AN ABSTRACT OF THE THESIS OF

Jessica N. Puccetti for the degree Honors Baccalaureate of Science in Bioresource Research with an option in Animal Reproduction and Development presented on August 14th, 2009. Title: Use of Artemisinin to Treat *Mycoplasma haemolama* Infection in Llamas.

Abstract Approved: _____

Susan J. Tornquist

Mycoplasma haemolamae is associated with mild to marked anemia in stressed, immune-suppressed, and debilitated animals, and may be found in low numbers in healthy animals. The continued presence of the organism, detectable by polymerase chain reaction (PCR)-based assay, may be associated with an underlying problem, such as stress or immune-suppression that prevents the immune system from completely clearing *M. haemolamae.*

Treatment with tetracycline can improve anemia, and decrease bacterial numbers, but it does not clear the infection in the majority of cases. Artemisinin (quinghaosu), an herbal remedy derived from wormwood, has been shown to successfully treat malaria in humans. The purpose of this research was to test use of artemisinin to treat *M. haemolamae* infection in camelids.

Six llamas, negative for *M. haemolamae* by PCR and blood smear examination, were experimentally infected with the bacteria by transfusion of an infected alpaca's blood. Once the llamas were positive for *M. haemolamae* as detected by PCR, they were treated with 200 mg of artemisinin given twice daily for a total of 20 days (5 days of treatment followed by 5 days of no treatment for four rounds). Blood was collected every other day during the treatment cycle and weekly for one month after the treatment cycle ended. PCR, packed cell volume, plasma protein, and blood smear diagnosis were performed on these samples. Four of the llamas remained positive by PCR after one month of treatment. Two llamas were negative at the end of the month

and were immune-suppressed by dexamethasone to determine if artemisinin had cleared the *M. haemolamae* infection. Both llamas became positive once immune-suppressed with the corticosteroid. These results suggest that artemisinin did not effectively clear *M. haemolamae* infection in the six llamas.

Key Words: *Mycoplasma haemolamae,* artemisinin, anemia, malaria, camelids

Corresponding e-mail address: gwpotts@comcast.net

Use of Artemisinin to Treat *Mycoplasma haemolamae* Infection in Llamas

By
Jessica N. Puccetti

A THESIS
submitted to
Oregon State University
University Honors College
and Bioresource Research

in partial fulfillment of
the requirements for
the degree of

Honors Baccalaureate of Science in Bioresource Research,
Animal Reproduction and Development (Honors Scholar)

Presented August 14th, 2009
Commencement June 2010

Honors Baccalaureate of Science in Bioresource Research thesis of Jessica Puccetti presented on August, 2009.

APPROVED:

Susan J. Wright 8/25/09

Mentor, representing Veterinary Medicine

8/24/09

Committee Member, representing Veterinary Medicine

8/25/09

Committee Member, representing BioResource Research

Dean, University Honors College

I understand that my project will become part of the permanent collection of Oregon State University, University Honors College and Bioresource Research. My signature below authorizes release of my project and thesis to any reader upon request.

Jessica N. Puccetti, Author

Acknowledgements

The course of this project has given me a great experience in the laboratory and handling llamas. The education that I have received at Oregon State University has not only been rewarding, with valuable experience, but has set me on path toward a bright future. First and foremost, I would like to thank, Dr. Susan Tornquist, my research mentor, for all of her guidance and support. Her knowledge and guidance has helped me to increase my passion for veterinary medicine. I would also like to give special thanks to Lisa Boeder, for her friendship, and patience and for helping me learn all of the skills in the laboratory and handling llamas. Thanks, Lisa for your knowledge and time.

I would also like to thank the other members of my undergraduate committee; Dr. Christopher Cebra and Dr. Kate Field for their assistance towards the completion of my thesis. Another thanks to Wanda Crannell, my Bioresource Research advisor, who helped me schedule my curriculum and kept me on track to make sure everything got completed. I thank the Bioresource Research program for making undergraduate research possible. Also thank you to the OSU vet school and veterinary students for helping geld the llamas and perform blood transfusions.

I give a special thanks to my parents, Mike and Kaye Puccetti. Thank you from the bottom of my heart for not only your financial support, but your unconditional love and support every day. Mom, your unending belief that I can make it through not only this research but through school was a tremendous help. Dad, your continuous support and push to do the best that I can has given me the drive and determination to succeed in all that I do. Finally, I would like to thank my boyfriend, George Potts. Thank you for your never ending faith in me, your support in everything that I do, and for just being there for me at the end of the day.

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Introduction:

Mycoplasma haemolamae (M. haemolamae) is a hemotropic bacteria that affects camelids (llamas and alpacas)[2]. Studies have shown that as many as 25% of camelids nationwide may be infected with *M. haemolamae* [4]. The organisms are generally less than 1 µm in size and may be rod-shaped, spherical, or ring-shaped[1]. The gram-negative bacteria attach to the surface of a red blood cell[3] without penetrating the plasma membrane of the erythrocyte[7]. Infection in the llama varies from asymptomatic to severe, depending on host susceptibility and probably other factors[3]. Clinical signs may be either acute or chronic and are associated with mild to marked anemia in stressed, immune-suppressed, and weakened animals. The infection is especially prominent in animals that are stressed, or simultaneously infected with other organisms. The infection may result in significant clinical signs and sometimes death. The signs may include fever, poor growth, acute collapse, chronic weight loss, depression or failure to thrive, and decreased fertility and lethargy[3,5,7]. *M. haemolamae* infection has also been reported in what appear to be healthy animals, but the bacteria are found in low numbers. Neonatal camelids may be more susceptible to acute infection, leading to more severe clinical signs of disease[3,10]. There is also good evidence that suggest that the bacteria can be transmitted in-utero, because the organism has been detected in crias as young as 24 hours of age and also diagnosed in early neonatal death in very young crias[14]. The most characteristic symptom of the infection is anemia, most likely due to erythrocyte destruction by the immune system[8,9]. The bacterium alters the shape and deformability of the affected red cell, which causes the immune system to destroy the erythrocytes[13]. The red blood cell with an attached organism is recognized as foreign and is thus removed from the circulation, causing anemia[11,12].

This bacterium, formerly known as *Eperythrozoon* species, was reclassified when its 16S ribosomal RNA gene was sequenced, showing that it belonged in the hemotropic mycoplasma (haemoplasmas) group[1]. Members of this *Mycoplasma* group infect a wide variety of vertebrate animals including and not limited to dogs, cats, lab animals, cattle, pigs, and wild animals[1,5]. *M. haemolamae* is most closely related to *M. haemosuis* (affecting swine) and next most closely related, genetically, to *M. wenyonii* (affecting cattle) [5]. Although, closely related to other mycoplasmas affecting other animals, the bacteria tend to be host-specific with cross-infections being rare[6]. The bacteria receive their nutritional support through surface parasitism of the erythrocyte. The haemoplasmas have not been grown successfully *in vitro*, possibly because of the inability to recreate the intricate nutritional support that is provided by the host, which the bacteria depend on for survival[1].

Blood smear examination was the primary method of diagnosis of these infections before a polymerase chain reaction (PCR)-based assay was developed for this bacterium. The recurring nature of the infection, as well as the low numbers of organisms present at times, makes blood smear a relatively insensitive method[5]. The camelid is frequently presumed negative when often times the bacteria are still present but cannot be seen on the slide. Also it is often difficult to confirm infection, as the organism resembles Howell-Jolly bodies and/or background debris, and because it tends to fall off the erythrocyte easily[8]. The PCR assay amplifies a unique section of the 16S rRNA gene of *M. haemolamae* using primers based on GenBank sequence accession AF306346 and blood from a naturally-infected alpaca[5]. The development of the PCR has led to a much more highly sensitive and specific diagnostic test than examination of a blood smear[16,17]. The reliability of the PCR is increased by stringent control measures, to prevent contamination with previously amplified products[17].

Currently, there is no treatment that clears the infection. This results in camelids becoming chronic carriers that may serve as a reservoir for the bacteria. Treatment with tetracycline can improve the anemia, reduce bacterial numbers, and improve clinical signs, if present. Treatment with tetracycline often clears the infection as detectable by blood smear, but does not clear it completely, as it is still detectable by PCR[5]. The search for an effective treatment that can clear the infection continues.

The continued presence of bacteremia suggests that the immune system cannot completely clear *M. haemolamae* infection. When the llamas become stressed from such factors as transportation, an environmental change, changes in social status, increased breeding use of males, parturition, immune suppression, or another recent or chronic infectious disease, it may result in the proliferation of the bacteria[4]. The cyclic nature of the infection suggests that infected llamas will probably remain chronic carriers even after clinical signs have resolved[1]. Chronic carriers have the potential to transmit the infection to other camelids. The carrier animals are most likely the source of infection for other camelids; however, the mode of transmission is currently unknown. It is suspected that it is through a biting insect vector, and spread only through contact with an infected animal's blood[12]. Since the mode of transmission is unknown, complete prevention of this disease is very hard. It is important to use a new needle on each llama when vaccinating or treating a herd of llamas, properly control insects (lice, mites, mosquitoes, and biting flies), to vaccinate and treat for other diseases, and to provide routine veterinary care, and proper husbandry[4].

A study of experimentally infected llamas tested the efficacy of injectable florfenicol in treatment of *M. haemolamae* infection. Florfenicol was less effective than tetracycline, as untreated llamas cleared the infection faster than those treated with florfenicol. Another study

examined the use of oral and injectable enrofloxacin to treat the infection[15]. Neither oral nor injectable forms were effective in clearing the infection, and there was no significant difference between the two forms. Currently the best treatment plan remains the tetracycline regimen, which only reduces the signs of clinical disease. It does not completely eliminate the organism leaving the chronic carriers who most likely serve as a reservoir for the infection.

Artemisinin (quinghaosu), an herbal remedy derived from wormwood, has been shown to successfully treat chloroquine-resistant malaria in humans[18-20]. Malaria is caused by *Plasmodium spp*, another disease agent that infects red blood cells[12]. Artemisinin has an endoperoxide linkage in the molecule, which breaks when in the presence of heme. Heme, which binds the oxygen molecule is a bound prosthetic group containing an organic component and a central iron atom, is found in hemoglobins, as well as myoglobins[21]. Malaria is located inside of the erythrocyte and consumes hemoglobin, which liberates the heme. Artemisinin has an affinity for the iron, which is present in high concentrations in the hemoglobin present in erythrocytes. As the endoperoxide linkage breaks it creates reactive free radicals that cause extensive damage and death to the infecting organism[22].

The objective of this study was to experimentally infect llamas with *M. haemolamae* and to determine if artemisinin had cleared the infection in at least one or more llamas. Following infection, the llamas were treated twice daily with 200 mg of artemisinin for a total of 20 days. The llamas that were negative after treatment, and one month post-treatment, for *M. haemolamae* by PCR assay were immune suppressed with corticosteroids to determine if they had truly cleared the infection. The central hypothesis of this study was that llamas that were experimentally infected with *M. haemolamae* would clear the infection following treatment with artemisinin at a standard dosage. The expected response that demonstrated this hypothesis would

be negative PCR results for *M. haemolamae* after the llama was immune suppressed. This was a preliminary study and if at least one llama after being treated with artemisinin was found negative for the *M. haemolamae* infection after immune suppression, a larger study with an infected, but non-treated control group would follow.

Materials and Methods:

Llamas

Six adult male llamas, including five geldings and one intact male, were used for this study. The llamas were obtained from private donors. The castrated llamas employed in this study had no significant health problems; however, the intact male had a heart murmur, which is the reason he was not gelded. The llamas were housed at OSU Veterinary Medicine Animal Isolation Laboratory (VMAIL) in an isolated stall. Castrated males were kept separated from the intact male. Free-choice water and hay were available to all animals. All procedures in this study were approved by Oregon State University's Institutional Animal Care and Use Committee.

All llamas were weighed and initial health screens were performed. These included physical exam, complete blood count and screening for *M. haemolamae* by PCR. All llamas were infected with *M. haemolamae* by blood transfusion from a naturally infected alpaca. This alpaca is clinically healthy but becomes bacteremic when immune suppressed with 2–mg/kg dexamethasone given intravenously. Following transfusion, this alpaca was treated with tetracycline and monitored until there was no detectable organism, anemia, or fever. This alpaca was housed in building 257 (an isolated stall) in VMAIL until bacterial numbers were reduced as detectable by blood smear. The blood transfusion was performed by injecting 50 ml of blood collected from the bacteremic donor into the jugular of the recipient.

The infected llamas were given daily health checks that included measurement of rectal temperatures. One ml of EDTA blood was drawn daily for the following tests: PCR, total protein (TP), packed cell volumes (PCV), and blood smears for the first week post infection. Once the organism was detectable through blood smear and PCR, llamas received treatment with artemisinin at a dosage of 200 mg in 2 cc warm water given rectally twice daily for 5 days,

followed by 5 days of no treatment for four consecutive rounds. Once treatment was completed the llamas were monitored for 1 month by measuring body temperature and performing PCV, TP, PCR, and blood smear exam. At the end of the month, they were immune-suppressed by administering 2 mg/kg dexamethasone intravenously injection for 3 consecutive days. They had daily blood draws for PCV, TP, PCR, and blood smear to determine if the organism was still present. Five of the six llamas were humanely euthanized and the other llama was donated to OSU—College of Veterinary Medicine for a different study.

PCV

The packed cell volume was determined by centrifuging blood at 10,000 RPM for five minutes. This separated the blood into packed cells, which are primarily erythrocytes, and plasma. The volume of packed red blood cells was divided by the total volume of the blood sample. The samples were in capillary tubes and the layers were measured by length to get the rough measure of anemia. Normal camelids have a reference range of 25-45% for the PCV.

Sample Collection

Blood samples were collected via jugular venipuncture from each llama. The blood was collected in EDTA tubes and 600 µL of sample was lightly centrifuged at 2 × 1000 for 2 minutes to separate the plasma fraction and surface layer of red cells. The plasma was transferred to a 2.0 ml screw top tube and was stored at -20 ˚C until DNA was extracted.

DNA extraction

DNA was extracted from plasma of the experimentally infected llamas and purified using the Qiagen Systems Generation® DNA Purification Capture Column Kit. Dry block heater was turned on to pre-heat to 99 ˚C. Two hundred µl of well-mixed sample was added to the Generation® Capture Column in the blue Waste Collection Tube and allowed to absorb at room

temperature for at least one minute or up to one hour. Then 400 µl of DNA Purification Solution (Solution 1) was added to the column and incubated for one minute at room temperature. The column was centrifuged for 10 seconds at 6 × 1,000 g. The Capture Column was transferred to the second blue Waste Collection Tube (the first blue Waste Collection Tube was thrown away with a waste volume of 600 µl). Another 400 µl of Solution 1 was added to the column and incubated at one minute at room temperature. The tube was centrifuged for 10 seconds at 6 × 1,000 g. Two hundred µl of DNA Elution Solution (Solution 2) was added to column, and no required incubation. The tube is centrifuged for another 10 seconds at 6 × 1,000 g, and there was another waste volume of 600 µl. The Capture Column containing the purified DNA should be white. The Capture column was transferred to clear DNA Collection tube and second blue Waste Collection tube was discarded. Added 100 µl of Solution 2 and incubated for 10 minutes at 99 ˚C in the dry block heater. The tube was centrifuged for 20 seconds at 6 × 1,000 g immediately following heating step to release purified DNA from Capture Column. The DNA solution appeared clear. The Capture Column was discarded. The purified DNA was ready for analysis or was stored at -20 ˚C until the next step, PCR, was performed.[23]

PCR

Reaction mixtures were prepared under a laminar flow hood that was irradiated by UV light. To avoid DNA contamination, a separate set of aerosol barrier tips and pipettes were used exclusively for preparation of reaction mixtures. A negative and positive control was included in each run along with the test samples. The negative control, PCR water, was used to ensure that reagents and/or extraction buffers were not contaminated. The positive control, *M. haemolamae,* was extracted alpaca DNA that was sequenced and verified as containing *M. haemolamae*

GenBank submission AF306346. The *M. haemolamae* specific primers, LIMH and R4MH, were used for 16s rRNA amplification. LIMH primer is 3' TAG ATT TGA AAT AGT CTA AAT TAA and R4MH primer is 5' AAT TAG TAC AAT CAC GAC AGA ATC A. Nested PCR was done after the conclusion of the study period on Llamas 2, 5, & 6. Nested PCR is intended to reduce possible contamination in products. Nested PCR reaction was done before the specific primers and used the universal primers Fhf1 and Rhf2. Thawed reagents were added to l.5 ml nuclease free microcentrifuge tube according to the appropriate volume (based on number of samples and controls) to make a master mix for PCR. Each reaction contained PCR water, 10X PCR buffer, dNTP mix (10 µM), 3' "LIMH" primer (20 µM), 5' "R4MH" primer (20 µM), platinum Taq DNA Polymerase (5 U/ µl), and $MgCl_2$ (50 µM). The master mix was lightly vortexed for 5 seconds to clear the cap of any liquid. Twenty-three µl of the master mix was put into each 0.2 ml PCR tube and then 2.0 µl of extracted DNA was added to each 0.2 ml PCR tube.

The 0.2 ml PCR tubes were placed in a mastercycler and standard amplification reactions were carried out. The thermal parameters consisted of an initial denaturation step for 1 minute at 94 ˚C, followed by 40 step cycles of 94 ˚C for 1 minute, 0 ˚C for 1 minute, and 72 ˚C for 2 minutes and a final extension step at 72 ˚C for 7 minutes. The products were held at 4 ˚C until the ethidium-bromide stained agarose gel was ran.[23]

The PCR products were separated by electrophoresis on 15% agarose gels, which were then stained with ethidium bromide. "Track-it", a 100 bp ladder (Invitrogen) used to measure the 318 bp positive amplicon, was also included in each run with the samples. They were photographed and analyzed for positive bands for each sample. A positive PCR product for *M. haemolamae* yielded a band at 318 base pairs (bp) with the specific primer set (Fig. 1).

Results:

PCR

PCR results were scored as positive for *M. haemolamae* if they had a positive band at 318 bp. The PCR based assay confirmed that all six llamas were positive for *M. haemolamae* both during the treatment with artemisinin and one month after treatment following immune suppression with dexamethasone. Llamas 1, 3, and 4 were positive for *M. haemolamae* by PCR over 85% of the study period. None of these llamas effectively cleared the *M. haemolamae* infection during the study period, and all remained positive even one month after treatment. The llamas did not have to be immune suppressed to determine if they had cleared the infection. Llama 5 and 6 did not initially become positive for *M. haemolamae* by PCR. By nested PCR (done after the study period), llama 5 was found positive on day 4. However, during the study period, he was not known to be positive until day 15 and was started on the artemisinin treatment during the second round. Llama 6 did not test positive until day 39; however, he was still treated with artemisinin before he became positive. Llama 5 was positive for 38% of the study period and llama 6 was positive for 30% of the study period. Both tested positive by PCR once immune suppressed one month after treatment. Llama 2 became positive on day 6 and began treatment with artemisinin on day 9. He became negative by PCR for *M. haemolamae* after the first treatment cycle. He was positive twice more during treatment and was negative for the rest of the study period. To determine if artemisinin effectively cleared llama 2's infection, he was immune suppressed with dexamethasone one month after treatment. He became positive by PCR after the first day of immune suppression and thus was found to have not effectively cleared the *M. haemolamae* infection. (Figures 2-7)

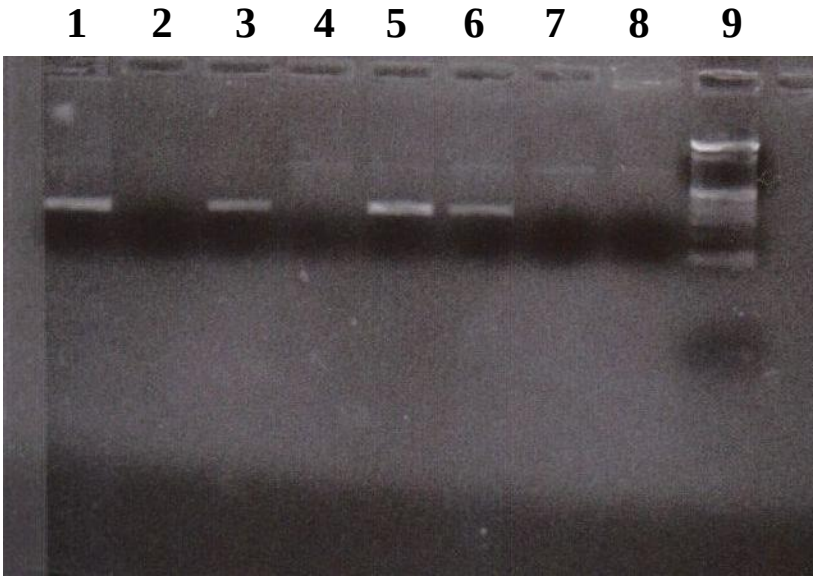

Figure 1. Representative gel showing amplification of bacterial 16S rRNA using specific primers LIMH & R4MH for *M. haemolamae.* Lane 1, DNA extracted from an alpaca that was sequenced and verified as containing *M. haemolamae* used as a positive control; lane 2, negative control (PCR distilled water); lane 3, DNA extracted from *M. haemolamae* experimentally infected llama 1 (Becker) blood; lane 4, DNA extracted from *M. haemolamae* experimentally infected llama 2 (Benito) blood; lane 5, DNA extracted from *M. haemolamae* experimentally infected llama 3 (Chestnut) blood; lane 6, DNA extracted from *M. haemolamae* experimentally infected llama 4 (Mouse) blood; lane 7, DNA extracted from *M. haemolamae* experimentally infected llama 5 (Randy) blood; lane 8, DNA extracted from *M. haemolamae* experimentally infected llama 6 (TreBon) blood; lane 9, "Track-it", a 100 bp ladder (Invitrogen).

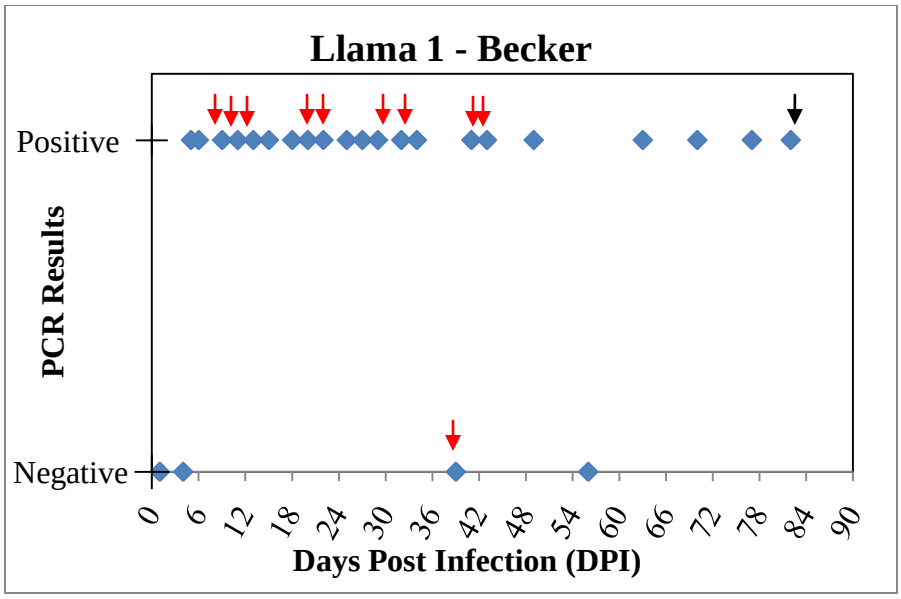

Figure 2. PCR results for llama 1 (Becker) for *M. haemolamae* infection. The data points represent the PCR results for the days post infection (days after transfused with infected alpaca blood). Red arrows indicate treatment days with artemisinin when blood was drawn and PCR assay performed. Black arrows indicate immune-suppression days.

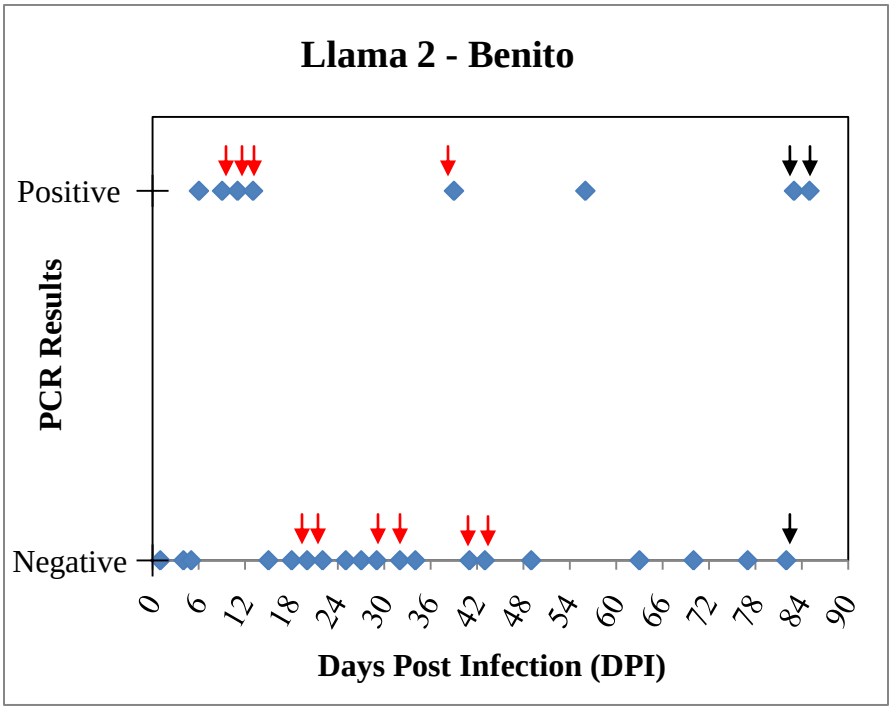

Figure 3. PCR results for llama 2 (Benito) for *M. haemolamae* infection. Red arrows indicate treatment days with artemisinin when blood was drawn and PCR assay performed. Black arrows indicate immune-suppression days.

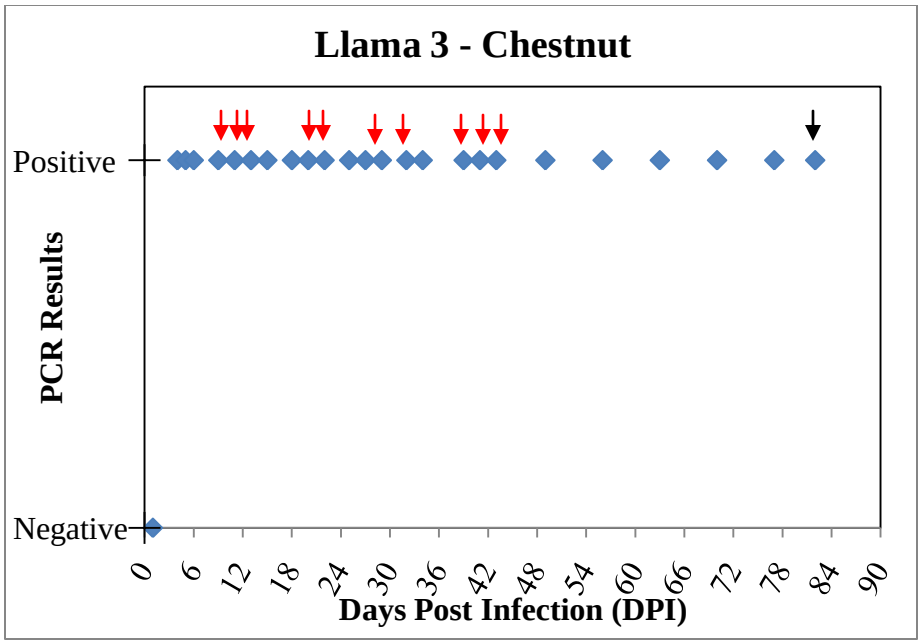

Figure 4. PCR results for llama 3 (Chestnut) for *M. haemolamae* infection. Red arrows indicate treatment days with artemisinin when blood was drawn and PCR assay performed. Black arrows indicate immune-suppression days.

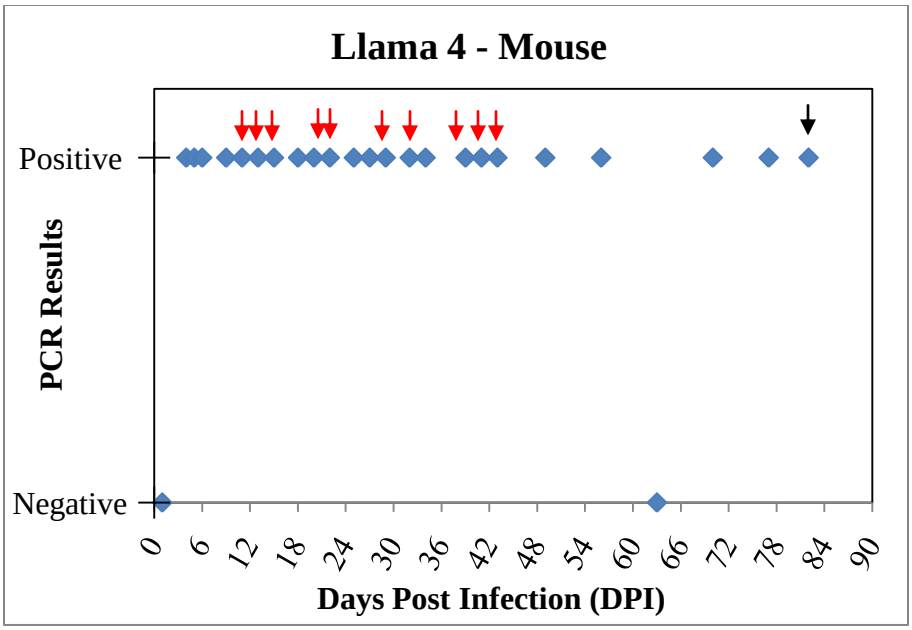

Figure 5. PCR results for llama 4 (Mouse) for *M. haemolamae* infection. Red arrows indicate treatment days with artemisinin when blood was drawn and PCR assay performed. Black arrows indicate immune-suppression days.

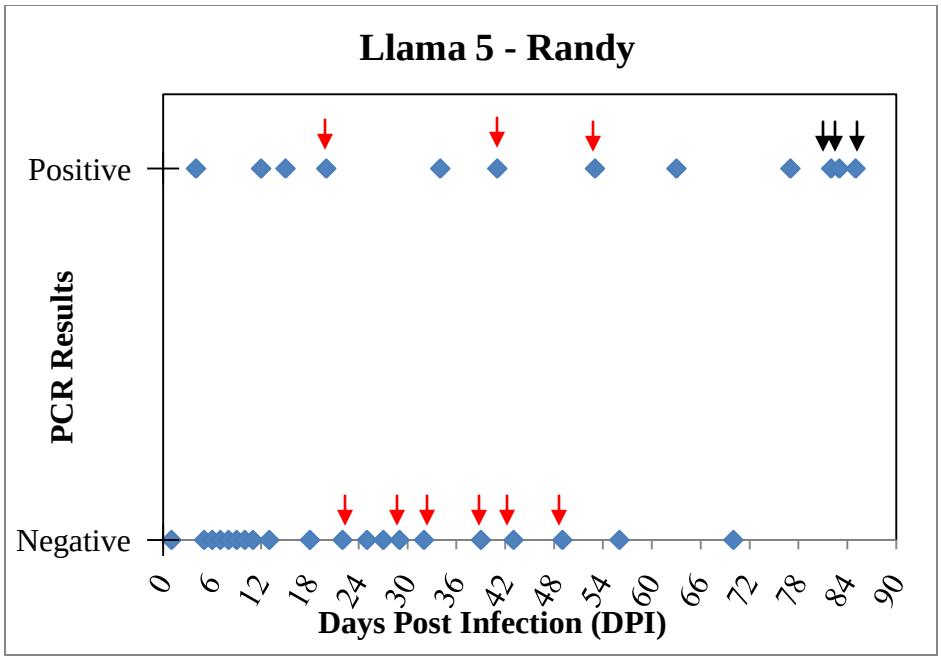

Figure 6. PCR results for llama 5 (Randy) for *M. haemolamae* infection. Red arrows indicate treatment days with artemisinin when blood was drawn and PCR assay performed. Black arrows indicate immune-suppression days.

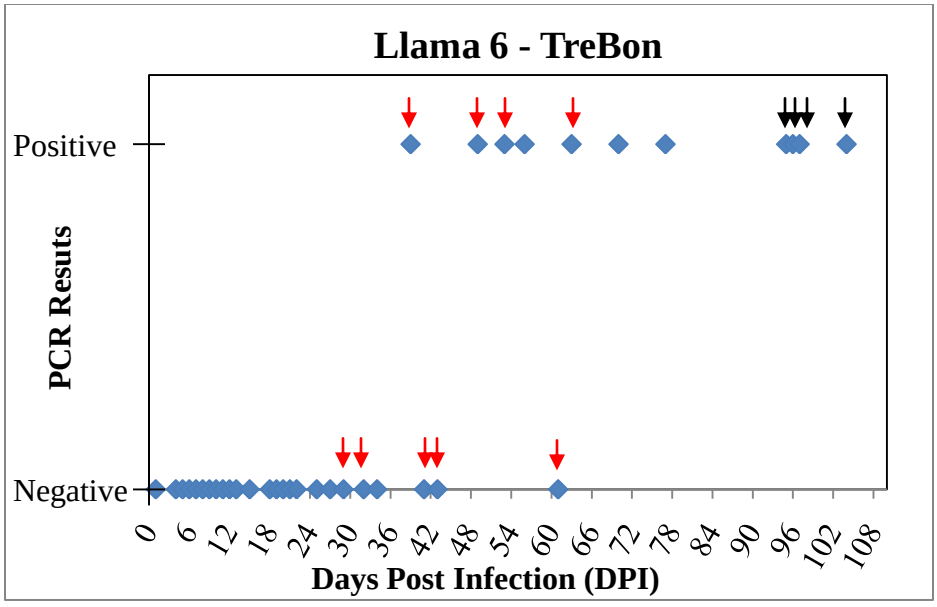

Figure 7. PCR results for llama 6 (TreBon) for *M. haemolamae* infection. Red arrows indicate treatment days with artemisinin when blood was drawn and PCR assay performed. Black arrows indicate immune-suppression days.

Packed Cell Volume (PCV)

Anemia is present when the PCV is less than 25%. This could be due to the infection, as infected red blood cells are destroyed by the immune system. Llama 5 had a higher initial PCV than all the other llamas in the study most likely related to his heart murmur. Llama 5 had an initial PCV of 40%. All llamas had a low PCV on day 4, when three of the llamas had a positive PCR result. The other llamas, excluding llama 6, had positive PCR result for *M. haemolamae* within 2 more days. On day 15, all llamas except llama 6 had a low PCV again and all were positive by PCR around this point. On day 39, there was another drop in all of the llama's PCVs. In this two day interval, days post infection (DPI) 39-41, all llamas were positive for *M. haemolamae*. Interestingly, all llamas were going through the artemisinin treatment day 39-43 and this was the last artemisinin treatment for llamas 1-4. At the end of one month, for llamas 1-5 were all immune-suppressed with dexamethasone, on day 81. All five of the llamas had a low PCV on day 83 and a positive PCR result for *M. haemolamae.* Llama 6 was immune-suppressed at the end of one month following his last treatment. He became positive by PCR once immune-suppressed and his PCV was also low during this period. Each llama's initial PCV could have been graphed to show the changes from the initial throughout the study period. This would probably have shown more similar changes in all the animals compared with the PCR result. (Figure 8)

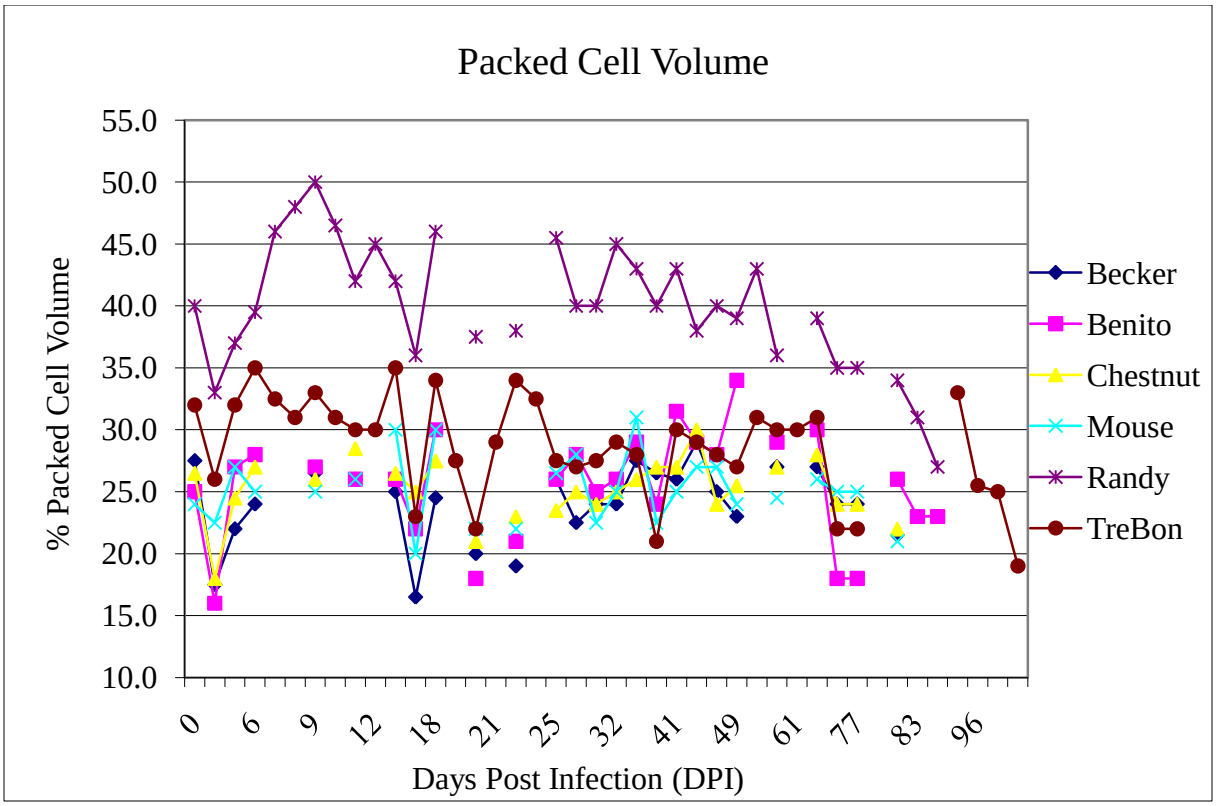

Figure 8. The packed cell volume (PCV) of each llama by day post infection.

Plasma Protein (PP)

The plasma protein (PP) indicates the concentration of total protein in the blood. The results of the PP test gives information on the hydration status, nutrition, and presence or absence of inflammation, as well as general health status of the animal[3]. The reference range for PP for llamas is 6-7 g/dl. Most llamas stayed in the 6-7 g/dl range almost the whole study period, llama 5, had a higher PP concentration possibly due to the heart murmur. Llama 1 and 4, were positive for *M. haemolamae* over 85% of the time, had lower PP concentrations than the other llamas. Llama 2 tended to have a low PP result on the same days that he had a positive PCR result. (Figure 9)

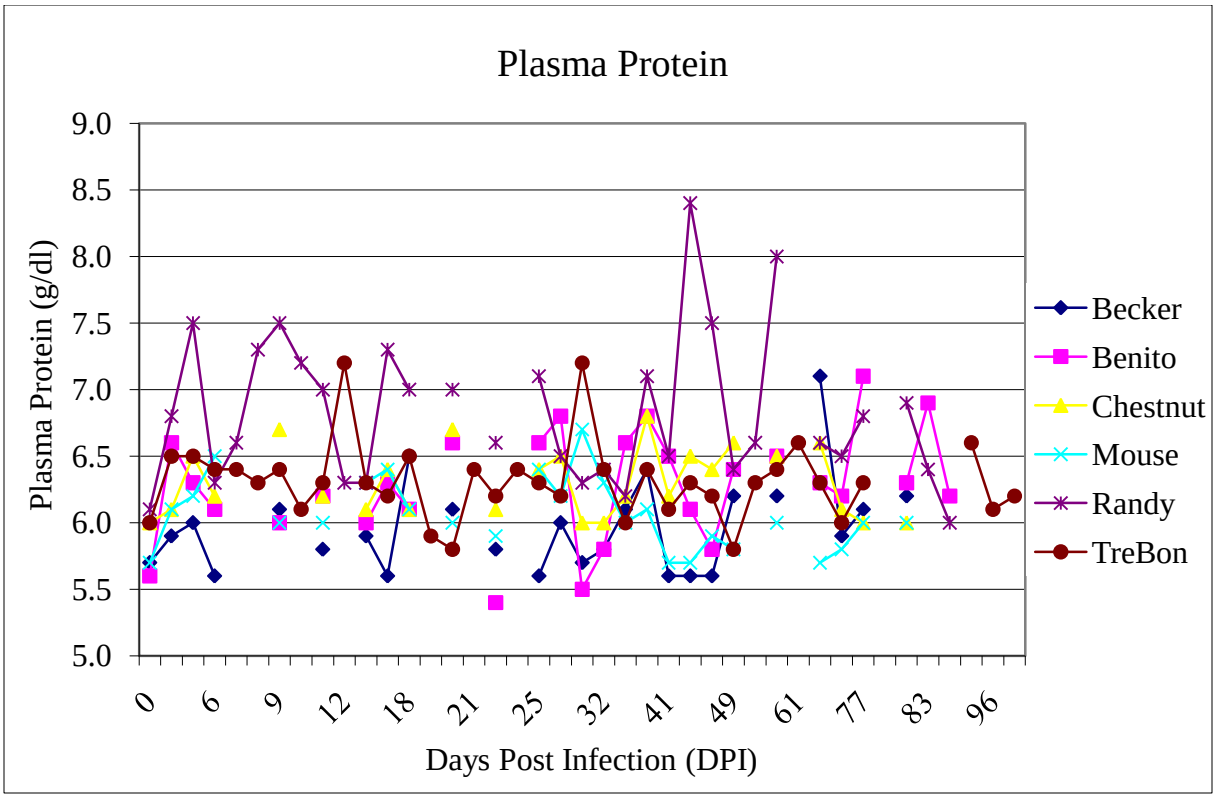

Figure 9. The plasma protein (PP) for each llama days post infection.

Body Temperature

The body temperature of each llama was taken because one of the common signs of other mycoplasmal infections is fever. The normal body temperature of camelids is 99-101.8 ˚F. A body temperature of over 102 ˚F is high; however, it may not necessarily correlate to the *M. haemolamae* infection and could be due to physical activity or the higher temperature of the day. Around day 41, almost all of the llamas had a high temperature. This time period, (DPI 39-41), correlated with a positive PCR result and a low PCV for all llamas. This was towards the end of the treatment regimen for most of the llamas. The llamas that were immune-suppressed at the end of the month all had a slight fever after the dexamethasone was given. (Figure 10)

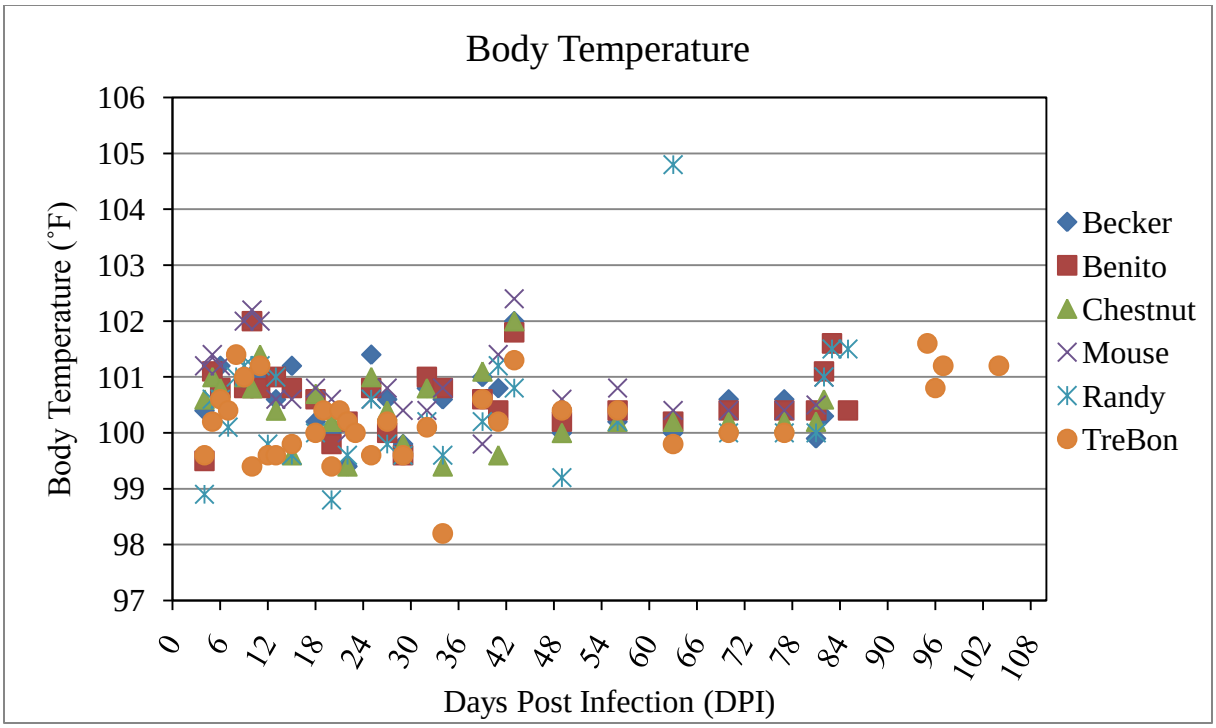

Figure 10. Body temperature, in degrees Fahrenheit, for each by llama day post infection.

Discussion:

The purpose of this study was to investigate the effectiveness of treatment with artemisinin on llamas experimentally-infected with *M. haemolamae*. All llamas were confirmed to be positive for *M. haemolamae* after transfusion with an infected alpaca's blood. They went through twice a day treatment for 20 days with artemisinin to test the hypothesis that artemisinin, at a dosage of 200 mg, would be effective in clearing the *M. haemolamae* infection. The results indicate that artemisinin was not a successful treatment at the dosage of 200 mg given twice daily for *M. haemolamae* and suggested that it was not as effective as the current tetracycline regimen.

The results for the PCR for *M. haemolamae* infection for llamas 1, 3, and 4, were positive for over 85% of the study period. These llamas were positive almost the whole time during the treatment regimen and positive one month after the conclusion of treatment. The PCR results for *M. haemolamae* infection in llama 5 and 6 were positive for about 38% and 30% of the days during the study period. Llama 5 was positive four times during the treatment regimen and llama 6 was positive five times during the treatment regimen. Both llamas 5 and 6 were positive at the end of the month following immune-suppression. The llama that appeared to respond the best to the treatment program was llama 2. He was positive about 28% of the study time. He was positive during the beginning of the study period, right after being transfused with infected blood and then negative for the majority of the treatment regimen. However, once he was immune-suppressed at the end of the month, he became positive for *M. haemolamae* infection by PCR. It is not clear whether his ability to suppress proliferation of *M. haemolamae* was due to the treatment with artemisinin or to his innate immunity.

The dosage of 200 mg given twice a day per animal was chosen based on anecdotal evidence of treatment success in several camelids. Because artemisinin is not a drug, but is rather an herbal treatment, pharmacokinetics have not been studied in camelids, nor is there knowledge of appropriate dosage for these species. Treatment at a higher or more frequent dosage, or a different method of administration, might provide different results.

Because this study was designed as a pilot study, there were only six experimental subjects. The low sample size makes it difficult to draw conclusions about infection and treatment. The low sample size may be associated with significant variability in immune responses between the llamas. However, this study was used to determine if a larger study of artemisinin would be warranted. The results of this study do not support further research into the use of artemisinin at the dosage of 200 mg, since all llamas were positive both during the treatment and one month after the treatment. If at least one llama had cleared the infection and stayed negative for *M. haemolamae* infection after immune-suppression it would have been good support for a larger study. A non-treated control group was not included in this study because previous studies of experimentally-infected llamas showed that non-treated llamas did not clear the infection spontaneously[2,5,15,23]. These previous studies were carried out in the same manner as this study with respect to infection, monitoring, and testing of animals.

In conclusion, this study does not support further research with artemisinin at the dosage used. The results of this study are part of an ongoing effort to understand the *M. haemolamae* infection and a possible treatment. Continued research on new treatments will be necessary to explore all of the unanswered questions of these bacteria. Due to the relatively good results with llama 2; although, he may have just been healthier or had a stronger immune system, it might be worthwhile to conduct a study using artemisinin for either a longer period of time or at a higher

dosage or different administration or a pharamacokinetic study on artemisinin and llamas. But it is unlikely that artemisinin will effectively clear the *M. haemolamae* infection in llamas or alpacas.

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