Elimination of Pasteurella pneumotropica from a Contaminated Mouse Colony by Oral Administration of Enrofloxacin

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Abstract: Enrofloxacin, a fluoroquinolone bactericidal antibiotic, was administered in an attempt to eradicate Pasteurella pneumotropica (P. pneumotropica) from a contaminated mouse colony. Contaminated mice, maintained within 4 animal rooms, were administered Enrofloxacin in drinking water at a daily dosage of 25.5 mg/kg for 2 weeks. Following one week of Enrofloxacin treatment, mice were selected randomly from each room and examined for P. pneumotropica. This procedure was repeated two or three times until all mice examined tested negative for the Pasteurella strain. With the exception of one room, treated mice consistently tested negative for P. pneumotropica for up to 45 weeks following completion of Enrofloxacin treatment. Thus, oral administration of Enrofloxacin significantly eliminated P. pneumotropica from a contaminated mouse colony.

Key words: Enrofloxacin, mouse, Pasteurella pneumotropica

Pasteurella pneumotropica, a gram-negative bacterium, is an opportunistic pathogen commonly found in rodents. Reports of natural outbreaks are rare and are generally limited to immunocompromised mice [6, 11]. Clinical disease caused by P. pneumotropica is generally confined to subcutaneous abscess formation, however isolation of pure cultures of P. pneumotropica from ophthalmitis, conjunctivitis, dacryoadenitis, and uterine infections has also been reported [7, 10–12]. Furthermore, recent reports have revealed that P. pneumotropica can frequently infect mice that are immunodeficient due to genetic manipulation [1, 5].

Hysterotomy and embryo transfer are presently the most effective procedures for elimination of P. pneumotropica, as well as other common pathogens, from infected laboratory animals. However, both methods are extremely time-consuming, particularly if numerous strains are being maintained in the same pathogen-contaminated environment. Instead, antibiotic treatment may provide a preferential method for overcoming the problem, because of its relatively easy procedure. Various antibiotic treatments have been used

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in attempts to eliminate infection by *P. pneumotropica* [3, 4, 6]. However, problems associated with such treatments include the influence of organisms resistant to the antibiotic, the influence of the antibiotic on the animals’ health, and the potential influence of the antibiotic on on-going animal experiments.

In our animal facility, isolation of *P. pneumotropica* from mice that had developed pneumonia revealed that our breeding mouse colony was contaminated by this pathogen. Goelz *et al.* [2] reported that oral administration of Enrofloxacin, a fluoroquinolone antimicrobial agent, was effective in eradicating *P. pneumotropica* from infected mice. This prompted us to treat our *P. pneumotropica* contaminated mouse colony with administration of Enrofloxacin in the drinking water.

The infected mice were transgenic and found, unexpectedly, to be immunodeficient. *Pneumocystis carinii* was also detected in pulmonary lesions in these mice. Other strains of mice in the same room, although immunocompetent and showing no clinical symptoms, were also infected with *P. pneumotropica*. Further examination revealed that four out of a total of 14 rooms containing breeding mice were contaminated with *P. pneumotropica*. Although the source of the contamination is unknown, it is worth noting that animals had been transferred among these four rooms for experimental purposes. In addition, regular microbiological monitoring demonstrated that the pathogen did not spread to the other mouse rooms (data not shown). Thus, Enrofloxacin treatment of animals housed within the four contaminated rooms commenced immediately in order to abolish *P. pneumotropica* from our mouse facility.

The Enrofloxacin (Baytril; Bayer Co. Ltd, Tokyo) treatment program used was based on that reported by Goelz *et al.* [2], with some modifications. Firstly, the antimicrobial activity of Enrofloxacin in the drinking water was monitored in order to determine the frequency at which the water bottles should be changed to ensure a continuous effective level of antimicrobial activity. Drinking water, supplemented with 170 mg/l Enrofloxacin to give a daily dosage of approximately 25.5 mg/kg per day, was given to 7 specific pathogen free (SPF) mice for 12 days. These mice were kept in polycarbonate cages lined with autoclaved wood shavings and fed on the commercial diet, MF (Oriental Yeast, Tokyo). These cages were placed in an animal room, artificially lit from 5:00 to 17:00, with a negative flow isolator and sustained at 23°C ± 2°C with a relative humidity of 55 ± 10%.

Water samples were collected every other day during the experimental period and examined *in vitro* for antimicrobial activity against *P. pneumotropica* (ATCC 35149). For each sample, two-fold serial dilutions were made and 0.1 ml was added to 0.9 ml of Trypan-soy broth (Eiken, Tokyo) supplemented with 5% horse serum, containing approximately 10⁴ colony-forming units (cfu) of *P. pneumotropica*. Following an 18 h incubation at 37°C, we noted the highest dilution factor for each sample that was capable of inhibiting the growth of the *Pasteurella* strain. This showed that the antimicrobial activity of Enrofloxacin in the drinking water did not decrease during the experimental period (data not shown). On the basis of this result, water bottles containing Enrofloxacin were changed every 4 or 5 days during the treatment programme.

Secondly, to evaluate the effectiveness of the treatment program, mice were experimentally infected with *P. pneumotropica*, then treated with Enrofloxacin. For this experiment, SPF female ICR mice, purchased from CLEA Japan (Tokyo), were inoculated oronasally with approximately 10⁷ cfu of *P. pneumotropica*. Fourteen infected mice were orally administered Enrofloxacin for 14 days at the same dosage described above. Oropharyngeal swabs were collected from these mice and from untreated controls for isolation of *P. pneumotropica* at 0, 7, and 14 days after the end of treatment. Each sample was directly plated onto blood agar and incubated at 37°C for 48 h. *P. pneumotropica* pathogen was identified by characterizing biological and biochemical properties with Gram-staining, agglutination testing with antiserum to *P. pneumotropica*, oxidase and catalase analyses, and a diagnostic kit (API 20 NE; bioMérieux, Tokyo), according to the methods previously described [8]. As shown in Table 1, *P. pneumotropica* no longer infected the treated mice at any time point after Enrofloxacin treatment, whereas the strain was consistently detected in all of the untreated mice. Moreover, *P. pneumotropica* was not detected in samples from the bulbar conjunctiva, vagina, and trachea taken from mice sacrificed 14 days after Enrofloxacin treatment (data not shown).

After confirming that Enrofloxacin treatment is in-
Table 1. Isolation of *P. pneumotropica* from experimentally infected mice following Enrofloxacin treatment

<table>
<thead>
<tr>
<th>Days after two weeks of treatment</th>
<th>Treated mice</th>
<th>Untreated mice</th>
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<tbody>
<tr>
<td>Prior to treatment</td>
<td>14/14 *</td>
<td>5/5</td>
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<tr>
<td>0</td>
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<td>14</td>
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Mice infected oronasally with 10^7 cfu of *P. pneumotropica* were treated with Enrofloxacin in drinking water for 2 weeks. Oropharyngeal swabs were collected from these mice and from untreated control mice in order to isolate *P. pneumotropica* at 0, 7, and 14 days after the treatment ended. The symbol * represents the number of positive mice/number of total mice examined.

Indeed an effective method for eradicating *P. pneumotropica*, the same treatment program commenced on the mice housed within the four contaminated rooms described above. In these rooms, approximately 8,000 mice were kept in 1,500 cages. Animal care was performed in accordance with the standard procedures of our facility described above. Regular microbiological monitoring confirmed that these mice were negative for the following agents: Sendai virus, mouse hepatitis virus, *Mycoplasma pulmonis*, *Clostridium piliforme*, *Corynebacterium kutscheri*, *Giardia muris*, *Spirochus muris*, *Aspiculirus tetraperta*, and *Syphasia* spp. All of the mice were administered Enrofloxacin in the drinking water at a concentration of 170 mg/l for 14 days. During treatment, breeding and transfer of mice were temporarily stopped. A further precaution was the prior removal and sacrifice of the above mentioned infected transgenic mice that had developed pneumonia. For the detection of *P. pneumotropica*, oropharyngeal specimens were collected from one mouse per cage sampled randomly in each room.

Before treatment, *P. pneumotropica* was detected in 11 to 46% of mice examined in each room, as shown in Figure 1. For each animal room, antibiotic treatment was repeated twice at an interval of two weeks, however an additional treatment was required in one room where *P. pneumotropica* was still present even after the first treatment. During treatment, none of the mice displayed any clinical symptoms. In the case of three out of four rooms, the bacterium was consistently undetectable for up to 45 weeks after the final treatment (51 and 55 weeks after the beginning of treatment). In one room (Animal room IV), *P. pneumotropica* was isolated from only one out of 56 mice examined at 45 weeks after the final treatment (51 weeks after the start of treatment), although it had not been detected by repeated examination up until then. In fact, after treatment ended in Animal room IV, several mice were introduced by hysterotomy from conventional mouse colonies belonging to other institutes. Thus, it is unclear whether the reinfection arose from the original outbreak or from contamination by an external source.

Antibiotic treatment by an oral route is easy to perform and is thus a potential practical procedure for the elimination of bacterial agents in laboratory animals. However, some issues must be considered when performing such therapy in laboratory animal colonies. Antibiotics may have an influence on the health of the animals or affect concurrent experimental results. When breeding colonies are treated with an antibiotic, there is a possibility that infant mice cannot take drinking water containing Enrofloxacin. Nevertheless, the drug is likely to be delivered indirectly to infants through lactation, since a higher concentration of Enrofloxacin was found in milk than in blood [9]. Goelz *et al.* [2] reported that Enrofloxacin eliminated all evidence of *P. pneumotropica* when administered at 25 or 85 mg/kg by either oral or subcutaneous routes. In the laboratory of Goelz, drinking water supplemented with Enrofloxacin was changed daily for 14 days of treatment. Whereas, in our facility, antibiotic treated water was changed every 4 or 5 days on the basis of results from preliminary experiments. *P. pneumotropica* was still successfully eradicated. There is no evidence, however, of complete elimination of *P. pneumotropica* from mice.

In conclusion, our bacteriological study suggests that
oral administration of Enrofloxacin may provide a practical alternative to hysterotomy or embryo transfer for elimination of P. pneumotropica from contaminated mouse colonies, especially when large numbers of mice are concerned.

Fig. 1. Isolation of P. pneumotropica from mice in contaminated rooms. Approximate 8,000 mice (1,700 mice/300 cages in Animal room I; 1,800/350 in Animal room II; 1,300/250 in Animal room III; and 3,200/600 in Animal room IV) were kept in these rooms. Enrofloxacin treatment was performed for two weeks ( ). Isolation of P. pneumotropica was examined repeatedly and is shown as positive rate (*: No. of positive/No. of examined mice). Note that no positive mice were detected after Enrofloxacin treatment, except for one mouse in Animal room IV (**).

References

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