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THE EFFECT OF OXYTETRACYCLINE ON EXPERIMENTAL *PASTEURELLA MULTOCIDA* INFECTION IN RABBITS

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SUMMARY

Thirty-six healthy New Zealand White rabbits were divided equally into four groups. Rabbits in group 1 were injected with a long acting oxytetracycline preparation at the rate of 20 mg/kg two days prior to the intratracheal challenge with 10^6 colony forming unit (cfu)/mL of *Pasteurella multocida* type A. Rabbits in group 2 were challenged with *P. multocida* type A before the long acting oxytetracycline was injected two days later. Rabbits in group 3 were challenged with the same strain of *P. multocida* but were not treated with the antibiotic while rabbits in group 4 were injected with the antibiotic without challenge with *P. multocida* type A. Three rabbits in group 2 and 8 rabbits in group 3 died within 5 days post challenge. The extent of lung lesions in rabbits in group 2 that survived decreased while the only rabbit that survived in group 3 showed severe and extensive lung lesions on post mortem. None of the rabbits in groups 1 and 4 died and on post mortem no lesions on the lung were seen. The serum level of oxytetracycline was highest at 6 h post-injection before the level gradually reduced to a low level as early as 48 h. Rabbits that survived were killed at days 3, 7, 11 and 14 post antibiotic treatment.

Keywords: Oxytetracycline, *Pasteurella multocida*, rabbits.

INTRODUCTION

Pasteurellosis or snuffles is one of the most common diseases of rabbits caused by either *Pasteurella multocida* type A or D. The agent has been recognised as a normal flora in the upper respiratory tract of many animals including rabbits (Dungworth, 1985). Under immunosuppressive conditions animals may succumb to respiratory tract infection leading to sneezing, coughing and death (Flatt, 1974; DiGiacomo *et al.*, 1989). Antibiotic therapy has been recommended for treatment and control of pasteurellosis in rabbits since vaccination has not been widely used (Cameron and Smit, 1970; Bapat and Sawhney, 1972; Harkness and Wagner, 1983). This experiment was designed to study the prophylactic and therapeutic effects of a long acting oxytetracycline preparation on experimental pasteurellosis in rabbits and to determine the level of this antibiotic in serum.

MATERIALS AND METHODS

Animals

Thirty six New Zealand White rabbits of about 4 months old were used in this study. They were divided into four groups with nine rabbits in each group and were kept in separate cages. Commercial rabbit pelleted ration and water were provided *ad libitum* to all rabbits. Nasal swabs were collected prior to and every alternate day post arrival for bacterial isolations. All rabbits were confirmed to be free from *P. multocida* in the nasal cavity for at least two weeks prior to the start of the experiment.

Bacterial inoculum preparation

To challenge the rabbits, a bacterial inoculum was prepared from the stock culture of *P. multocida* serotype A isolated earlier from lungs of a rabbit with snuffles. The organism was initially grown onto blood agar at 37°C overnight. Thirty colonies were then inoculated into brain heart infusion broth (BHI) and incubated overnight at 37°C. The concentration of the bacterial inoculum was determined by the standard plate count method (Lenette *et al.*, 1974) and diluted to a final concentration of 3.27×10^6 colony forming units (cfu)/mL.

Experimental procedure

Rabbits in group 1 were prophylactically treated with an intramuscular injection of a long acting oxytetracycline preparation (Terramycin; Pfizer) at the rate of 20 mg/kg two days prior to experimental intratracheal challenge with a 2 mL inoculum of *P. multocida* prepared earlier. Animals in group 2 were therapeutically treated with the intramuscular injection of the same antibiotic at the rate of 20 mg/kg two days after the intratracheal challenge with 2 mL

P. multocida. Rabbits in group 3 were challenged with *P. multocida* but without oxytetracycline treatment while those in group 4 were treated with oxytetracycline without *P. multocida* challenge.

Sample collection and processing

Serum samples were collected from the ear vein of all animals prior to and serially thereafter at three-hour intervals for the first three days after the antibiotic treatment. The concentrations of oxytetracycline in serum were determined using a modified high performance liquid chromatography (HPLC) method as described previously (Martinez and Shimoda, 1987). Rabbits that survived were slaughtered at days 3, 7, 11 and 14 post antibiotic treatment. Necropsy examinations were carried out on all animals and the extent of the lung lesions were scored according to the method of Zamri-Saad *et al.* (1996). Trachea and lungs tissues, and heart blood swabs were collected from all rabbits during necropsy for isolation and identification of *P. multocida* (Lenette *et al.*, 1974).

RESULTS

Clinical observations

During the 14-day infection period, a total of 3 (33%) rabbits in group 2 and 8 (89%) rabbits in group 3 were found dead within five days post challenge with *P. multocida*. Of these rabbits, one animal from group 2 and 3 animals from group 3 (35%) died within 24 h post challenge. Prior to death, the affected rabbits showed fever, cyanosis of the skin around the muzzle and inner thigh, laboured breathing and recumbency. The remaining rabbits survived without showing any clinical abnormality.

Pathological observations

The entire lungs and tracheal mucosa of the rabbits that died within 5 days post challenge were severely congested and oedematous with patches of haemorrhages particularly at the anterior lobes of the lungs. Severe congestions were also observed in the liver, small intestines and the entire musculatures.

The lungs of the rabbits that survived in group 2 showed lung lesions affecting as much as 25% of the lung area. The therapeutically treated rabbits in group 2 that were slaughtered early during the course of the infection showed severe lesions as compared to those that were slaughtered later. The rabbits that survived in group 3 and challenged with *P. multocida* but untreated showed severe and extensive lung lesions. None of the rabbits in groups 1 and 4 died or showed lung lesions.

The challenged but untreated rabbits in group 3 showed the highest average lung lesion score followed by rabbits in group 2 while rabbits in groups 1 and 4 showed no gross lung lesions (no lesion score). The difference in the average lung lesion score between group 2 and 3 was significant ($p<0.05$). Similarly, lung lesion scores of groups 2 and 3 were significantly ($p<0.05$) higher when compared to the average lung lesion score for the rest of the groups (Table 1).

Table 1. Average gross lung lesion scores of the different groups

Group	Average lung lesion score
1	0 ± 0.0^{a}
2	8 ± 4.8^{b}
3	19 ± 1.6^{c}
4	0 ± 0.0^{a}

[a,b,c]Values with same superscript do not differ significantly ($P>0.05$)

Microbiology

P. multocida type A was successfully re-isolated from the lungs, trachea, and heart blood of the rabbits, that died, and from the lung lesions of slaughtered rabbits from groups 2 and 3. None of the rabbits in groups 1 and 4 had *P. multocida* type A in their lungs following the re-isolation attempts.

Serum oxytetracycline level

Following a single intramuscular injection at the rate of 20 mg/kg, the serum concentration of oxytetracycline reached its peak level at 6 h post-injection and gradually returned to the low levels as early as 48 h post injection. The highest peak value of oxytetracycline in rabbits of group 4 was 1.4 µg/mL compared to the peak values of 0.4 µg/mL and 0.3 µg/mL in rabbits of groups 1 and 2 respectively. The concentrations in groups 1 and 2 were later reduced to less than 0.1 µg/mL at 72 h post injection (Figure 1).

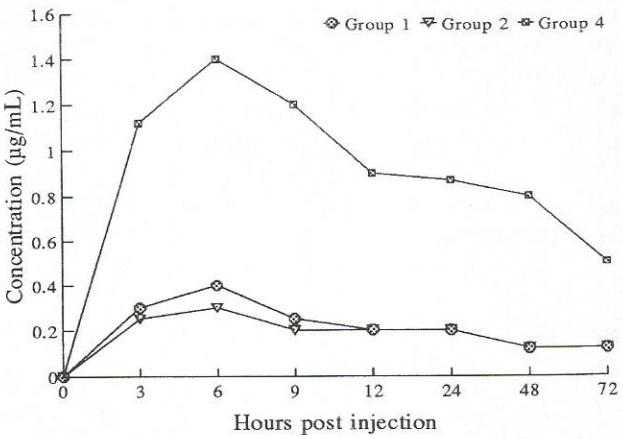

Figure 1. The serum concentration of oxytetracycline in the treated groups

DISCUSSION

The long acting oxytetracycline (Terramycin LA; Pfizer) was found to be able to effectively control *P. multocida* infection in rabbits since there was a significant reduction in the death rate and the severity of lesions following therapeutic oxytetracycline treatment of the infected rabbits. The fact that only 3 challenged rabbits in group 2 died following therapeutic oxytetracycline treatment while the remaining rabbits of the same group showed a decreasing severity of lung lesions provided further evidence that oxytetracycline was able to effectively control *P. multocida* infection. Similar effectiveness of oxytetracycline has been observed in *P. haemolytica* infection of sheep (Gilmour *et al.*, 1982; Appleyard and Gilmour, 1990).

The prophylactic treatment, however, gave the most successful control of *P. multocida* infection when none of the animals in group 1 that were treated prior to the *P. multocida* challenge showed neither clinical signs, pneumonic lesions nor deaths. This suggested that the long acting oxytetracycline can be used prophylactically to prevent the development of pneumonia due to *Pasteurella multocida* infection in rabbits. Gilmour *et al.* (1982), however, found that prophylactic treatment of lambs using similar oxytetracycline preparation prior to *P. haemolytica* challenge would just postpone the appearance of clinical signs for four days before death appeared at days 5 to 6 post challenge. It was suggested that the lesions developed after the effect of oxytetracycline had waned (Gilmour *et al.*, 1982). The differences in the prophylactic effect was probably due to the fact that *P. multocida* strain used in this study was more sensitive to oxytetracycline compared to the *P. haemolytica* strain used in lambs.

The serum concentration curves indicated that the oxytetracycline levels in serum reached its peak value level at 6 h post injection and returned to low levels between 48 to 72 h post injection. These findings are in agreement with the findings by Escudero *et al.* (1994) who proposed repeat treatment every 48 h using the same long acting oxytetracycline preparations but was not in total agreement with the recommendations made by the manufacturer to repeat the dose every three days. Since the therapeutic levels of oxytetracycline was between 6 to 48 h post injection, treatment of infected animals requires about 6 h after injection before the therapeutic effects of the drug can be observed. Therefore, prophylactic treatment must be given between 6 to 48 h prior to infection, particularly following stressful conditions.

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RINGKASAN

KESAN OKSITETRASIKLIN TERHADAP JANGKITAN PASTEURELLA MULTOCIDA *UJIKAJI DALAM ARNAB*

Tiga puluh enam ekor arnab New Zealand White sihat telah dibahagikan kepada empat kumpulan. Arnab dalam kumpulan 1 telah disuntik dengan persediaan oksitetrasiklin bertindak lama pada kadar 20 mg/kg dua hari sebelum cabaran intratrakea dengan 10^6 unit pembentuk koloni (cfu)/ml Pasteurella multocida tipe A. Arnab dalam kumpulan 2 telah disuntik

P. multocida *tip A sebelum oksitetrasiklin bertindak lama disuntik dua hari kemudian. Arnab dalam kumpulan 3 telah dicabar dengan strain* P. multocida *sama tetapi tidak diperlakuan dengan antibiotik, sambil arnab kumpulan 4 pula disuntik antibiotik tanpa dicabar dengan* P. multocida *tip A. Tiga ekor arnab dalam kumpulan 2 dan 8 dalam kumpulan 3 mati mengejut dalam tempoh 5 hari pascacabaran. Kadar lesi peparu menjadi kurang dalam arnab kumpulan 2 yang terselamat sambil arnab kumpulan 3 terselamat menunjukkan lesi peparu yang teruk dan luas. Tidak seekorpun arnab dalam kumpulan 1 dan 4 mati ataupun menunjukkan lesi peparu. Aras oksitetrasiklin serum adalah paling tinggi pada 6 j pascajangkitan sebelum aras ini beransur kurang mencapai aras rendah seawal 48 j. Arnab yang terselamat dibunuh pada hari 3, 7, 11 dan 14 pascarawatan antibiotik.*