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Investigation of the efficiency and safety of tilmicosin phosphate in treating experimental mycoplasmal infections in pigs

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Abstract: In order to evaluate the in vivo therapeutic effect of oral administration of tilmicosin phosphate on mycoplasmal pneumonia in swine and its safety for the infected swine, the model of mycoplasmal pneumonia disease in swine was established by artificial infection, and indices such as efficiency, cure rate, death rate, the score of lung lesion, and the routine blood tests, blood biochemistry, and routine urine tests of tested swine were determined. The results showed that all of the dosage groups of 100 mg/L, 80 mg/L, 60 mg/L of 10% tilmicosin phosphate soluble powder demonstrated distinct therapeutic effects on mycoplasmal pneumonia in pigs, significantly reducing the scores of lung lesions in diseased pigs, and that the treatment effect of 60–80 mg/L tilmicosin phosphate soluble powder was equivalent to that of 10% tilmicosin soluble powder. Administration of 10% tilmicosin phosphate soluble powder improved the weight gain of the diseased swine. Treatment of infected pigs with 60–100 mg/L of 10% tilmicosin phosphate soluble powder did not seem to influence the routine blood tests, biochemical index, and routine urine tests, and is therefore safe for diseased swine. In conclusion, administration of 10% tilmicosin phosphate soluble powder is effective and safe to treat artificially infected swine with mycoplasmal pneumonia.

Key words: Tilmicosin phosphate, treatment effect, safety, mycoplasmal pneumonia, pigs

1. Introduction

Mycoplasmal pneumonia is a chronic respiratory disease involved in porcine respiratory disease complex (PRDC), which causes considerable suffering for the animals and enormous economic losses for the pig-farming industry; the main reasons for the losses are costs for treatment and vaccination, decreased performance, and increased mortality from secondary infections (1). *Mycoplasma hyopneumoniae* (*M. hyopneumoniae*) is one of the primary agents involved in PRDC (2) and is primarily located on the mucosal surface of the trachea, bronchi, and bronchioles, and affects the mucosal clearance system by disrupting the clearing actions of the cilia on the epithelial surface. Additionally, the pathogen can also influence the immune system of the respiratory tract (1,2). Because of its slow growth and potential overlap with other swine mycoplasmas, the pathogen is very difficult to isolate for routine diagnostics using bacterial cultures (2). Infections with *M. hyopneumoniae* are clinically characterized by an intermittent and dry cough whose intensity is variable (3). The virtual lung lesions consisting of purple to grey consolidated areas affecting the apical and middle lobes and even diaphragmatic lobes in affected animals are responsible for the cough (4). With the addition of poor air quality to the *M. hyopneumoniae* strain, clinical symptoms may be aggravated, including labored breathing, pyrexia, anorexia, lethargy, and even death (5). Laboratory testing for a conclusive diagnosis includes bacterial isolation, polymerase chain reaction (PCR), and real-time PCR (6,7). In the detection process of sampling, samples collected from bronchi and bronchioles in the lower respiratory tract show a higher sensitivity compared to tissues obtained from the upper respiratory tract (8).

As far as the therapeutics of mycoplasmal pneumonia are concerned, an effective antibiotic is still the first choice to address this disease. Antimicrobial agents against *M. hyopneumoniae* include tetracyclines, 15- and 16-membered ring macrolides, pleuromutilins,

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florfenicol, and aminoglycosides, which interfere with the polymerization of cell wall precursors (8). Tilmicosin is a semisynthetic 16-member macrolide antibiotic widely used in veterinary medicine, such as in the treatment of mycoplasmal pneumonia (9). The antimicrobial mechanism of tilmicosin inhibits the protein synthesis of susceptible bacteria by combining simultaneously with the 50S subunits in the ribosome to block transpeptidation and/or mRNA displacement (10). In clinical application, tilmicosin has shown good efficacy and pharmacokinetic properties for porcine pulmonary infectious diseases such as mycoplasmal pneumonia (11,12). The particular effectiveness of tilmicosin is attributed to its low inhibitory concentration, broad antimicrobial spectrum, large volume of distribution, and its long elimination half-life (11,12). Tilmicosin preparations used in previous reports have included injections, food premixes, and solid lipid nanoparticles (13). However, *M. hyopneumoniae* is intrinsically resistant to the aforementioned antibiotics, and acquired resistance has been documented for 16-membered ring macrolides (tylosin, tilmicosin) (14). Meanwhile, the insolubility and bitter taste of tilmicosin limits its permeability into pathogens and palatability for animals, respectively. In order to overcome this disadvantage and improve efficacy, a phosphate group was added to the chemical structure of tilmicosin to form tilmicosin phosphate (15). Our previous study in vitro illustrated that tilmicosin phosphate demonstrated similar and even more active antibacterial effects on *M. hyopneumoniae* and *M. gallisepticum* compared to tilmicosin, and that tilmicosin phosphate within the dosage of 600 mg/L water was harmless for healthy pigs. However, the in vivo efficacy of tilmicosin phosphate for *M. hyopneumoniae* was not known. Some properties of a drug can be impacted by pathophysiological conditions during an infection (16,17). For example, as previously reported, physiological and biochemical differences in healthy and diseased animals can result in changes to a drug's PK parameters (18). Therefore, the safety of tilmicosin phosphate for infected pigs in the clinical application needs to be further studied.

The aim of this study was to investigate the therapeutic effect in vivo of oral administrations of tilmicosin phosphate on mycoplasmal pneumonia through building an infection model of *M. hyopneumoniae* in pigs. Additionally, the effects of tilmicosin phosphate on some hematological, biochemical, and urinary parameters of infected pigs were studied following oral administrations of different dosages in swine.

2. Materials and methods

2.1. Materials

Tested drugs: 10% tilmicosin phosphate soluble powder (20110401) provided by Ningxia Tairui Pharmaceutical Co., Ltd. (China; 10% tilmicosin soluble powder (11020601) provided by Ringpu (Tianjin) Biological Pharmaceutical Co., Ltd. (China).

Experimental strain: *M. hyopneumoniae* virulent strain (lyophilized AH strain) was provided by Dr. Guoqing Shao, Veterinary Research Institute, Jiangsu Academy of Agricultural Sciences.

Laboratory animals: 60 healthy weaning Landrace pigs (30 male and 30 female), which were about 40 days old, free of mycoplasmal pneumonia (ELISA detection, data not shown), and weighing 7 kg ± 2 kg, were purchased from Dingshan Pig Farm (Nanjing, China). The selected pigs were not vaccinated against *M. hyopneumoniae* before the experiment. During the periods of acclimatization and experiment, all the tested pigs received routine feeding and had free access to drinking water. No antibiotics were given. The environmental temperature and relative humidity in the animal housing were maintained at 15–20 °C and 80%– 90%, respectively.

All experimental protocols concerning the handling of pigs were in accordance with the requirements of the experimental animal ethics guidelines of the Ethics Committee at the laboratory animal center of Nanjing Agricultural University (license number: SYXK(Jiangsu)2011-0036), and all efforts were made to minimize any stress to the animals.

2.2. Experimental groups

Before the experiment, all of the tested pigs were weighed and grouped randomly (half male and half female in each group) according to similar body weight (Table 1). Excluding the positive and negative controls, the high dose group received 100 mg/L of 10% tilmicosin phosphate soluble powder; the medium dose group, 80 mg/L of 10% tilmicosin phosphate soluble powder; the low dose group, 60 mg/L of 10% tilmicosin phosphate soluble powder. Meanwhile, the drug control group received 75 mg/L of 10% tilmicosin soluble powder as recommended by the instructions. The tested drugs for each group were administered once daily for 7 d. The different groups of swine were segregated from each other by raising them in isolated houses of different Specific Pathogen Free (SPF) levels to avoid cross-influence.

2.3. Artificial infection of experimental animals

Lyophilized *M. hyopneumoniae* virulent AH strain was dissolved and diluted, with normal saline, to reach eventually 1×10^9 ccu/mL. The experimental pigs from the high-, medium-, and low-dosage groups, drug control group, and positive control group were inoculated with a solution of *Mycoplasma hyopneumoniae* into the trachea at a dosage of 5 mL/head. Experimental pigs in the negative control group were injected with normal saline into the trachea at the dosage of 5mL/head. After artificial infection, clinical observations such as the behavior, appetite, and

Table 1. The experimental groups and corresponding treatments.

Group	Quantity(number of pigs)	Processing method
High-dose group	10	Infected with *M. hyopneumoniae*, drinking water mixed with 100 mg/L of 10% tilmicosin phosphate soluble powder
Medium-dose group	10	Infected with *M. hyopneumoniae*, drinking water mixed with 80 mg/L of 10% tilmicosin phosphate soluble powder
Low-dose group	10	Infected with *M. hyopneumoniae*, drinking water mixed with 60 mg/L of 10% tilmicosin phosphate soluble powder
Drug control group	10	Infected with *M. hyopneumoniae*, drinking water mixed with 75 mg/L of 10% tilmicosin soluble powder
Positive control group	10	Infected with *M. hyopneumoniae*, no treatment
Negative control group	10	No infection, no treatment

breathing of the pigs were conducted and recorded every day (19).

2.4. Drug treatment

Seven to fourteen days after inoculation, apart from the negative control group, all infected pigs began to show typical symptoms of asthma, sneezing, coughing, depression, loss of appetite, and fever, and were given drugs in accordance with Table 1. Daily observation of clinical reactions in the pigs after administration of the drugs was conducted for consecutive 15 days. All pigs were weighed at the end of the experiment.

2.5. Evaluation of therapeutic effect

2.5.1. Death rate

Typical clinical symptoms of a mycoplasmal pneumonia infection were observed, and necropsy revealed typical pancreatic islet lesions of lungs. If *Mycoplasma hyopneumoniae* could be isolated from the lung tissue, we concluded that the death was caused by the experimental infection. The death rate was calculated from the number of infected dead pigs divided by the number of tested pigs in the corresponding group.

Five grades were set for the typical clinical symptoms (forced breathing, sneezing, cough, depressed behavior, diminished appetite), namely 0, 1, 2, 3, 4, and 5, which represented corresponding severity: normal, very slight, slight, moderate, severe, and very severe, respectively (19). A grade was given to each pig in all groups at the end of the experiment; a comprehensive comparison of the scores above was then conducted by experts to reach a conclusion for cure, effective treatment, or no effect, and then the cure rate was determined.

2.5.2. Cure rate

During the test period, after medication was administered, a certain number of pigs returned to normal health. The clinical symptoms of infection disappeared, and behavior, appetite, respiration, and body temperature returned to normal. No recurrence took place after withdrawal of treatment. The cure rate was calculated from the number of diseased pigs that were cured divided by the number of tested pigs in the corresponding group.

2.5.3. Effective treatment rate

Both the cured pigs and the animals which showed a marked improvement in behavior and increased appetite after drug treatment but still displayed mild abdominal breathing or cough were considered to be effectively treated. The total efficacy was calculated from the number of effectively treated pigs divided by the number of tested pigs in the corresponding group.

2.5.4. Score of lung lesions

Each lung lobe of all pigs was assessed according to Table 2. Scoring comprised only the ventral side of the accessory lung lobe; both the dorsal and ventral sides in the other lobes were scored and the results given as an average. The scores from 7 lung lobes were added together to form the final number of scored points for each individual pig. The maximum possible number of points was 28. Reduction rate of lung injury following the administration of tilmicosin was calculated according to the following formula (19):

$$\text{Reduction rate of lung injury (\%)} = \frac{\text{Score (Positive control group)} - \text{Score (experimental group)}}{\text{Score (Positive control group)}}$$

2.5.6. Weight gain rate

The weight gain per pig was calculated from the difference in body weights at the beginning and end of the experiment. The average weight gain of the group was calculated accordingly. The weight gain rate of each group was calculated by dividing the average weight gain per pig by the average body weight before the experiment.

2.6. Blood sampling and the evaluation of drug safety

At the end of the experiment, anticoagulant whole blood samples of all tested pigs were taken from the anterior

Table 2. The score standard for lung lesions.

Specific lesion areas of the lungs (%)	Score
0	0
1–25	1
26–50	2
51–75	3
>75	4

The score standard for lung lesions was cited from Shao et al. (19).

chamber vein and stored at 4 °C for routine blood analysis. Serum of procoagulant whole blood from the anterior chamber vein was collected after 1 h clotting time after collection at room temperature and centrifugation for 20 min at 4000 rpm and 4 °C. Serum was stored at −80 °C for analysis of blood biochemistry. The urine of pigs was sampled and stored at 4 °C for the determination of routine urine tests. All analyses were conducted at Nanjing Integrated Traditional Chinese and Western Medicine Hospital.

2.7. Statistical analysis

Differences of mortality, cure rate, effective rate, and relative weight gain rate between all infected groups and the healthy control group were tested for significance using the t-test method. Significance of differences of the final lung lesion score, physiological and biochemical index, and urine index between all infected groups and the healthy control group were analyzed with one-way ANOVA (Duncan multiple comparison test), using the Statistical Package for Social Sciences (SPSS version 16.0 for Windows). Results were expressed as the mean ± standard deviation. Confidence levels were set at 95% ($P < 0.05$) or 99% ($P < 0.01$) for statistical significance.

3. Results

3.1. Establishment of an artificial infection model of mycoplasmal pneumonia

Before inoculation with *Mycoplasma hyopneumoniae*, all experimental pigs were in good condition, had no signs of panting, coughing, fever, or similar signs of respiratory disease. They displayed normal behavior and they ate and drank freely and proactively. Six days following infection with *M. hyopneumoniae*, the infected swine started to show forced breathing, sneezing, cough, depressed behavior, diminished appetite, and fever of various degrees, while all negative control pigs remained clinically normal throughout the experiment. At the end of the experiment, lung tissues of all experimental pigs were collected and sent to the veterinary laboratory of Jiangsu Academy of Agricultural Sciences for examination. *M. hyopneumoniae* species were isolated, cultured, and identified by conventional methods from the infected lung tissues. The etiological diagnosis was confirmed by polymerase chain reaction (PCR), showing that the pigs displaying pneumonia were suffering from an infection of *M. hyopneumoniae*.

3.2. Clinical symptoms and pathological necropsy findings after drug administration

None of the 10 tested pigs in the negative control group showed clinical symptoms of mycoplasma pneumonia. The lungs were pink, without any suspected lesions of pneumonia. Meanwhile, in the positive control group, all 10 tested pigs developed the typical symptoms of pneumonia and coughing. They lost appetite, were lying on the floor most of the time, and had typical purple to grey consolidated areas in the heart and apical, middle, and diaphragmatic lobes of the lungs. In the drug-treated groups, typical clinical symptoms of mycoplasmal pneumonia were observed before treatment. After administration of 60 mg/L, 80 mg/L, and 100 mg/L 10% tilmicosin phosphate soluble powder and 75 mg/L 10% tilmicosin soluble powder, the pathological condition of the pigs improved significantly to varying degrees (Figure 1a). The highest reduction of clinical symptoms and lung lesions was reached after administration of 100 mg/L of 10% tilmicosin phosphate soluble powder (high-dose group). The reduction of symptoms caused by 60 mg/L and 80 mg/L of tilmicosin phosphate soluble powder (medium-dose and low-dose groups) were almost equivalent to that from 75 mg/L of 10% tilmicosin soluble powder (drug control group).

3.3. The final lesion scores of the lungs of tested pigs

The final average scores of lung lesions in all groups are shown in Figures 1b and 1c. Compared with the negative control group, the islet-like lesion score of the positive control group was significantly ($P < 0.01$) higher. After drug administration, the lesion level of the high-dose group was reduced significantly ($P < 0.01$); meanwhile, the medium-dose, low-dose, and drug control groups also had lesion scores which were significantly ($P < 0.05$) lower than that of the positive control group. The scores among the medium-dose, low-dose, and drug control groups were statistically equivalent.

3.4. Therapeutic effect of tilmicosin phosphate on mycoplasmal pneumonia

The therapeutic effect of 10% tilmicosin phosphate soluble powder and the control drug (10% tilmicosin soluble powder) on pigs infected with *M. hyopneumoniae* is summarized in Table 3. No deaths occurred due to *M. hyopneumoniae* infection in any test group, and both cure

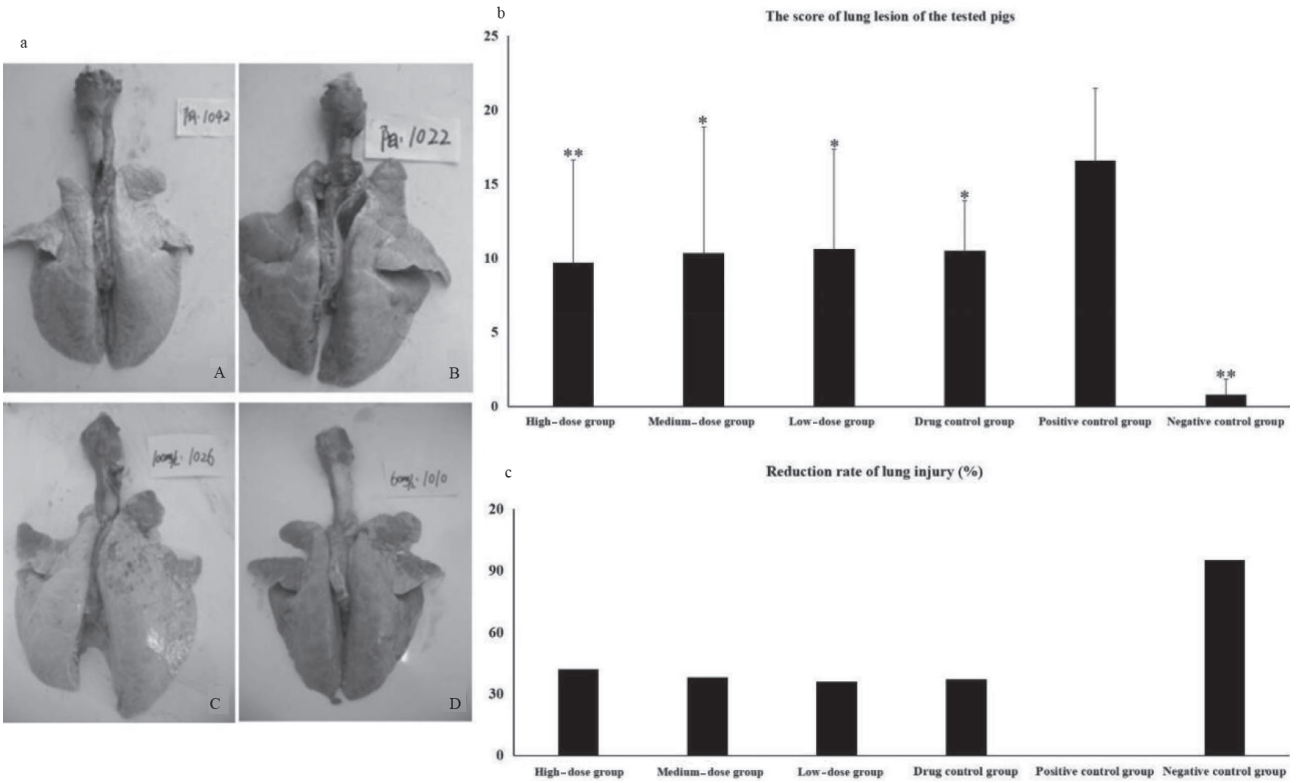

Figure 1. The anatomical pathological changes of tested pigs. a: The lungs are anatomically representative of the pigs in the test groups. A. Negative control group, no lesions in lungs; B. Positive control group, obvious and extensive islet-like lesions in the apex lobe and heart lobe of lungs; C. High-dose group, mild and localized islet-like lesions in lungs; D. low-dose group, mild and localized islet-like lesion in lungs. b, c: ** $P < 0.01$ and * $P < 0.05$ indicate a significant difference between the positive control group and all other groups.

Table 3. The therapeutic effect of 10% tilmicosin (mg/L water) phosphate soluble powder on mycoplasmal pneumonia disease in pigs.

Group	Dosage (mg/L water)	Death rate	Cure rate	Effective rate
High-dose group	100	0	3/10	8/10
Medium-dose group #	80	0	2/9	6/9*
Low-dose group	60	0	2/8	6/8
Drug control group #	75	0	3/10	7/10*
Positive control group	—	0	0	0
Negative control group	—	0	—	—

#: 1 pig from the medium-dose group and 2 pigs from the drug control group died of nonmycoplasma disease during the experiment. "—" represents not being involved. The difference of the effective rate between the high-dose group and that of the other drug-treated groups is indicated by * $P < 0.05$.

rate and effective rate were zero in the positive control group. As shown in Table 3, the high-dose and drug control groups showed the best cure rate; however, this was not significantly different ($P > 0.05$) from those of the medium- and low-dose groups. Meanwhile, there was also no significant difference ($P > 0.05$) between the cure rates of the medium- and low-dose groups. The effective rate of the high-dose group was significantly higher ($P < 0.05$) than those of the medium-dose and drug control groups and was equivalent to that of the low-dose group. At the same time, the differences were not significant ($P > 0.05$) among the effective rates of the medium-dose group, drug control group, and the low-dose group. It could be seen that, like the control drug, all tested dosages of 10%

Table 4. The weight gain of 10% tilmicosin phosphate soluble powder on tested pigs infected with mycoplasmal pneumonia.

Group	Weight before test (kg/head)	Weight after test (kg/head)	Average weight gain (kg/head)	Weight gain rate (%)
High-dose group	7.73 ± 1.57	10.51 ± 2.11	2.78 ± 0.69†	36
Medium-dose group	7.03 ± 1.81	10.07 ± 2.79	3.03 ± 1.09†	43
Low-dose group	8.10 ± 1.39	10.63 ± 2.00	2.53 ± 0.90*	31
Drug control group	8.15 ± 2.50	11.04 ± 1.87	2.89 ± 1.03†	36
Positive control group	7.82 ± 1.94	9.33 ± 1.53	1.51 ± 1.32**	19
Negative control group	6.87 ± 1.35	10.57 ± 0.83	3.70 ± 0.97††	54

** $P < 0.01$ and * $P < 0.05$ indicate a significant difference between negative control group and all the other groups. †† $P < 0.01$ and † $P < 0.05$ indicate a significant difference between the positive control group and all the other groups.

Table 5. The detection results of blood panels of the tested pigs.

Index	Neg. control	Pos. control	Drug control	High-dose	Medium-dose	Low-dose
RBC ($\times 10^9$/L)	7.34 ± 0.88	7.53 ± 0.66	6.91 ± 0.62	7.18 ± 0.41	7.25 ± 0.53	7.19 ± 0.48
HTC (L/L)	0.46 ± 0.05	0.45 ± 0.05	0.42 ± 0.02	0.45 ± 0.04	0.43 ± 0.02	0.44 ± 0.04
HGB (g/L)	129.0 ± 11.87	123.1 ± 14.54	116.6 ± 13.06	130.0 ± 14.36	120.6 ± 2.75	123.7 ± 7.23
MCV (fL)	62.19 ± 1.96	59.97 ± 1.56	59.51 ± 2.64	61.89 ± 2.55	59.90 ± 3.37	61.01 ± 2.28
MCH (pg)	17.66 ± 0.840	16.64 ± 0.58	16.86 ± 1.24	17.29 ± 1.03	16.70 ± 1.32	17.21 ± 0.76
MCHC (g/L)	283.9 ± 6.91	276.3 ± 5.22	283.1 ± 10.70	286.6 ± 13.24	278.9 ± 10.19	282.1 ± 10.57
RDW (%)	16.06 ± 0.96	16.70 ± 1.30	15.07 ± 1.40	15.16 ± 1.10	15.77 ± 1.07	15.83 ± 2.84
WBC ($\times 10^9$/L)	21.04 ± 6.39	31.09 ± 5.37**	28.11 ± 4.04	22.51 ± 4.14	27.70 ± 1.91	29.95 ± 2.29
PLT ($\times 10^9$/L)	475.4 ± 134.4	480.7 ± 75.16	436.1 ± 99.86	515.0 ± 163.6	397.3 ± 86.10	515.4 ± 76.81

** $P < 0.01$ indicates a significant difference between negative control group and all the other groups.

Table 6. The detection results of the blood biochemical index of the tested pigs.

Index	Neg. control	Pos. control	Drug control	High-dose	Medium-dose	Low-dose
TP (g/L)	69.63 ± 4.40	70.79 ± 3.72	66.39 ± 3.66	67.30 ± 4.38	67.27 ± 4.058	70.01 ± 4.92
ALB (g/L)	28.60 ± 3.36	29.79 ± 4.49	29.76 ± 2.38	30.64 ± 1.77	30.91 ± 3.13	31.16 ± 6.03
GLO (g/L)	41.61 ± 5.46	42.44 ± 2.08	40.94 ± 3.76	39.53 ± 4.80	39.23 ± 3.85	38.86 ± 2.34
ALT (U/L)	50.86 ± 9.87	46.43 ± 12.23	52.00 ± 11.82	48.57 ± 5.74	50.00 ± 12.56	47.71 ± 17.53
AST (U/L)	100.71 ± 20.06	95.57 ± 14.51	81.00 ± 21.56	91.86 ± 20.31	76.00 ± 20.98	86.86 ± 39.10
BUN (mmol/L)	4.11 ± 0.36	3.90 ± 0.88	3.93 ± 0.35	3.98 ± 0.24	4.02 ± 0.12	4.06 ± 0.37
CREA (μmol/L)	48.56 ± 9.36	56.10 ± 6.10	56.29 ± 5.41	53.87 ± 8.38	48.30 ± 11.91	49.31 ± 5.16
GLU (mmol/L)	3.75 ± 1.04	4.21 ± 0.51	4.50 ± 0.96	4.06 ± 0.38	4.87 ± 1.61	4.021 ± 1.65
TBILI (μmol/L)	1.14 ± 0.36	1.24 ± 0.43	1.11 ± 0.33	0.97 ± 0.21	0.93 ± 0.34	1.13 ± 0.14

Table 7. The detection results of routine urine tests of the tested pigs.

Index	Neg. control	Pos. control	Drug control	High-dose	Medium-dose	Low-dose
WBC	0/10	0/10	0/10	0/10	0/10	0/10
BLD	0/10	0/10	0/10	0/10	0/10	0/10
Ph	8.29 ± 0.49	8.00 ± 0.00	8.14 ± 0.38	8.29 ± 0.49	8.29 ± 0.49	8.14 ± 0.38
SG	1.00 ± 0.00	1.00 ± 0.00	1. 00 ± 0. 00	1.00 ± 0.00	1.001 ± 0.00	1.00 ± 0.00
KET	0/10	0/10	0/10	0/10	0/10	0/10
BIL	0/10	0/10	0/10	0/10	0/10	0/10
NIT	0/10	0/10	0/10	0/10	0/10	0/10
URO	0/10	0/10	0/10	0/10	0/10	0/10
Vc	0/10	0/10	0/10	0/10	0/10	0/10
PRO	0/10	0/10	0/10	0/10	0/10	0/10
GLU	0/10	0/10	0/10	0/10	0/10	1(±)/10

± indicates a suspicious positive result.

tilmicosin phosphate soluble powder showed excellent therapeutic effect on mycoplasmal pneumonia in pigs.

3.5. Effect of tilmicosin phosphate on weight gain in pigs subjected to *M. hyopneumoniae*

Table 4 shows the effect of tilmicosin phosphate on weight gain of pigs infected with *M. hyopneumoniae*. The average weight gain of the tested pigs in the positive control group was the lowest, and was significantly ($P < 0.01$) decreased compared with that of the negative control group. Average weight gain in the low-dose group was the second lowest, which was still higher by 12% than that of the positive control group. Weight gain in all other drug-treated groups was significantly ($P < 0.01$) higher than that of the positive control group, and weight gain rates were greater by 17% to 24%. The medium-dose group showed the best weight gain effect. The results also showed that despite the use of 10% tilmicosin phosphate soluble powder and 10% tilmicosin soluble powder, the highest weight gain of the *M. hyopneumoniae*-infected pigs was only 43% which is still 11% lower than that of the negative control group, demonstrating that the body weight of the infected pigs was lower and could not be completely corrected by the treatment.

3.6. Effect of tilmicosin phosphate on routine blood tests of tested pigs

The effect of tilmicosin phosphate on the routine blood indices of tested pigs is shown in Table 5. Except for a significant ($P < 0.01$) increase of white blood cells (WBC) in the positive control group, there were no obvious differences for the other indices among all groups. In particular, there was no significant difference for all tested indices between the drug-treated groups and the negative control group.

3.7. Effects of tilmicosin phosphate on blood biochemical indices of tested pigs

The effect of tilmicosin phosphate on the serum biochemical indices of the tested pigs is shown in Table 6. No significant differences among all experimental groups were observed at the end of testing. There was also no significant difference between the measured value of each drug-treated group and that of the negative control group.

3.8. Effect of tilmicosin phosphate on routine urine tests of tested pigs

The effect of tilmicosin phosphate on the routine urine tests of the tested pigs is shown in Table 7. The routine urine indices of tested pigs among all groups showed no significant differences at the end of testing. Compared with the results of the negative control group, the routine urine indices of all drug-treated groups did not change.

4. Discussion

Swine mycoplasmal pneumonia induced by *M. hyopneumoniae* has been one of the most serious health issues plaguing the world's pig industry. The pathogen can be spread by the aerial route and causes the common porcine respiratory disease complex (PRDC) (20). In addition to the development of new vaccines that are compatible with the serotype of the pandemic strain and that provide preventative immunity, the development of more effective antibiotics is still preferred for treating this disease (21). Tilmicosin has been widely used as a medicated premix for control of *M. hyopneumoniae*. However, the poor solubility of tilmicosin negatively affected its intake quantity and pharmacokinetic features (13), which resulted in the development of soluble tilmicosin phosphate. The efficacy of tilmicosin phosphate in eliminating *Actinobacillus*

pleuropneumoniae from carrier pigs has been reported (15). Considering the seriousness of *M. hyopneumoniae* infection, it seemed useful to study the clinical effects of tilmicosin phosphate and its safety in pigs experimentally infected with *M. hyopneumoniae*.

In the present study, an animal model of mycoplasmal pneumonia disease in pigs was successfully established through the endotracheal injection of *M. hyopneumoniae*. Obvious clinical symptoms of respiratory disease such as coughing, panting, pyrexia, and anorexia were observed in all infected animals. In necropsy, tested pigs in the positive control group had obvious consolidated areas of the lungs. These features are typical for pneumonia and consistent with previous reports (4,3). In contrast, all negative control pigs remained clinically normal throughout the experiment. Although the blood biochemical and urinary indices in the infected pigs did not show significant differences (Table 5), there was a significant ($P < 0.01$) increase of white blood cells in the positive control group compared to that of the negative control group, which was probably due to the cellular immune defense response caused by *M. hyopneumoniae* infection, and which further verified our model. In the other drug-treated groups, the numbers of leukocytes also increased nonsignificantly ($P > 0.05$) at different levels.

On the basis of the presented animal model of mycoplasmal pneumonia induced by *M. hyopneumoniae*, using mortality, cure rate, effective rate, weight gain, and lung injury scores of the infected pigs, this experiment comprehensively evaluated the clinical treatment efficacy of 10% tilmicosin phosphate soluble powder with the dosages of 60, 80, and 100 mg/L in water, and made a comparison with a commercial control drug (10% tilmicosin soluble powder at the dosage of 75 mg/L in water).The results of this study show the improvement of clinical signs and best therapeutic effect on mycoplasmal pneumonia in pigs after the oral treatment of 100 mg/L 10% tilmicosin phosphate soluble powder. The oral administration with 60 mg/L and 80 mg/L also proved to be effective to treat experimental mycoplasmal pneumonia, and the efficacy was equivalent to that of 10% tilmicosin soluble powder. Moreover, there was no obvious difference between the efficacy of 60 mg/L of 10% tilmicosin phosphate soluble powder and that of 80 mg/L. The score results for lung injury in this study also showed that 100 mg/L of 10% tilmicosin phosphate soluble powder had the best inhibiting effect in diseased pigs on lung lesion levels, which was significantly ($P < 0.01$) different from that of the positive control group. Meanwhile, 60 mg/L and 80 mg/L of 10% tilmicosin phosphate soluble powder and 10% tilmicosin soluble powder also showed a significant ($P < 0.05$, compared to that of the positive control group) and approximate reduction of lung injury degree. The results were in accordance with previous studies which had also shown the efficacy of tilmicosin for the treatment and control of *M. hyopneumoniae* infections in terms of improved clinical signs and decreased lung lesions (8,22).

In addition to disturbing the physiological function of pathogens, tilmicosin has the unique ability to concentrate and retain neutrophils and macrophages to migrate preferentially to infection sites in swine in vivo (23), which further strengthens its antibacterial effects. The dose of 80 mg/L of 10% tilmicosin phosphate soluble powder showed equivalent therapeutic effect to that of 10% tilmicosin soluble powder at 75 mg/L, implying that the active ingredient of tilmicosin phosphate was still tilmicosin. Interestingly, 60 mg/L of 10% tilmicosin phosphate soluble powder still showed the approximate effect of the control drug, illustrating that the improvement of solubility of tilmicosin phosphate effectively promoted the intake, assimilation, and function of the active constituent, tilmicosin. Therefore, for the treatment of mycoplasma pneumonia in swine, 60 mg/L to 80 mg/L of 10% tilmicosin phosphate soluble powder can be recommended as a clinical application dose. Because oral administration of tilmicosin phosphate in drinking water is more convenient and effective than tilmicosin, 10% tilmicosin phosphate soluble powder is promising for clinical use.

In the present study, the average weight gain of the tested pigs in the positive control group was 11% lower than that of the negative control group, illustrating that the infection of *M. hyopneumoniae* could severely disturb the growth of diseased pigs by reducing action and intake. A previous study found that with tilmicosin treatment the average daily weight gain of infected pigs was significantly better (24). In the present study, 10% tilmicosin phosphate soluble powder could considerably but not completely improve the weight gain of pigs infected with *M. hyopneumoniae* by 12% to 24% compared to that of the positive control group, which was similar to study results for tilmicosin phosphate for control of pneumonia caused by *Actinobacillus pleuropneumoniae* in swine (25). The improvement in weight gain of 10% tilmicosin phosphate soluble powder at the dose of 60 mg/L and 80 mg/L on infected pigs was comparable with that of 10% tilmicosin soluble powder. However, the weight gain at 100 mg/L was slightly lower than that at 80mg/L, the reason for which needs further study.

The present study also investigated the influence of tilmicosin phosphate on the principal physiological functions of the infected pigs by analysis of routine blood tests, blood biochemistry, and routine urine tests. Hematological constituents of the animal usually reflect the condition of physiological responsiveness to its external and internal environments, which serves as a tool for monitoring the physiological or pathophysiological

status of the body (26). It was reported that tilmicosin could cause temporary decreases in the RBC and WBC counts and pack cell volume concentration (PCV), and that tilmicosin administration achieved high levels of phagocytes in avian, porcine, and bovine subjects (27). The present study demonstrated that tilmicosin phosphate did not significantly affect the levels of all tested hematological constituents of the infected pigs, which was different from results of previous studies. The reason may be that tilmicosin phosphate was safer for clinical treatment, or that the effect of tilmicosin phosphate/tilmicosin on hematological parameters was temporary and then returned to normal. It is worth mentioning that after administration of tilmicosin phosphate, the white blood cell counts in the drug-treated groups decreased compared to that of the positive control group, which verified the mitigative effect of tilmicosin phosphate/tilmicosin on inflammation in the infected pigs.

The analysis of clinical biochemical indexes is a fundamental tool used in veterinary medicine to monitor the effects of therapeutic, nutritional, and environmental management (28). In a previous study on chickens, it was observed that tilmicosin did not make changes in biochemical parameters except for temporary significant decreases in total protein and albumin concentrations (29). The examination of routine urine tests is often used to monitor nephropathy and kidney damage (30). The present study demonstrated that all tested parameters of blood biochemistry and urine were not influenced significantly by tilmicosin phosphate/tilmicosin, demonstrating the drug's safety for infected pigs.

In conclusion, this study has demonstrated the excellent therapeutic effect of tilmicosin phosphate in vivo in pigs experimentally infected with *M. hyopneumoniae*. The weight gain of the pigs improved and no negative effects of tilmicosin phosphate on biochemical and physiological parameters were observed. Tilmicosin phosphate can be widely used in animal production for therapeutic purposes due to its treatment potency and safety.

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