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

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Objective

The purpose was to determine if artemisinin would be effective in clearing *Mycoplasma haemolamae* infection in llamas.
Why is this important?

• Llama/Alpaca owners
  – Show
  – Raise
  – Business
• Affects selling and moving animals, health certificates, treatment, and insurance
• Easy answer: health of animal
Mycoplasma haemolamae

• Hemotropic bacteria
• Very small (<1 μm)
• Rod-shaped, spherical, or ring-shaped
• Infects camelids
• Infection varies from asymptomatic to severe
Mechanism of Action

- Lacks a peptidoglycan layer
  - Penicillin inhibits production of layer
- Attaches to erythrocyte plasma membrane
- Unable to culture *in vitro*
- Immune system recognizes infected cells as foreign
- Removes from circulation
Mycoplasma Species

• Formerly known as *Eperythrozoon* species
• Reclassified as hemotropic *mycoplasmas* after 16S ribosomal RNA gene sequenced
• *Mycoplasma* infects wide variety of species
• Most closely related to *M. haemosuis* (affecting swine) and *M. wenyonii* (affecting cattle)
Clinical Signs

• Anemia
• Fever
• Edema
• Mild to severe hypoglycemia
• Acute collapse
• Chronic weight loss
• Depression
• Decreased fertility and lethargy
Mode of Transmission

• Unknown
  – Believed spread through contact with infected animals blood (known as chronic carriers)
  – Lice, ticks, mosquitoes, and other vectors

• Prevention
  – New needle for each animal
  – Vaccinate and treat other diseases
  – Routine veterinary care
  – Proper husbandry
In-utero Transmission

4-day-old female alpaca:
• Born 2 weeks premature
• Within 48 hours
  – Loss of appetite
  – Inability to stand
  – Weakness
  – No longer suckled dam
• Fed 75 and 45 mL of alpaca colostrum
In-utero continued

• Cria was given IV fluid therapy, dextrose, and plasma transfusion
  – After had interest in food, responsive to external stimuli, and stand unassisted

• Couple hours later
  – Developed seizures
  – Dyspnea
  – Died
In-utero continued

• Dam was non-parasitemic
  – Ran PCR on both dam and cria found positive for *M. haemolamae*

• Suggests *M. haemolamae* was transferred in-utero from dam to cria

Current Treatment

• Tetracycline regimen reduces numbers of infecting organisms
  – Inhibit bacterial translation
  – Binds reversibly to prokaryotic 30s ribosomal subunit and blocks attachment of aminoacyl tRNA

• No known treatment that clears infection from “carrier” animals
Artemisinin

• Herbal remedy from wormwood
• Used by Chinese herbalists since 200 B.C.
• Currently used to treat malaria
• Further studies for possible cancer treatments

http://www.socbioscience.org/bioengdoc9C.jpg
Artemisinin Mechanism of Action

- Affinity for iron
- Linkage breaks creating reactive oxygen species (ROS)
- Damage to infecting organism leading to death
Tests to determine *M. haemolamae* infection

- **Packed Cell Volume (PCV)**
  - 25-45%
  - Lowered indicates anemia
- **Plasma Protein (PP)**
  - 6-7 mg/dl
  - General health status of animal
- **Body Temperature**
  - 99-101.8 °F
  - Fever—possible sign of infection
Tests to determine *M. haemolamae* infection

- Blood smear diagnosis

- Polymerase Chain Reaction (PCR) assay
  - Positive amplicons at 318 bp

PCR assay

- Sensitive test
  - Detectable by PCR <2 days before seen on blood smears
- Diagnose low numbers of hemoplasma
- Amplify 16s rRNA gene
Materials & Methods

• Six male adult llamas
  – Becker, Benito, Chestnut, Mouse, Randy, and TreBon

• Initial health screening
  – Physical exam: weighed and found 5 of 6 intact
    • Randy had heart murmur
  – Complete blood count
  – PCR for *M. haemolamae*
    • All llamas found negative
Methods continued

• Immune-suppressed donor alpaca (known chronic carrier)
• Llamas transfused with blood from infected donor
  – Mixed with sterile acid-citrate-dextrose (ACD)
Methods continued

• After first week, post-transfusion daily health checks
  – Rectal temperatures and 1 ml of EDTA blood drawn for PCR, PCV, TP, and blood smear

• Once bacteria was detected by blood smear and PCR, treatment began
  – TreBon, only llama that did not become positive
Materials & Methods

- Artemisin dosage 200 mg per 2 cc of water rectally
- Rounds of treatment: twice a day for 5 days and 5 days off
Rectal Treatment

• Drugs given orally are broken down by ruminal flora in ruminant animals

• Camelids are modified ruminants

• Given rectally, the intestinal mucosa absorbs drug rapidly
Methods continued

• One month after treatment

  – Llamas immune-suppressed
    • 2 mg/kg dexamethasone (a corticosteroid) IV
    • 3 consecutive days

  – Monitored by PCR, PCV, TP, and blood smear
Results

• All llamas were positive at least one time during treatment and one month after treatment
Llama 1 - Becker

PCR Results

Positive

Negative

Days Post Infection (DPI)
Llama 3 - Chestnut

PCR Results

Days Post Infection (DPI)
PCR Results

Positive

Llama 4 - Mouse

Negative

Days Post Infection (DPI)
Llama 2 - Benito

PCR Results

Positive

Negative

Days Post Infection (DPI)
Conclusions

• Artemisinin at a dosage of 200 mg did not clear the *M. haemolamae* infection

• Each llama was positive both during treatment and after treatment of artemisinin
Possible reasons of why it didn’t work?

• Malaria infects blood cell and consumes hemoglobin
  – Liberates free heme (iron-porphyrin complex)
  – Cascade of reactions produces ROS
  – ROS damages and kills parasite
• *M. haemolamae* may not liberate heme
• Artemisinin may not have made it past the intestinal microbes
What next?

• Pharmacokinetic testing of artemisinin on camelids

• Further studies on artemisinin
  – Use different dosage
  – Longer amount of time
  – Different administration

• Keep looking for another possible treatment
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• Tornquist, SJ. Willamette Valley Llama Foundation Grant Proposal Request; Use of artemisin to treat *Mycoplasma haemolamae* infection in llamas. Corvallis, OR, 2008.


Questions?

References