Preliminary Study of Enrofloxacin as an Axolotl Antibiotic

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Infectious disease is a concern in any animal facility and at the Axolotl Colony we make every attempt to prevent the spread of disease by isolating and treating animals at the first signs of illness. Most commonly found in the colony are species of *Pseudomonas, Aeromonas* and other gram negative rods (Brothers, 1977; Boyer et al., 1971). Several different antibacterial drugs have been used throughout the history of the colony. These include gentamicin, amikacin, nitrofurozone and penicillin (see Duhon, 1989a). The colony has also used tetracylcine and its derivatives, which cause severe skin irritation in amphibians. We started systematically testing enrofloxacin, packaged as Baytril and manufactured by Bayer, in July of 2000 upon recommendation of the IU veterinarian. Dr. Farrar felt enrofloxacin was a potential axolotl antibiotic because it reaches so many different tissues, is less prone to resistance and has a low incidence of side effects. The colony already had preliminary toxicity data on enrofloxacin from its use as a treatment of Hemorhagic Bleeding Syndrome in 1995 (Duhon, 1996). From that experience we knew enrofloxacin wasn't harmful but we didn't have enough data to determine positive effectiveness. Enrofloxacin belongs to the fluroquinolone class of antibiotics. These drugs act by deactivating bacterial DNA gyrase thereby preventing uncoiling and transcription. Enrofloxacin is broad spectrum and effective against most gram-negative bacteria including *Pseudomonas* and *Aeromonas* species.

Animals were chosen for treatment of the basis of several symptoms commonly considered signs of infection. These included anorexia, floating or twisted posture, loss, of gill filaments or spindly gills, gill enlargement, anemia, cutaneous hemorrhaging, edema and ascites. These symptoms and their implications are discussed in more detail in Issue 28 of the Axolotl Colony Newletter. Each animal was rated on a scale of 0-6, zero meaning no symptoms, 1 being likely to recover on its own and 5 having little if any chance of survival without immediate treatment. Some of the symptoms used to classify animals falling within each score are described in Table 1. Animals that died during the study were given a score of 6. Typically, enrofloxacin is given intramuscularly (IM) but due to apparent skin and muscle irritation, we switched to interperitoneal (IP) injections. Each animal received a dose at 5-10mg/kg given IP every other day for a total of 5-7 doses. One month after treatment, each animal's health rank was again assessed based on the 1-5 scale. For the conclusion of the study, each animal's health rank was reexamined along with its post-treatment mating history. Successful mating behavior is another way we determine health because sick animals are unlikely to put their energy into reproduction (Duhon, 1989a).

0	no symptoms
1	loss of filaments but otherwise healthy
2	extensive loss of filaments, spindly gills, slightly pale, some floating
3	tail float, not eating/regurgitating, bleeding gills, irritated skin, enlarged gills
4	extreme pallor, floating, green bile
5	adema/ascites, floating, green bile, severe hemor-rhaging, skin appears green (d/d, a/a animals)
6	death

Table 1 Symptoms Typical of Each Health Score

Of the 206 animals treated in the last year many were sent out to researchers and were not counted in the final follow-up. 70% of the animals treated and still in the colony for follow-up showed an improvement in symptoms, 12% stayed the same and 8% are either dead or their symptoms have worsened (Table 2). Of the animals tried in matings after treatment, 80% mated successfully. Figure 1 shows that the average health score from pre-treatment to final follow-up improves significantly. Figure 2 compares the percent distribution of health scores between pre-treatment, one month and final analysis. The pre-treatment curve peaks at a health score of 3, while 1 is the score of highest frequency for the one-month follow-up. 60% of animals scored for the final follow-up had zero symptoms and none had scores of 4 or 5.

Health Score	Pre-treatment	1-Month	Final
0	0	47	77
1	19	51	26
2	54	45	15
3	79	27	5
4	43	9	0
5	11	0	0
6	0	7	7

Table 2 Health Score Data
Number of animals injected and their health score.

Thurty-five of the animals treated were given an initial rating of 4 or 5, indicating that recovery without treatment was highly unlikely. Of these animals, 74% showed an improvement in symptoms and of the 13 tried in matings 11 were successful. This suggests that enrofloxacin is effective in treating severe cases as well as controlling minor infections.

The results of our treatments, including symptom improvement as well as mating success, point strongly to enrofloxacin's effectiveness in controlling the bacterial infections most commonly found in the IU Axolotl Colony. Before drawing any definite conclusions, however, we must ask ourselves whether there is an alternative explanation for the high percentage of improvement. Perhaps the animals are fighting off the infections on their own. We did not have the space, nor could the colony afford to set aside an equivalent number of control animals having similar symptoms but receiving no treatment. However, the fact that after treatment, 74% of the animals rated the sickest and least likely to recover on their own showed improvement adds confidence that enrofloxacin is effective in controlling the infections most commonly found in the colony. Also of interest is the large increase in healthy animals not only for the one month but also for the final follow-up. This suggests that for many animals, enrofloxacin may keep fighting the infection for several months and in some cases, its effects are not even seen within one month's time. Of the 8% that died, the majority had been rated as 4 or 5 suggesting the infection was too far along and reminding us of the importance of acting upon symptoms early. Also, no tests were done on any of the animals to find out if a gram negative bacterial infection was actually the culprit. If parasites or viruses were the cause of illness then we wouldn't expect enrofloxacin to have any effect except on secondary symptoms. Although the symptoms of 12% of the treated animals appeared unchanged for the final follow-up, many of them had mated successfully by that point. This suggests that they had either recovered without regenerating the damaged tissue or that they weren't truly sick to begin with. Although axolotls are well known for their regenerative abilities, mature animals often fail to regrow filaments lost from past infections.

This preliminary study of enrofloxacin can act as a base reference for future studies or simply as another option for treatment. We didn't control for age, sex, exact dosages or amount of time elapsed before the final follow-up and these variables could all be taken into consideration for further study. Because the colony tends to send out our older adult animals for research, we weren't able to get data for the final follow-up on a large percentage of animals. However, we don't usually ship out unhealthy animals unless they are specifically requested. The probability is low that the animals sent out had worsened since treatment. These shipments limited our ability to do any kind of age comparison. In fact, nearly half of the animals treated over the age of three were shipped before the final follow-up. In the future it would be interesting to see whether antibiotic treatment is more effective on certain age groups. Between the ages of 6-14 months juveniles tend to be more susceptible to infection. Finding out how these juveniles respond to enrofloxacin may help more of them emerge from that vulnerable period as healthy adults. Experimenting with dosage, treatment intervals, and method of administration could also prove useful. Does optimal dose vary with symptom severity and disease advancement? Graham Crawshaw of the Toronto Zoo recommends cool water amphibians be treated every 2 days, which was the frequency we used. However, frequency and dose should be manipulated together to find the most effective combination. Since amphibian skin is permeable to liquids, many antibiotics are administered as baths. It would be very helpful to find out if enrofloxacin is as, if not more effective when added to water, and what concentrations provide the best therapy. The University of Liverpool recommends enrofloxacin as a preliminary treatment at a concentration of 30ppm for up to 5 hours every other day (Jepson, 1999). Many young animals suffer from bloating which is likely to be caused by bacterial metabolism. Some of them merely float for a short time then overcome the infection. Others, however, may float for weeks before they stop eating and eventually die. We are currently testing bath doses now on young floaters. Although there is still much to learn about enrofloxacin's effectiveness under various conditions, we can now say that it is safe and merits further study to determine the optimal dose and range of effectiveness.

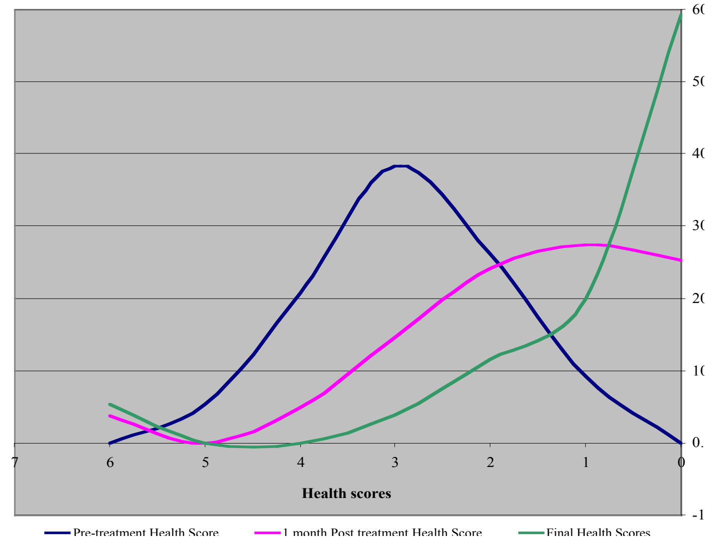

Figure 1 – Health score distributions of 3 groups.

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