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Research Article

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Clinical trial on the efficacy of compound sulfadiazine suspension

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ABSTRACT

The Efficacy of Compound Sulfadiazine Suspension in the treatment of Escherichia coli and Salmonella was investigated. One hundred eighty 15-day-old chickens were randomly divided into six groups, which were blank control group, negative control group, Trisulmixos gavage group, Trisulmixos in drinking water group, Sufuning gavage group, Sufuning in drinking water group resppectively. Each group contained three repeating treatments and each treatment contains 10 chickens. Results indicated that the efficacy of both Trisulmixos gavage group and Sufuning gavage group on treating Escherichia coli and Salmonella were better than other groups. The death rates of the two groups decreased by 10 percent, and their cure rates reached 30% and 40%.

Keywords: Compound Sulfadiazine Suspension; Efficacy; Trisulmixos; Sufuning

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INTRODUCTION

With the development of poultry industry, it has become the main tendency of poultry epidemic that poultry were infected by Escherichia coli and Salmonella [1, 2]. High morbidity rates, mortality rates become the main feature of this tendency, which caused serious economic losses in poultry industry [3-6]. Because of being many serotypes and depending on different regions, these two bacteria cannot be prevented by vaccine [7, 8]. Drug prevention is still the main method used in the clinical practice [9, 10]. However, the two kind of bacteria have produced resistance and cross-resistance for many commonly used antibiotics at present, which increased the difficulty of treatment [11-13]. The main ingredients of compound sulfadiazine suspension are sulfadiazine and trimethoprim. This drug can be used for prevention and treatment of pullorum caused by Escherichia coli and Salmonella [14, 15]. Sufuning is a kind of product of compound sulfadiazine suspension produced by Qingdao KDN Pharmaceutical Co., Ltd. For the purpose of evaluation the clinical efficacy of Sufuning, the following clinical trials were conducted.

EXPERIMENTAL SECTION

Drugs used in clinical trial

The targeting drug is Sufuning. The formulation of Sufuning is compound sulfadiazine suspension. This drug was provided by Qingdao KDN Pharmaceutical Co., Ltd. Control drug is Trisulmixos. The formulation of Trisulmixos is also compound sulfadiazine suspension, produced by France Vic Co. Ltd.

Experimental animals

Two hundred twenty one-day-old broilers bought from Chia Tai avian field. Before the test, these broilers were fed according to conventional breeding, feeding the complete feed without any drug, with ad libitum and free access to water. At 7 day old, these broilers were immunized using Newcastle disease LaSota vaccine by intranasalling. When growing to 15 day old, these broilers were used in clinical trials.

Experimental bacteria and reagents

Bacteria used in experiment were Escherichia coli and salmonella which were reserved in our laboratory.

The reagents used in experiment were MacConkey, LB agar and nutrient broth medium. All of them were purchased from Beijing Luqiao Technology Co., Ltd.

Preparation of bacterial liquid used in experiment

At first, preserved Escherichia coli and Salmonella bacteria were inoculated on LB agar and MacConkey agar. After being cultivated for 24 hours at 37℃, typical colonies were selected to inoculate on 100ml Broth medium and then to cultivate in desktop bath oscillator for 6~8 hours at 37℃. Then 0.1ml bacteria liquid cultivated was gradiently diluted for 10-fold and then was coated on a common flat medium. Finally, colony forming units were counted after being cultivated for 24 hours at 37℃. The ratio of Escherichia coli and Salmonella in mixed bacteria liquid was 3:1.

Pre-experiment for determination of dosage

Bacteria liquid used in experiment was gradiently diluted for 10-fold. Forty 10-day-old broilers were divided into 4 groups, that is, original liquid group, 1×10^{-1} group, 1×10^{-2} group, 1×10^{-3} group. There are ten broilers in every group. These broilers were injected 1ml corresponding bacteria liquid through pectoral respectively. After that, these broilers were observed for 5 days and then the quantity of dead broilers was counted to calculate LD_{50} by Karber method. Finally, the dosage of bacteria liquid was determined.

Treatment experiment

One hundred eighty healthy 15-day-old broilers were weighed one by one and were divided into six groups. There were three repeat treatments in every group and ten broilers in every treatment. Blank control group were isolated and fed normally without any processes. Broilers in other groups were injected 1ml bacteria liquid through pectoral respectively and were fed normally. Other processes of different groups were shown in Table 1. After that, these broilers were observed twice every day and the quantity of the sick and the dead in each group were recorded. The dead were weighed and were dissected to observe pathological changes. The bacteria in corresponding tissues was separated and observed for ten days. After that, remaining living broilers were weighed one by one. Average daily weight gain, average daily feed intake and feed conversion ratio of each group were calculated. Finally, all living broilers were culled and were dissected to observe pathological changes. And morbidity rates, mortality rates, effective rate and cure rate of each group were calculated.

Table 1. Processes of different groups

No	Groups	Samples	Different processes
1	blank control	30	1ml Normal saline gavage for 5 days。
2	negative control	30	1ml Normal saline gavage for 5 days。
3	Trisulmixos gavage	30	After typical clinical symptoms appeared, each broiler was given Trisulmixos 0.08ml/kg by gavage for 5 days
4	Trisulmixos in drinking water	30	After typical clinical symptoms appeared, each broiler was given Trisulmixos 0.4ml/L by centralized watering three hours for 5 days.
5	Sufuning gavage	30	After typical clinical symptoms appeared, each broiler was given Sufuning 0.08ml/kg by gavage for 5 days
6	Sufuning in drinking water	30	After typical clinical symptoms appeared, each broiler was given Sufuning 0.4ml/L by centralized watering three hours for 5 days.

Evaluation criteria for efficacy

Morbidity rates

In pre-experiment, all broilers were sick in 24 hours after being injected mixed bacteria liquid and died centrally in 72 hours. According to this characteristic, when the broiler showed clinical symptoms such as fever, lassitude, loose feathers, two wings droop, necking lethargy, sudden death, severe diarrhea, dysentery, fluffy feathers, stop eating, it was judged to be sick. Therefrom, the quantity of sick broiler was counted and morbidity rates were calculated.

Mortality rates

When broilers died with typical symptoms of Escherichia coli and Salmonella disease and their necropsy showed typical lesions characteristics such as pericarditis or perihepatitis, and Escherichia coli and Salmonella were separated from liver, the broiler was judged to die because of Escherichia coli and Salmonella. Therefrom, the quantity of dead broilers was counted and mortality rates were calculated.

Effective rate

When 5-days observation period ended, if the broiler recovered to normal and stop diarrhea, the broiler was judged to be effective. There, the quantity of effective broilers was counted and effective rate was calculated.

Cure rate

When 5-days observation period ended, if the broiler recovered to normal and stop diarrhea, and if there were no pericarditis or perihepatitis after necropsy, the broiler was judged to be cured. There, the quantity of cured broilers was

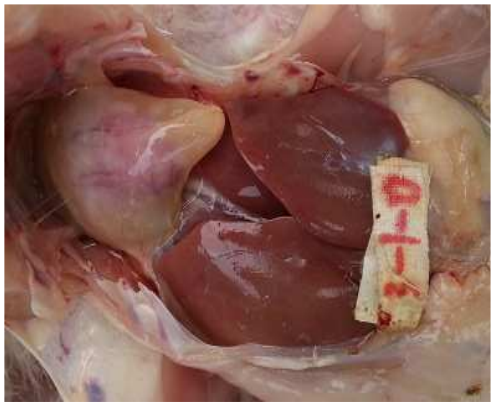
counted and cure rate was calculated.

RESULTS AND DISCUSSION

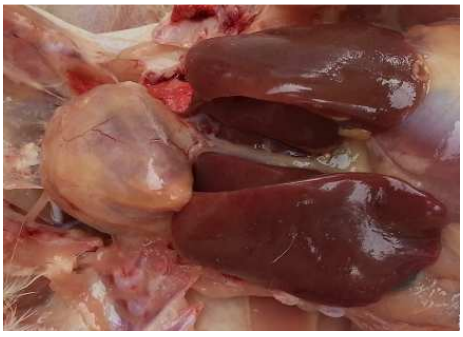
Clinical symptoms and pathological changes after injection bacteria

Through pre-experiment for dosage determination, the dosage for injecting was determined to be 5.6×10^7CFU/ml. After being injected bacteria, broilers showed typical clinical symptoms such as severe diarrhea, lassitude, fever, loose feathers, necking lethargy, two wings droop. All broilers which died in experiment period were conducted necropsy. When their enterocoelias were opened, it smelled stench. And pericarditis or perihepatitis in different extent appeared, shown as Figure 1. Heart and liver were collected to isolate and cultivate pathogens in laboratory. In the early stage of the experiment, Escherichia coli were advantage bacteria. Till late stage of the experiment, Salmonella was the advantage bacteria.

a. Severe pericarditis and perihepatitis



b. Liver is normal but heart has pericarditis



c. Pericarditis, liver has water and punctate exudates

d. Pleural effusion, pericarditis and mild perihepatitis

Figure 1. The photos of heart and liver of broiler after necropsy

Results of effective experiment

As shown in Figure 2, Trisulmixos gavage group and Sufuning gavage group played an important role in treating Escherichia coli and Salmonella. Mortality rates in both of the two groups decreased 10 percent than negative control group. However, the performance of two in water groups wasn't satisfactory. Mortality rates in both of the two groups were consistent with negative control group. It hadn't any efficacy. As shown in Figure 3, the cure rates of Trisulmixos gavage group and Sufuning gavage group reached 30% and 40% respectively. Although the effective rates of Trisulmixos in drinking water group and Sufuning in drinking water group were comparatively high, the cure rates of the two groups are lower than two gavage groups.

When injected mixed bacteria liquid, all of the broilers in negative control group were sick and mortality rate reached to 23.3%. However both the morbidity rate and mortality rate of blank control group were zero. All these results indicated that this artificial attack drug onset modeling was successful. In this experiment, two kind of giving drug method were used, being gavage and drinking respectively. The mortality rate of two gavage groups decreased 43% than that of two drinking groups, which indicated that the method gavage was better than drinking method. The reason, by analyzing, was that the method of gavage can give drug accurately. On the one hand, while drug was given by drinking, broiler individuals got different dosage of drug. On the other hand, when Trisulmixos or Sufuning mixed with water, sulfadiazine and trimethoprim tend to sink to the bottom. Therefore, broilers can only absorb a little drug actually, which cannot assure efficacy.

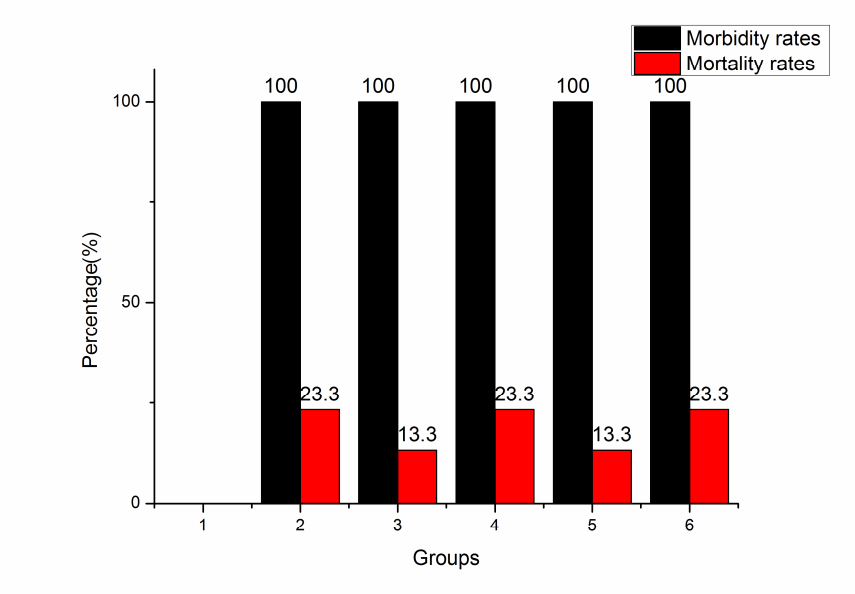


Figure 2. Morbidity rates and mortality rates of every group

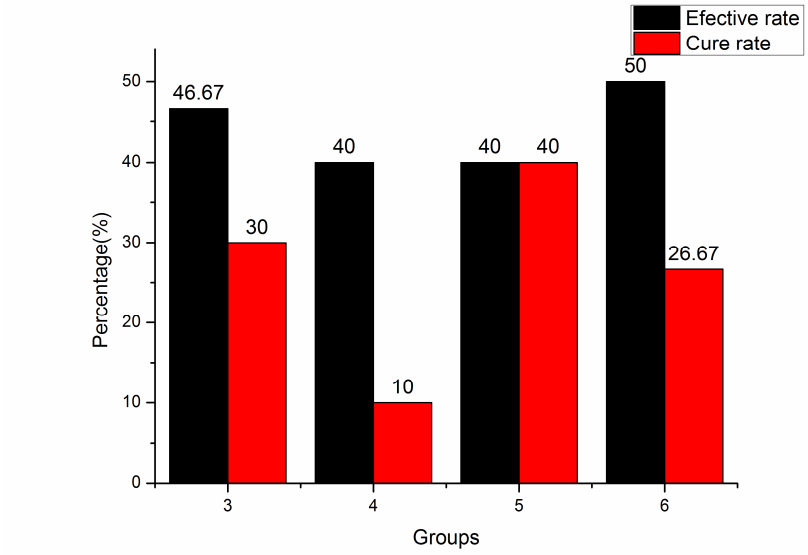


Figure 3. Effective rates and cure rates of groups being injected bacteria

CONCLUSION

From all above-mentioned results, it can be concluded that when broiler was infected by Escherichia coli and Salmonella, Sufuning can be utilized to cure sick broiler. Moreover, the efficacy of giving drug by gavage is better than that of giving by drinking. From the results of treatment experiment, it can be concluded that Sufuning can effectively decrease mortality rate, relieve symptoms and improve the cure rate. For the purpose of developing therapeutic drugs of bacterial or viral infectious diseases for livestock, the mechanism of anti-bacterial of Sufuning needs further study.

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