IMMUNE EFFICACY OF INACTIVATED *BORDETELLA BRONCHISEPTICA* VACCINE IN RABBITS

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ABSTRACT

Vaccination is considered to be an effective way to prevent and control *Bordetella bronchiseptica* (*B. bronchiseptica*) disease. However, licensed vaccines are not available for rabbits in China at present time. In order to determine the efficacy of whole-bacterium inactivated vaccine in rabbits, immunization trials were conducted by using New Zealand white rabbits. The results showed that a single dose of subcutaneous immunization with 1.6×10^{10} CFU inactivated whole cells (CZJ-1 strain) resulted in high levels of specific IgG antibody, and high protection rate against *B. bronchiseptica* challenge in rabbits (2.0×10^{10} CFU) during 21 to 120 days post immunization (protection rate $\geq$ 87%). In this study, we reported a success in preparation of a potent and efficacious inactivated *B. bronchiseptica* vaccine which is a promising approach for controlling *B. bronchiseptica* disease in rabbits.

Key words: *Bordetella bronchiseptica*, inactivated vaccine, immune efficacy

INTRODUCTION

B. bronchiseptica is a highly transmissible respiratory pathogen that infects diverse animal species and occasionally humans. It is the cause of respiratory diseases such as rhinitis and pneumonia in swine, kennel cough in dogs, and snuffles in rabbits (Long *et al*., 2010). *B. bronchiseptica* often causes acute death in young rabbits and rhinitis, bronchitis and pustular pneumonia in adult rabbits. *B. bronchiseptica* infections are endemic in commercial rabbitries, and it is difficult to be controlled due to the rapid spread and persistence of the infection . A group of free-range rabbits were studied for five consecutive years, the incidence of the disease is between 88% and 97%. *B. bronchiseptica* can also coinfect with other pathogens such as toxigenic *Pasteurella multocida* which can result in huge economic losses (Magyar *et al.*, 2011). There are commercial vaccines for swine and dogs, including *B. bronchiseptica* as inactivated vaccine antigen (Ellis *et al*., 2014; Scott-Garrard *et al*., 2018) while the immunogenicity of inactivated *B. bronchiseptica* Ag has not yet been elucidated in rabbits and there was no commercial vaccine for rabbits in China. The efficacy of inactived *B. bronchiseptica* vaccine *in* rabbits were studied in this study.

MATERIALS AND METHODS

Animals and experimental design

The *B. bronchiseptica* strain CZJ-1 originally isolated from a rabbit with infectious rhinitis and identified by Animal Husbandry and Veterinary Institute, Zhejiang Academy of Agricultural Sciences. Mineral oil was provided by Connaught times Wei Biotechnology Co., Ltd. Thirty-five-day-old healthy New Zealand white rabbits were purchased from Zhejiang Animal Center of Animal Husbandry Institute, Zhejiang Academy of Agricultural Sciences. The animals were observed for 7 days prior to use. The animal experiments were approved by the ethics committee of the Zhejiang Academy of

Agricultural Sciences (ethics protocol no. 001012 and no. 002067). Animal studies were conducted following the principles and guidelines of the Zhejiang Farm Animal Welfare Council of China.

Preparation of oil adjuvant inactivated antigen

B. bronchiseptica antigen was prepared by Connaught Times Wei Biotechnology Co., Ltd. *B. bronchiseptica* (CZJ-1 strain) was cultured through GMP certified fermentation, sterilized with 0.2% formalin for 36 h, and kept at 4°C after a sterilization test. The inactivated *B. bronchiseptica* antigen was concentrated with PBS to 6.4×10^{10} CFU /mL. Then the concentrated antigen was mixed with mineral oil adjuvant at 1:3 (vol/vol) and the final vaccine preparation is about 1.6×10^{10} CFU /mL.

Vaccination and challenge trial

Experiment A.

Thirty-five days old male New Zealand rabbits were prepared for the vaccination trial. Twenty rabbits were immunized subcutaneously in the neck with 1 mL of inactivated *B. bronchiseptica* vaccine on Day 1, fifteen rabbits were injected with 1 mL of PBS. Blood samples were collected before immunization and 6, 13, 20, 27, 41, 57, 89, 117, 148, 184 days post-immunization from the ear vein and stored at 4 °C for serum separation. Whole bacteria protein-specific IgG antibodies in serum were detected by an indirect ELISA. Whole cell protein was prepared by our laboratory for ELISA assay. The optimal conditions for the indirect ELISA were determined to be 1 ìg/mL of *B. bronchiseptica* bacteria whole protein, a 1: 400 serum dilution, and a 1: 5000 dilution of secondary antibody. Specific IgG antibody was detected by *B. bronchiseptica* bacteria whole protein-based indirect ELISA as previously described (Xiao *et al*., 2016).

Experiment B.

A total of two hundred and ten 35 days old male New Zealand rabbits were prepared for the vaccination trial. Seventy rabbits were immunized subcutaneously inactived *B. bronchiseptica* vaccine, the other seventy rabbits were injected with PBS on Day 1. 14, 21, 28, 60, 90, 120 and 150 days post immunization, 10 rabbits were randomly selected from immunization group and control group, and challenged with *B. bronchiseptica* (1mL, 2.0×10^{10} CFU/mL) through ear vein injection. After the challenge, the animals were closely monitored for 15 days to determine their survival rate and to monitor whether the animals were moribund.

Statistical Analysis

Statistical differences between groups were determined using IBM SPASS statistics 19.0 (SPASS Software).

RESULTS AND DISCUSSION

The titers of antibodies were analyzed by SPASS 19.0 statistical software and LSD-t test. Data were expressed as the mean ± S.D. The results showed that the vaccine induced a strong antibody response in all vaccinated rabbits two weeks after the immunization, the specific IgG antibodies levels increased to the highest value by day 13, then stabilized at high levels and last until day 148 after immunization. By day 184 after the immunization, the titer of antibodies decreased, which was similar to the titer of antibodies by day 7 after immunization.

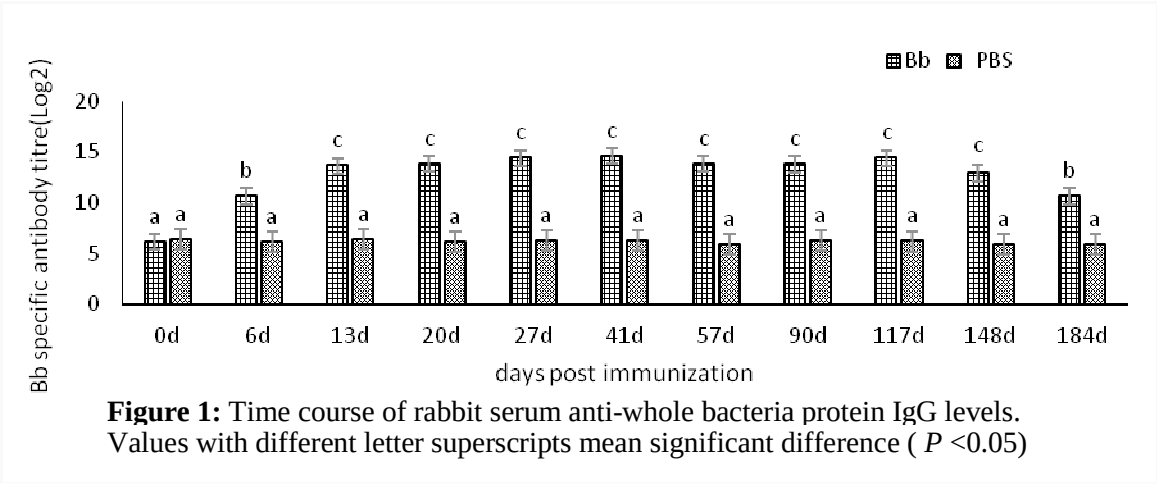

Figure 1: Time course of rabbit serum anti-whole bacteria protein IgG levels. Values with different letter superscripts mean significant difference (P <0.05)

The survival rate post *B. bronchiseptica* challenge are shown in Fig. 2. 14, 21, 28, 60, 90, 120 and 180 day after vaccination, the protective rate in immunization group was significantly higher than that in control group. The results showed that protective rate of the vaccine was 73% at 14 day after vaccination, and 87% protective rate at 21 day after vaccination, 67% at the end of 6 months after vaccination. So it concluded that the inactivated vaccine had a high effective protection within 4 months. The immunity protection term can be defined as 4 months.

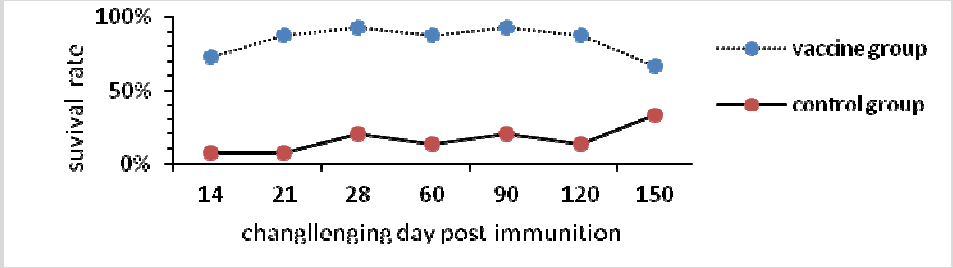

Figure 2: Survival rate of rabbits in vaccinated and control group challenged with live *B. bronchiseptica*

CONCLUSIONS

In this study, CZJ-1 strain isolated in laboratory was used as the vaccine strain. The inactivated oil adjuvant antigen was prepared, and the intravenous injection was as the challenging route. The study proved that the whole bacterial antigen of *B. bronchiseptica* had high immune protection for rabbits, and the immune protection could last for 4 months. The experiments provides a technical basis for the study of the whole-bacterium inactivated *B. bronchiseptica* vaccine for rabbits .

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