ESTABLISHMENT OF INFECTION MODEL OF PATHOGENIC *ESCHERICHIA COLI* IN RABBITS BY ORAL ADMINISTRATION

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ABSTRACT

In order to evaluate the efficacy of antibiotic substitutes for pathogenic *E.coli* in rabbits, it is necessary to establish an oral *E.coli* infection model. After pathogenic *E.coli* and concentrated bacterial solution were prepared, four different challenge ways were adopted, including intramuscular, intraperitoneal, intravenous injection and oral administration. The dose range from 7.3×10^8 CFU/ml to 175.2×10^8 CFU/ml according to the design requirements. The results showed that all of the four routes could cause diarrhea and death in the experimental rabbits, but the oral route with a dose of 175.2×10^8 CFU had the best effect, the most typical symptoms and pathological changes, Thus, it is the most suitable way for the actual needs in evaluation of antibiotic substitutes. Therefore, the model of diarrhea caused by oral administration of pathogenic *E.coli* was successful in this study, which laid a foundation for the evaluation of antibiotic substitutes of pathogenic *E.coli*.

Key words: Rabbit, *Escherichia coli*, Diarrhea, Model

INTRODUCTION

Rabbit colibacillosis is a common and multiple enteric infectious disease caused by pathogenic *Escherichia coli*(*E.coli*) and its toxin. It is most likely to occur in 1-3 months old young rabbits. Once the disease occurs, it often leads to a large number of deaths in rabbits. The morbidity and mortality are very high, which can reach 10-30% and 30-50% respectively, causing serious economic losses in rabbit breeding industry (Guo et al., 2009; Li et al., 2016).

In order to control the disease, antibiotic treatment is the first choice, but China will stop adding antibiotics to feed from 2020. Therefore, we must look for antibiotic substitutes. In the process of searching, how to pass clinical examination and evaluate the effect of these substitutes? Therefore, the establishment of pathogenesis model is very important, but there is no ready-made. Therefore, we have carried out research in this area, and successfully established a disease model of *E.coli*.

MATERIALS AND METHODS

Preparation of strains and bacterial solutions

Pathogenic *E.coli* isolated and identified from a rabbit farm in Zhejiang Province (Wei et al., 2017). After culture on TSA plate, a single colony was selected and transplanted into TSB liquid culture medium. After 18 hours of shaking at 37℃, 200rpm, the bacterial content was measured. Part of the bacterial solution is 5000 rpm, concentrated through centrifuged for 10 min, Suspended sediment to the required concentration for standby.

Experimental rabbits and feed
40 days old New Zealand experimental rabbits fed with no antibiotic feed, or 40 days old SPF New Zealand experimental rabbits fed with no antibiotic feed.

Experimental group
Experiment 1: 16 New Zealand experimental rabbits were divided into four groups, 4 in each group: three experimental groups (intramuscular, intraperitoneal, intravenous injection) and one control group. The dose of challenge was 38.0×10^8 CFU, and the control group was injected with 1ml of TSB sterile culture solution. It was observed for 4 weeks after challenge, the dead rabbits were dissected and bacteria were isolated.

Experiment 2: 20 SPF experimental rabbits were divided into five groups, 4 in each group: abdominal cavity, oral group 1, oral group 2, oral group 3 and control group. The dose of intraperitoneal challenge group was 14.6×10^8 CFU; Oral 1 group was 7.3×10^8 CFU; oral 2 group was 89.2×10^8 CFU; oral 3 group was 175.2×10^8CFU; control group: 2ml TSB sterile culture solution. The dead rabbits were dissected and the bacteria were isolated. Each experimental group was forbidden to eat 12 hours before challenge.

RESULTS AND DISCUSSION

Results of test 1:
Experiment rabbits in each group suffered from mental depression and loss of appetite 24 hours after challenge; 3 days later, all the rabbits of the group challenged in the intravenous were died. Only one of them had mild diarrhea symptoms, the autopsy showed mild intestinal inflammation. The rest of the dead rabbits did not have intestinal lesions; 2/4 of the rabbits of the group challenged via intramuscular injection suffered from diarrhea and died 6 days later, the autopsy showed that The intestinal inflammation was obvious, the mesenteric lymph nodes were swollen, and other organ lesions were not obvious. 2 of this group survived, but were obviously emaciated; All the patients challenged in the group of intraperitoneal died 6 days later, and 2/4 of them had diarrhea. Autopsy of the dead rabbits: ascites and cellulose exudate were found in the abdominal cavity, a thin envelope was found on the surface of the liver, and mesenteric blood vessels were obviously congested. No abnormality was found in the control group. The original challenged bacterial can be isolated from the mesenteric lymph nodes, liver and blood of all of the dead rabbits. The results are shown in Table1 which showed that the rabbits challenged in intravenous were died too fast. Diarrhea was found challenged via intramuscular injection, but only 2/4 of them died. Diarrhea was found in 2/4 of rabbits challenged via intraperitoneal injection, and 4/4 of them died. Among these three routes, intraperitoneal challenge is relatively suitable as a model, but unfortunately, diarrhea is not typical.

Table 1: Results of challenge

Exp. group	No. exp.	No. Diar.	No. dea.
Intramuscular	4	2	2
Intraperitoneal	4	2	4
Intravenous	4	1	4
Control	4	0	0

Results of test 2:
The results are shown in Table2.
Two rabbits died within 24 hours after challenged in the intraperitoneal group, without any symptoms, mainly due to septicemia. The other two died 72 hours later, with mild diarrhea, cellulosic exudation in the abdominal cavity, and cellulose adhesion on the surface of the liver and spleen.

*In the oral group 1***:** One rabbit began to suffer from anorexia, abdominal distention and soft feces on the 4th day after challenge; On the 8th day, this rabbit began to pull mucus stool, the shape was gelatinous, emaciated, fasted on the 12th day, and died on the 13th day. The other three were normal

all the time. Autopsy of dead rabbits: emaciation, dehydration, fecal adhesion in anus, abdominal distention, obvious inflammatory lesions in various segments of small intestine, filled with viscous liquid in intestinal cavity.

Oral group 2: On the 4th day after oral challenge, the appetite of two rabbits decreased, the feces became thin and small, and the feces became soft on the 5th day; diarrhea began on the 6th day, and gradually became serious: from soft stool to paste, jelly-like, and then to bloody stool, the spirit became poor, and thin; died shortly after hunger strike. The other two rabbits were the loss of appetite, emaciation, and then, become gradually improved and survived. The pathological changes of the dead rabbits were similar to those of the oral group 1.

Oral group 3: On the 4th day after oral challenge, the appetite of all experimental rabbits decreased, and the feces showed obvious changes: the feces of three rabbits became thin and small, and gradually began to become paste, jelly-like, turned to bloody stool, died shortly after the fast; The other one's feces became soft, but gradually improved after three days, and survived. The pathological changes of the dead rabbits were similar to those of the oral group 1.

Table 2: Results of challenge

Exp. group	No. exp.	Dose（CFU/mL）	No. Diar.	No. dea.
Oral 1	4	7.3×10^8	1	1
Oral 2	4	89.2×10^8	2	2
Oral 3	4	175.2×10^8	3	3
Intraperitoneal	4	7.3×10^8	2	4
Control	4	2ml TSB	0	0

The results of test 2 showed that 2/4 of the rabbits challenged via intraperitoneal injection suffered from mild diarrhea and atypical symptoms, the main pathological changes of the dead rabbits were acute septicemia. The typical pathological changes could be reproduced in the three test groups of oral routes: After challenged, firstly, the spirit of the rabbits became poor, the appetite decreased, then the form of feces began to become soft and thin gradually, until pyogenic stools were produced, and finally died. Pathological anatomy showed typical inflammatory changes in each segment of intestine. All the dead rabbits in each experimental group were able to isolate and identify the original challenged bacteria from liver, heart blood and mesenteric lymph nodes.

Therefore, oral administration can reproduce typical diarrhea, but the oral dose needs to be large, 175.2×10^8 CFU is appropriate.

CONCLUSIONS

An oral infection model of pathogenic *E.coli* was successfully established in this study: SPF New Zealand experimental rabbits of 40 days old were challenged in oral with a dose of 175.2×10^8 CFU, fasting for 12 hours before challenged, and fed without antibiotics. The experimental results laid a good foundation for the evaluation of the effect of oral antibiotic substitutes in the prevention and treatment of diarrhea.

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