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Article

Analysis of monotherapy and combination therapy on *Helicobacter felis*

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SUMMARY

Helicobacter felis **is a bacterium that infects the gastrointestinal systems of small animals (most commonly cats), causing stomach gastritis with symptoms of vomiting, dehydration, poor appetite, pain and weakness. The goal of this study was to determine which antibiotics would be most effective at eradicating *H. felis*, therefore promoting healing in afflicted animals. It was hypothesized that, in a 24-hour period, a combination of high-performing antibiotics would be more effective in combating infection than monotherapy with a high-performing antibiotic. In the current study, we gathered various antibiotics that have been proven to successfully fight bacterial infections, including ampicillin, gentamicin, streptomycin, tetracycline, chloramphenicol, and oxacillin. We also created a combination of gentamicin and streptomycin, the two most effective monotherapy antibiotics, in addition to a combination of ampicillin and tetracycline, the two most ineffective monotherapy antibiotics. The *H. felis* was grown on tryptic soy and subjected to the Kirby-Bauer antibiotic test with eight different antibiotic treatments. After 24 hours of incubation, we measured the inhibition zones of the stand-alone monotherapy antibiotics (i.e., how effective they were against the *H. felis*). The treatment that proved an overall most effective average eradication rate was the monotherapy with streptomycin. The combination of gentamicin and streptomycin, while second most effective in comparison to the monotherapy, proved to be less effective. This research suggests that it would be best to use a monotherapy in treating animals infected with *H. felis*.**

INTRODUCTION

H. felis is a bacterium that is strongly associated with *Helicobacter heilmannii*, the provisional name of a tightly coiled gram-negative bacteria occurring in 0.2 to 2.4% of human gastric pathologies. *H. felis*, *H. bizzozeronii* and *H. salmonis* are associated species that are naturally occurring in the stomachs of dogs and cats. The bacteria can potentially live inside of the animal for its entire lifetime which may cause gastric issues and symptoms that need veterinary care. This can affect the pet owner negatively in terms of time spent, costly medication, and visits to the veterinarian. The treatment difficulties veterinarians face is when these bacteria are abundant and present mucosal inflammation, causing clinical symptoms such as chronic vomiting and gastritis, ultimately leading to dehydration, poor appetite, abdominal pain, weight loss, and weakness (1, 2). Cats commonly acquire this easily transmitted infection shortly after they are adopted and have to be rehomed. According to the ASPCA, 42% of cats are rehomed due to costly health problems. Many times, they are denied a home as a result of their illness and are rehomed to shelters where they will infect other cats (as the bacteria is passed through saliva) and be put down if not adopted. The shift in host may be prevented by antibiotic-specific treatment to these infected animals. It is important to determine how to treat these cats and other infected animals effectively and rapidly (5).

The purpose of the study is to test the effects of various antibiotics on *H. felis*. The antibiotics that we will use during the experiment have properties that eradicate the bacteria. Antibiotics taken over a long course of treatment are known to become ineffective because the specific bacteria develop immunity as a result of the timed exposure. The mechanism of action is different between various types of antibiotics. Ampicillin and oxacillin break down bacterial cell walls by inhibiting enzyme proteins necessary for the third stage of cell wall formation. Streptomycin and gentamicin inhibit the regulation required to metabolize and repair bacterial DNA. Chloramphenicol prevents protein synthesis by diffusing through the bacterial membrane and binding to bacterial ribosomes.

Other researchers have found that *H. felis* can grown in the presence of some antibiotics, namely: vancomycin, trimethoprim, bacitracin, polymyxin B, flucytosine, and amphotericin B (4). However, an extensive number of antibiotics remain to be tested for their effect on *H. felis*. Combination therapy treatment of *H. felis* with antibiotics has not been tested either. Antibiotic combination therapy has proven effective in other studies such as Evaluation of Antibiotic Therapies for Eradication of *Helicobacter hepaticus*. In that study, a triple therapy combination of antibiotics (amoxicillin-metronidazole-bismuth (AMB) and tetracycline-metronidazole-bismuth (AMD)) proved effective in eradicating *H. hepaticus* from mice. *H. hepaticus* is a practical model when researching helicobacter-related gastric disease (3).This study will test the resistance of *H. felis* bacteria to antibiotics. Our experiment will test six different antibiotics used to treat bacterial infections, as well as two combinations of these antibiotics: ampicillin, chloramphenicol, gentamicin, oxacillin, streptomycin and tetracycline. We hypothesize that if *H. felis* resistance is tested over 24 hours with various individual antibiotics (monotherapy) and a combination of antibiotics, then the combination of antibiotics will eradicate

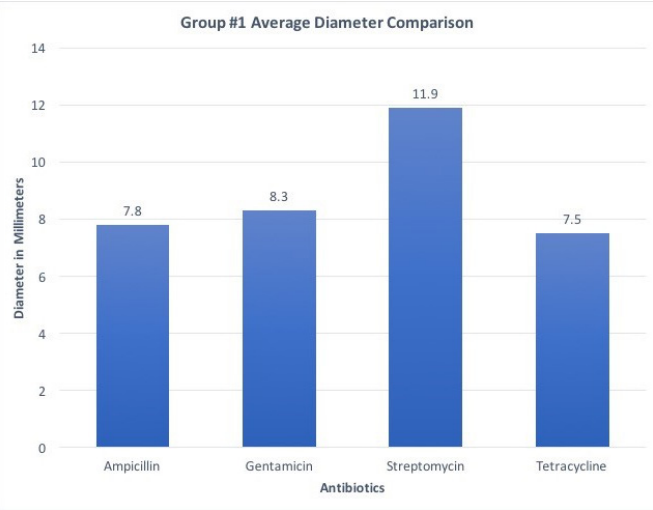

Figure 1. Group #1 Average Diameter Comparison. This result of this graph expresses the most effective mono-therapy antibiotic for eradicating *H. felis* was streptomycin in this test group. The least effective nontherapy antibiotic for eradicating *H. felis* was Tetracycline.

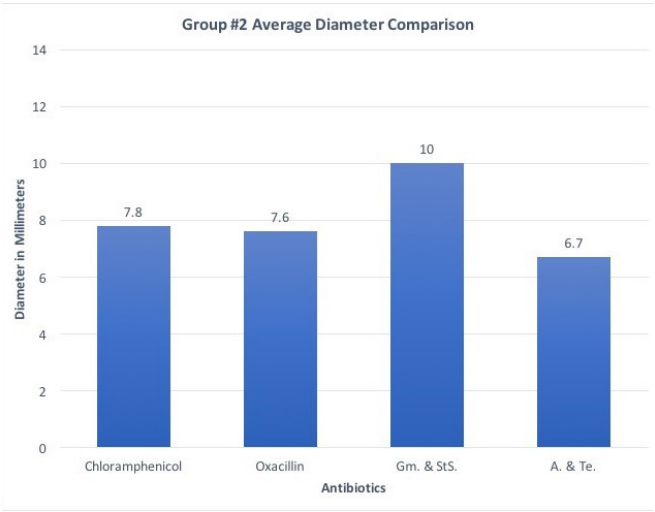

Figure 2. Group #2 Average Diameter Comparison. This result of this graph expresses the most effective combination-therapy antibiotic for eradicating *H. felis* was Gentamycin and Streptomycin in this test group. The least effective combination-therapy antibiotic for eradicating *H. felis* was Ampicillin and Tetracycline.

a higher number of bacteria.

RESULTS

We conducted this research in order to determine if a combination of antibiotics would be more effective at eradicating *Helicobacter felis* than a monotherapy of an antibiotic. In order to test antibiotic effectiveness, *H. felis* was plated on tryptic soy and subjected to the Kirby-Bauer antibiotic test with eight different antibiotic treatments. After 24 hours we measured the inhibition zones of the stand-alone monotherapy antibiotics, then decided to test the two strongest antibiotics out of the results in combination and the two weakest in combination to see if the paired antibiotics would prove effective.

The results showed that streptomycin, which proved to be the most effective antibiotic in a 24-hour period, was superior as a monotherapy as well as when combined with gentamicin. Alone, streptomycin had a resistance diameter of 11.9 mm (**Figure 1**). When combined with gentamicin, its effectiveness weakened, as it had a diameter of 10 mm (**Figure 2**). Ampicillin had a resistance diameter of 7.8 mm, while gentamicin had a diameter of 8.3 mm, and tetracycline had a diameter of 7.5 mm. Chloramphenicol had a diameter of 7.8 mm, and oxacillin had a diameter of 7.6 mm (**Figure 1**). The combination of ampicillin and tetracycline had a resistance diameter of 6.7 mm (**Figure 2**).

DISCUSSION

The results showed that streptomycin, which proved to be the most effective antibiotic in a 24-hour period was superior as a monotherapy treatment, but the data suggest it is less effective when combined with other antibiotics, as compared to the other combination treatment trials.

Our data indicate that streptomycin was effective against *H. felis* growth in vitro, suggesting that streptomycin could be a useful treatment option for veterinarians seeking to treat infected animals. It is hypothesized that the reason the antibiotic streptomycin was more effective in monotherapy rather than in combination therapy is because of the weakening effect that the antibiotic streptomycin was combined with leading to an increased amount of bacteria found with the combination therapy trials. As seen in **Figures 1** - **4** there is a direct correlation to how the bacteria performed in monotherapy and in combination therapy. The streptomycin had an average inhibition size of 11.9 mm, and the gentamicin (the antibiotic in combination therapy with the streptomycin) had an average inhibition size of 8.3 mm. The combination therapy including these two antibiotics had an average inhibition of 10 mm. This suggests that the gentamicin had a direct impact on inhibiting the antibiotic performance of the streptomycin. Similar observations were seen for the other two antibiotics that were tested in combination therapy, with the combination therapy average size being roughly equivalent to the mean of the two individual component antibiotics.

Our ability to draw conclusions from the results is limited by our experimental conditions. With more time, in a different medium and perhaps in animal tissue, we would have more biologically relevant results. We did not use the recommended tryptic soy agar liquid medium to grow the *H. felis*, instead we used another medium, lysogeny broth, to grow the bacteria. We only tested results after 24 hours. If we had extended testing, we would have had additional insight into the bacteria's infection processes. Finally, we only used an *in vitro* method to analyze our results. Alternatively an *in*

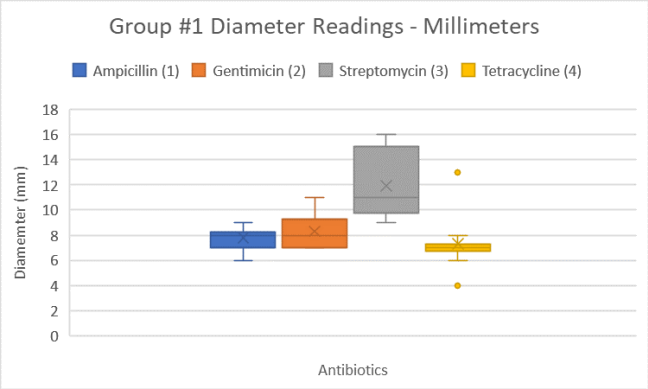

Figure 3. Group #1 Antibiotic Diameter Bar Graph Readings. This bar graph expresses the average readings that were taken from the monotherapy therapy antibiotic zone of inhibition in Group #1 on day one of testing. Recorded in millimeters (mm).

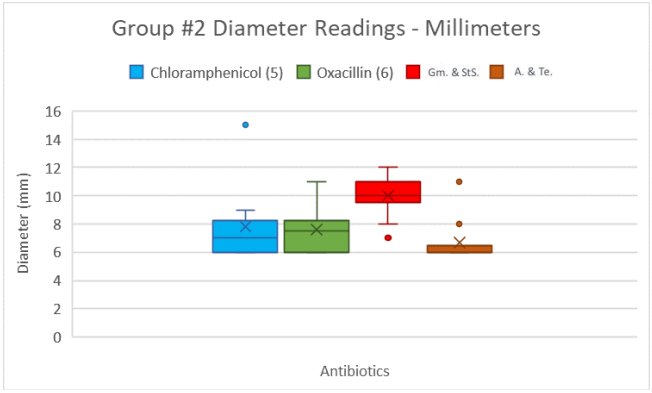

Figure 4. Group #2 Antibiotic Diameter Bar Graph Readings. This bar graph expresses the average readings that were taken from the combination therapy antibiotic zone of inhibition in Group #2 on day two of testing. Recorded in millimeters (mm).

vivo method could be tested to better simulate the biological conditions of an organism suspectable to these infections.

A previous experiment on *H. felis* called for incubation at 37ºC. We aimed to replicate this condition in our experiment; however we incubated at 35ºC due to incubator availability. We observed which petri dishes had this error directly affecting the growth of the bacteria and found no significant effect on the growth of the bacteria. Measurement error may have occurred in the experiment while measuring of diameter of the resistance spots. The spots had been scattered in various parts of the dish and this made it challenging to measure the spots with a ruler.

In the future, it may be useful to test the resistance of the *H. felis* to antibiotics other than those tested in this study, to determine their effectiveness. Using a different medium or a live animal subject in the experiments would be another area worth exploring. It may also be useful to test a probiotic after the antibiotic to assess the animal's gastric inflammation as well any decrease in symptoms. Another interesting experiment would be to see how various concentrations of each antibiotic affect the *H. felis*, in order to determine the optimal concentration and amount that could be given to a patient with this *H. felis* infection. The results of my experimentation contribute to future research in making a effort in helping animals who get *H. felis*.

MATERIALS AND METHODS

Nine hundred microliters of lysogeny broth was mixed with 100 microliters of *H. felis* to expand the sample size of the bacteria. *H. Felis* was incubated for 24 hours while shaking. Ninety microliters of distilled water were placed inside each antibiotic cell to rehydrate the various antibiotics to be used for testing. Each hydrated antibiotic solution was placed in a 2 ml tube. The streptomycin and gentamicin were combined by mixing 10 microliters of each antibiotic within a 2 ml tube. This process was repeated to combine tetracycline and ampicillin. The filter disks where then soaked in each antibiotic to apply on designated plate divisions among then tryptic soy agar plates with 5% sheep's blood plates, spread with *H. Felis*. Each dish was placed in the incubator at 35ºC for 24 hours. After the 24-hour period, we measured the diameter of the zone of inhibition in millimeters using a ruler. After testing the stand-alone monotherapy antibiotics, we tested combinations of the two strongest antibiotics and the two weakest ones to determine if these combinations of antibiotics would be effective.

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