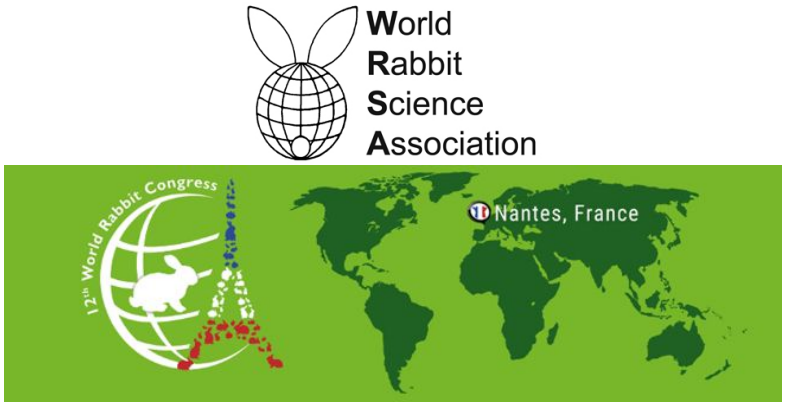

PROCEEDINGS OF THE 12th WORLD RABBIT CONGRESS

Nantes (France) - November 3-5, 2021

ISSN 2308-1910

Session

PATHOLOGY & HYGIENE

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How to cite this paper

Wei Qiang, Qian Wei, Xiao Chenwen, Liu Yan, Ji Quan'an, Huang Ye'e, Li Ke, Bao Guolian, 2021. Establishment of pathogenesis model of *Bordetella bronchiseptica* in rabbits. Proceedings 12th World Rabbit Congress - November 3-5 2021 - Nantes, France, Communication P-39, 4 pp. + presentation

ESTABLISHMENT OF PATHOGENESIS MODEL OF *BORDETELLA BRONCHISEPTICA* IN RABBITS

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ABSTRACT

In order to establish the pathogenetic model of *Bordetella bronchiseptica(B.b.)* in rabbits，rabbits were challenged with a virulent strain FX-1 which has been screened out by the way of rabbit intravenous, intrapleural, intraperitoneal, nose drop and subcutaneous injection. And the most suitable way has been screened out. The half lethal dose of FX-1 strain to rabbit is 6.61×10^9 CFU. The pathogenetic model of *B.b.* in rabbits has been established. The model was used to test the immune effect of the inactivated vaccine of *B.b.*, and the ideal result was obtained. It proved that the established model was accurate and reliable.

Key words: rabbit, *Bordetella bronchiseptica* in rabbit, pathogenesis model

INTRODUCTION

Bordetella bronchiseptica (*B.b.*) is one of the main pathogens of infectious rhinitis. Its main symptoms and pathological changes are inflammation of respiratory tract. The morbidity and mortality were 10-25% and 5-10% respectively. Due to the chronic course of the disease and the weak virulence of the pathogen, it is difficult to establish an ideal pathogenesis model. In recent years, we isolated and identified several strains of *B.b.*, infected mice through intraperitoneal injection, and have screened out the strain FX-1 with relatively strong virulence (Qian, 2013). This strain will be used to infect experimental rabbits through different routes to screen out the most suitable route of infection; Through this route, the half lethal dose of the strain was determined, the disease model was established. Further, through the test of the efficacy inactivated vaccine of *B.b.*, the reliability of the disease model was confirmed. The results lay a good foundation for evaluating the immune effect of the pathogen vaccine.

MATERIALS AND METHODS

Preparation of experiment strain and bacterial solution

FX-1 strain was preserved by freeze-drying, The LD_{50} was 3.22×10^6 CFU to mice. It was cultured and resuscitated on TSA medium at 37℃. After the incubation, a single colony was selected and transplanted into TSB liquid medium. After 18-20 hours of culture in shaking bed at 200rpm, the OD value and bacterial content were determined.

Experimental animals

The experimental rabbits were 40-45 days old , which were purchased from the experimental rabbit farm of Zhejiang Academy of Agricultural Sciences. Their antibody of *B.b.* were negative.

Pathogenetic characteristics of different challenge ways in experimental rabbits

The bacterial content was adjusted to about 7×10^9 CFU/ml, 1ml per dose of a rabbit. 48 rabbits were divided into 5 experimental groups, which were divided into 5 ways: nose dropping, intravenous, intrapleural, intraperitoneal, and subcutaneous injection. 8 rabbits in each way, 8 rabbits in control

group; The dose of nose dropping is double. Observe for 3 weeks after challenge, the morbidity and mortality of each experimental rabbit were recorded. The pathological changes of the dead rabbit were observed by autopsy, and the bacteria were isolated and identified from the heart blood, liver and other organs. The most suitable way to challenge was screened out.

Determination of LD_{50} in experimental rabbits

The bacterial content was adjusted to 4.5×10^{10} CFU/mL by centrifugation，and diluted to 4.5×10^{6} CFU/mL continuely for 10 times, with total 5 dilution and 1ml dose per of a rabbit. According to the best challenge way which was selected. 36 rabbits were divided into 6 groups, 6 experimental rabbits in each group, one of them was the control group. Observed three weeks after challenge, the morbidity and mortality of the rabbits were recorded. The dead rabbits were dissected and the bacteria were isolated and identified from heart blood, liver and other organs. The LD_{50} was calculated by Kohl's method (Gu et al., 2009; Tan, 2010) and processed by Excel software. $LgLD_{50} = \Sigma 1/2\ (X_i+X_{i+1})(P_{i+1}-P_i)$, where X_i is the dose logarithm and P_i is the mortality.

Determination of immune effect of inactivated vaccine of *B. b.* in rabbit

In order to test and evaluate the practical application effect of the model, 40 of 64 experimental rabbits were immunized with 1ml of *B.b.* inactivated vaccine (antigen content 1.50×10^{10} CFU/ml), the other 24 rabbits were used as control simultaneously. At 7, 14, 21 and 31 days after immunization, the doses of challenge were more than $2LD_{50}$. Observe for 2 weeks after challenge, the morbidity and mortality of each experimental rabbit were recorded.

RESULTS AND DISCUSSION

Results of different challenge ways in experimental rabbits:

The test results are shown in Table 1, in which there is no death in the experimental rabbits of nasal and subcutaneous ways; 87.5% of death rabbits in intrapleural injection, and 100% of death rabbits in intravenous and intraperitoneal injection, respectively; Peritonitis was found in the dead rabbits through intraperitoneal injection, no pathological changes were found in the chest and lung, and the original challenge bacteria were not isolated; While in the rabbits of intravenous injection, there were typical respiratory symptoms and pathological changes, and the original bacteria *B.b.* can be isolated from the heart blood and liver. However, only focal pneumonia can be seen in intrapleural injection, and the operation was not easy. Therefore, intravenous was selected as the challenge route of the rabbit pathogenic model.

Table 1: The results of challenge with FX-1 strain in rabbits by different routes

Challenge route	Dose(CFU/mL)	Rabbit No.	Dead No.	Dead rate(%)
Nose dropping	14×10^{9}	8	0	0
Intrapleural	7×10^{9}	8	7	87.5
Intraperitoneal	7×10^{9}	8	8	100
Subcutaneous	7×10^{9}	8	0	0
Intravenous	7×10^{9}	8	8	100
Control	TSB1ml	8	0	/

The results of determining the LD_{50} of strain FX-1 to rabbit:

After challenge, all the rabbits in Group 1 died 24-48 hours later, and a large number of bleeding spots were found in the lungs, and enlarged spleen. Group 2: 2 rabbits died on the 4th day after challenge. On autopsy, pustules of different sizes were found in the lungs and kidneys. In the other 4 rabbits, they recovered gradually after 4 days. Group 3: one died on the 10th day after challenge, and a large number of purulent lesions were found on the lung and kidney, spleen atrophy was found on the autopsy; the other 4 rabbits gradually recovered their appetite and survived. There was no death rabbits in groups 4 and 5. In the control group, there was no significant change in spirit and appetite. The original bacteria *B.b.* could be isolated from liver and heart blood in all the dead rabbits. The results were shown in Table 2. The LD_{50} of *B.b.* was calculated to be 6.61×10^{9} CFU by using Excel

software and Kohl's method (Gu et al., 2009; Tan, 2010). The dose of challenge was a little large, which meant that the virulence of bacteria was relatively weak.

Table 2: Determination results of half lethal dose of *B.b.* to rabbit

Groups	Dose(CFU/mL)	No. Of rabbit	Dead No.	Dead rate
1	4.5×10^{10}	6	6	6/6
2	4.5×10^{9}	6	2	2/6
3	4.5×10^{8}	6	1	1/6
4	4.5×10^{7}	6	0	0/6
5	4.5×10^{6}	6	0	0/6
Control	TSB1ml	6	0	0/6

The results of immune effection with inactivated vaccine of *B.b.* :

The test results were shown in Table 3. It showed that the protection rates of the experimental rabbits on the 7, 14, 21 and 31 day after immunization were 60%, 80%, 90% and 80% respectively, while the mortality rates of each group in the control group were 100%, 83.3%, 66.7% and 66.7% respectively. The protective effect of the vaccine was very good, and the test results correctly detected the immune effect of the vaccine. Although the dose of each experimental group was more than $2LD_{50}$, the ideal results were obtained. Therefore, the challenged dose was appropriate, and the challenged route of intravenous were reliable. The model is ideal.

Table 3: Results of immune effect with inactivated vaccine of *B.b.*

Days after immu.	No. Of exp. rabbit		Challenge dose (CFU/mL)	Live No. After chall.		Pro. rate (%)	
	immu.	control		immu.	control	immu.	control
7	10	6	14.25×10^{9}	6/10	0/6	60	0
14	10	6	15.0×10^{9}	8/10	1/6	80	16.7
21	10	6	16.05×10^{9}	9/10	2/6	90	33.3
31	10	6	14.28×10^{9}	8/10	2/6	80	33.3

CONCLUSION

The rabbits of 40 days old were used as the experimental animal, and FX-1 strain was used as the model of *B.b.* infection through intravenous injection. The half lethal dose was 6.61×10^{9} CFU. The results can effectively evaluate the immune efficacy of the inactivated vaccine of *B.b.* .

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Establishment of pathogenesis model of *Bordetella bronchiseptica* in rabbits

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Context : Bordetella bronchiseptica (*B.b.*) was used to infect experimental rabbits to screen out the most suitable route of infection; Through this route, the half lethal dose was determined. Then, the disease model was established.

Methods :(1)Determine infection routes:The *B.b.* bacterial content was adjusted to about 7×10^9 CFU/ml. 40-45 days old rabbits were challenged via 3 ways: intravenous, intrapleural, intraperitoneal.(2)Determine of LD_{50}:The bacterial content was adjusted to 4.5×10^{10} CFU/mL and diluted to 4.5×10^6 CFU/mL continuely for 10 times, with total 5 dilution. And the best challenging way was selected .

Result1: There were typical respiratory symptoms, pathological changes, and 100% of death in the intravenous injection rabbits. Therefore, intravenous injection was selected as the challenge route of the rabbit pathogenic model.

Chall. route	Dose(CFU)	Rabbit No.	Dead No.	Dead rate(%)
Intrapleural	7×10^9	8	7	87.5
Intraperitoneal	7×10^9	8	8	100
Intravenous	7×10^9	8	8	100

Result 2 : The LD_{50} of B.b. was calculated to be 6.61×10^9 CFU by using Excel software and Kohl's method. The dose of challenge was high, which meant that the virulence of bacteria was relatively weak.

Groups	Dose(CFU/mL)	No. Of rabbit	Dead No.	Dead rate
1	4.5×10^{10}	6	6	6/6
2	4.5×10^9	6	2	2/6
3	4.5×10^8	6	1	1/6
4	4.5×10^7	6	0	0/6
5	4.5×10^6	6	0	0/6

Conclusions :The model of B.b. infection:the rabbits of 40 days old was used as the experimental animal,and the challenge route was intravenous injection. The half lethal dose was 6.61×10^9 CFU.

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ASFC www.asfc-lapin.com
12th World Rabbit Congress November 3-5 2021 - Nantes France
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