lc-pPG-VP3 vaccine was constructed in this study, providing a novel vaccine to be used to prevent Derzsy's disease in the future. We proved that the Lc-pPG-VP3 could efficiently induce mucosal and humoral responses against GPV. The advantages of recombinant *Lactobacillus casei* in animal infectious disease vaccines are very obvious, and they can provide a new direction for the prevention of waterfowl infectious diseases. In follow-up research, it is necessary to optimize and improve the immune dose, the immune cycle, and concentration of immune recombinant *Lactobacillus casei,* to further improve the immune effect of recombinant *Lactobacillus casei*.

Conclusions

We successfully constructed a recombinant *Lactobacillus casei* system expressing the GPV VP3 protein. After immunizing goslings, it was proven that recombinant *Lactobacillus* had a certain preventive effect against Derzsy's disease. This study laid a foundation for the further development of oral vaccines for waterfowl infectious diseases.

Ethics statement

Every experiment procedure was completed as per the Guidelines for Animal experiments of our university (JLAU08201409).

Financial support statement

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Declaration of Competing Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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SAŽETAK

Parvovirus gusaka (GPV) jest patogen koji uzrokuje Derzsyjevu bolest. Bolest dovodi do velikih ekonomskih šteta u uzgoju vodenih ptica u Kini. Stopa smrtnosti od Derzsyjeve bolesti doseže i do 90% u tjedan dana. *Lactobacillus casei* (*L. casei*) zajednički je vektorski kandidat u oralnim cjepivima. Široko se primjenjuje u sprečavanju zaraznih i nametničkih bolesti životinja kao i prevenciji tumora. *L. casei* je obećavajući vektor u razvoju novih živih bakterijskih vektorskih cjepiva koja se primjerice mogu koristiti za oralnu imunizaciju guščića. U ovom je istraživanju načinjen rekombinantni *Lactobacillus casei* koji eksprimira gen *VP3* parvovirusa gusaka, a imunogenost i zaštitna učinkovitost Lc-pPG-612-VP3 procijenjene su u SD štakora i guščića. Nakon peroralne imunizacije Lc-pPG-612-VP3 kolonizirao je crijevo približno 34 dana i imao je dobru imunogenost. Rezultati su pokazali da Lc-pPG-612-VP3 može inducirati humoralni i mukozni imunosni odgovor u guščića te povisiti transkripcijske razine citokina u različitim tkivima. Na taj je način utvrđeno da je rekombinantni *Lactobacillus casei* imao stanoviti preventivni učinak protiv Derzsyjeve bolesti (30%), koji je bio jednak učinkovitosti komercijalnog cjepiva u zaštiti od te bolesti.

Ključne riječi: parvovirus gusaka; Derzsyjeva bolest; živo rekombinantno bakterijsko cjepivo; *Lactobacillus casei*