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

Jessica Puccetti BioResource Research,
Susan Tornquist DVM, PhD.
Biomedical Sciences, College of Veterinary Medicine

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

http://www.apacapacas.com/images/09-16-04-female-cria-02.jpg

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

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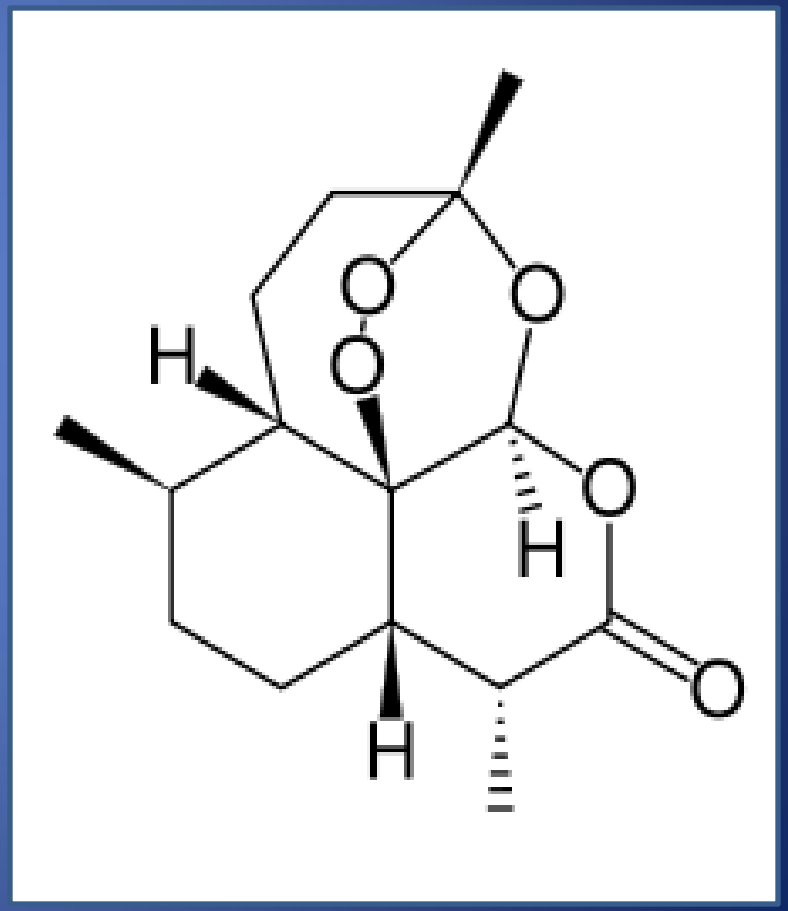
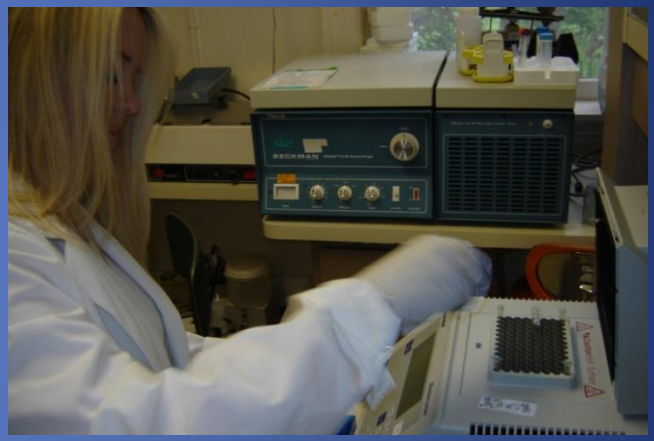
Artemisinin Mechanism of Action

- Affinity for iron
- Linkage breaks creating reactive oxygen species (ROS)
- Damage to infecting organism leading to death



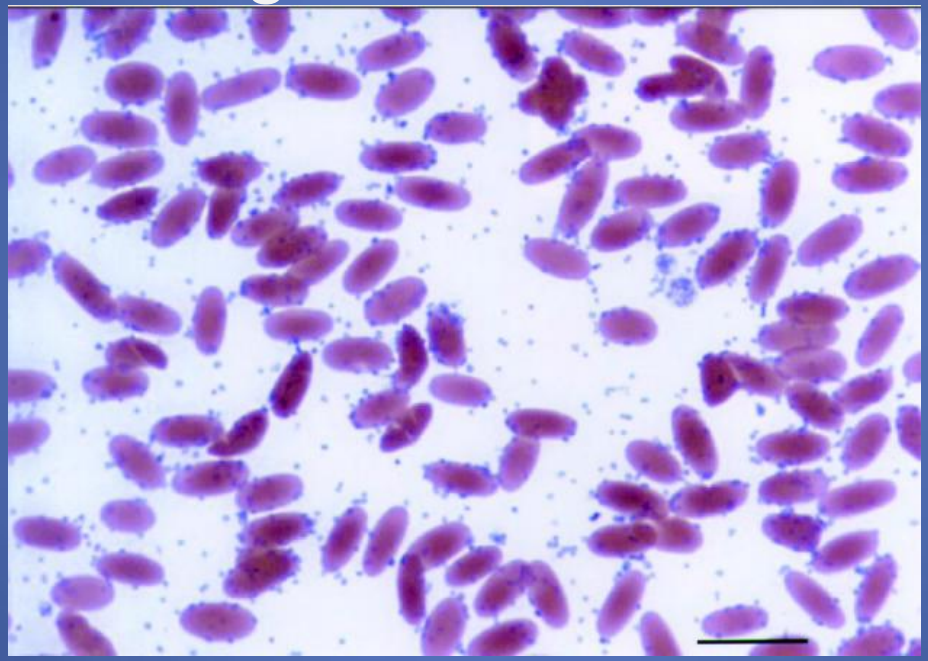
Tests to determine *M. haemolamae* infection

- 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



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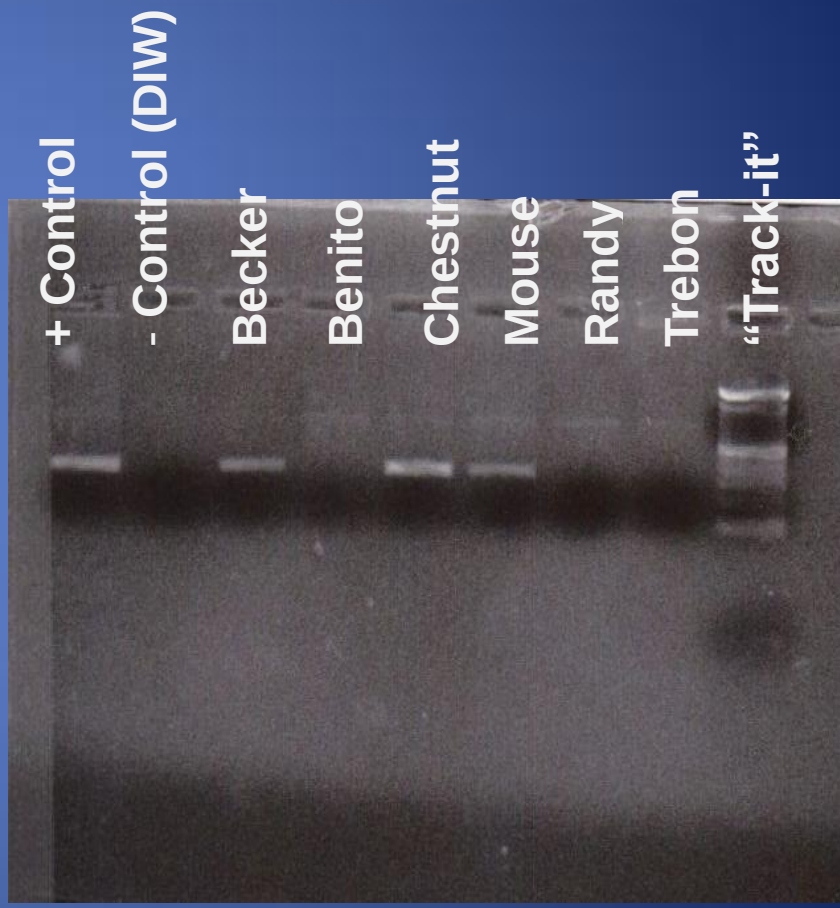
- Polymerase Chain Reaction (PCR) assay
  - Positive amplicons at 318 bp

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

Treatment Days

Llama	9-13	19-23	29-33	39-43	49-53	59-63
Becker	X	X	X	X		
Benito	X	X	X	X		
Chestnut	X	X	X	X		
Mouse	X	X	X	X		
Randy		X	X	X	X	
TreBon			X	X	X	X

Rectal Treatment

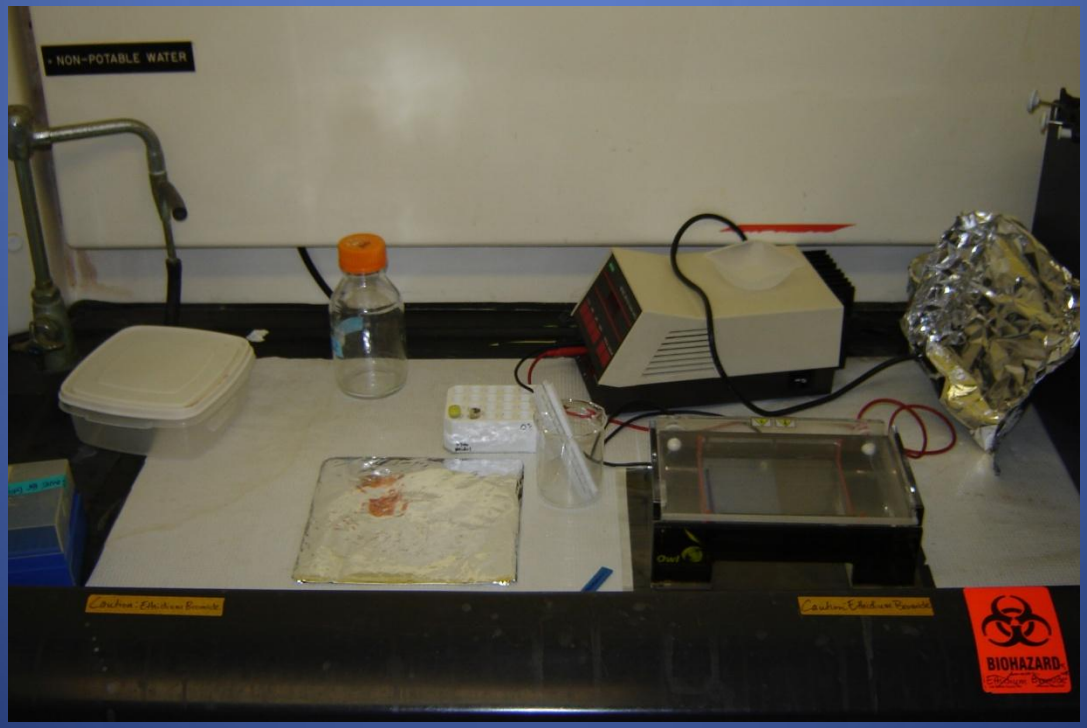
- Drugs given orally are broken down by ruminal flora in ruminant animals
- Camelids are modified ruminants
- Given rectally, the intestinal mucosa absorbs drug rapidly

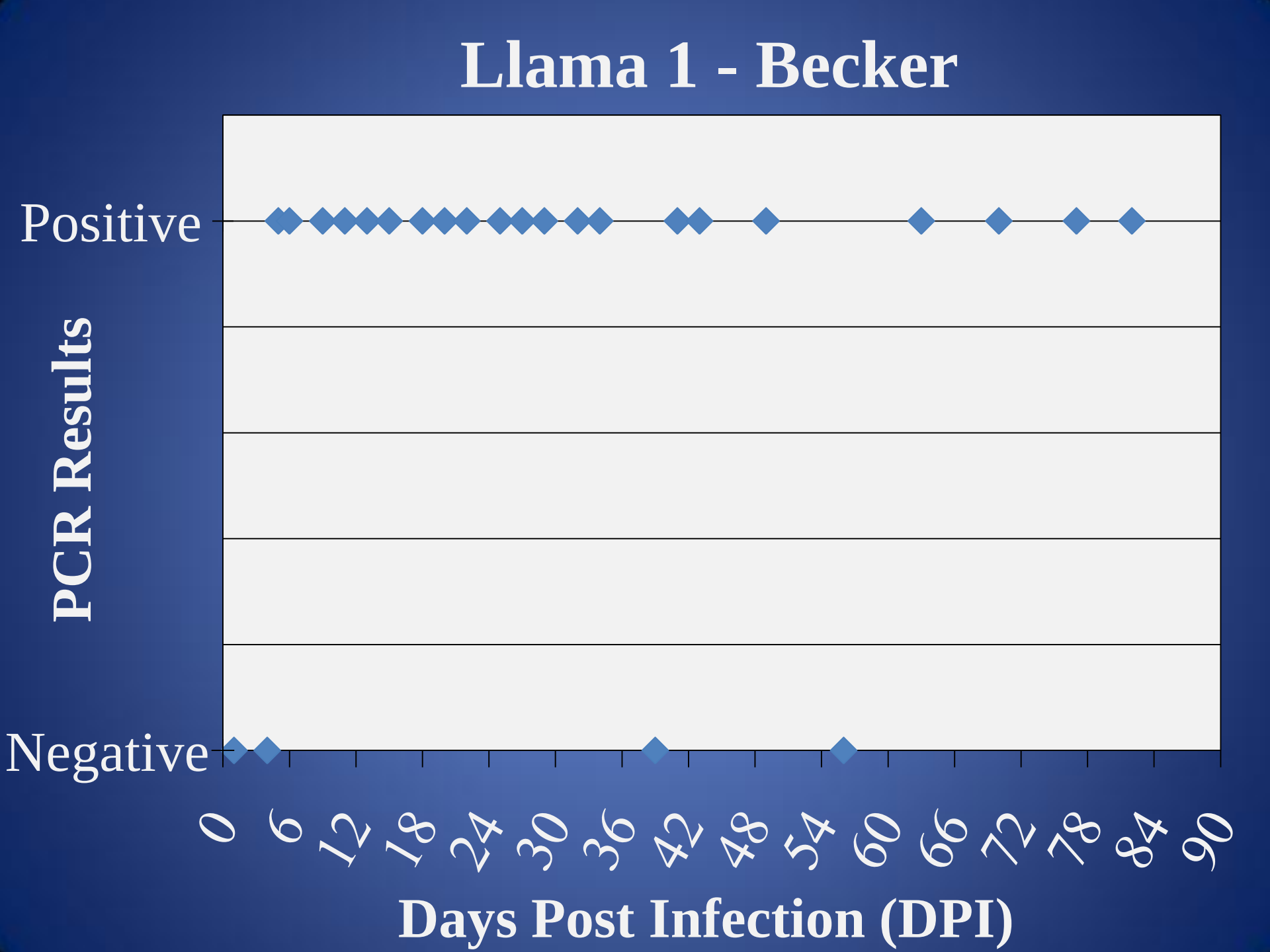
Methods continued

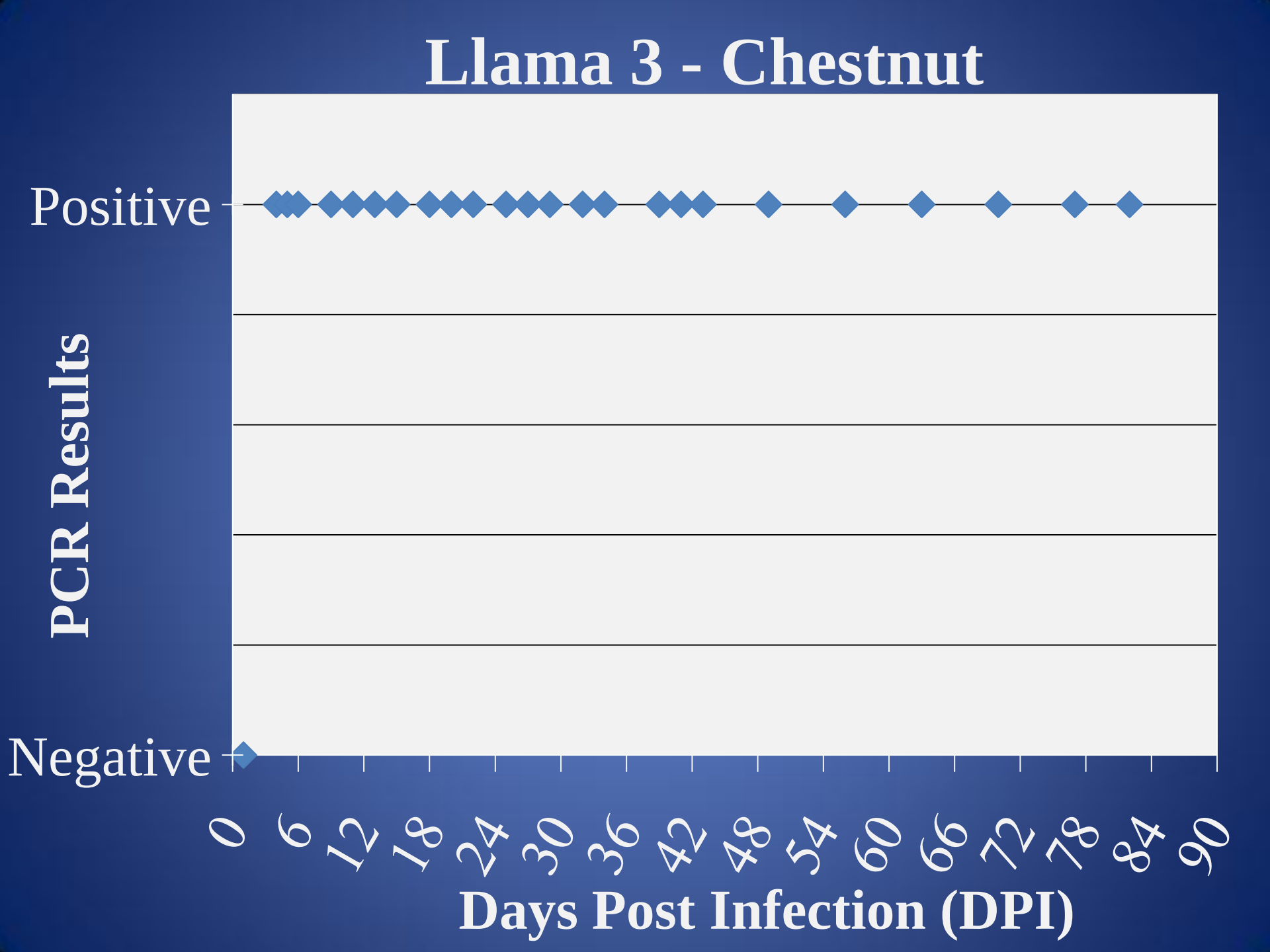
- One month after treatment
  - Llamas immune-suppressed
    - 2 mg/kg dexamethasone (a corticosteroid) IV
    - 3 consecutive days
  - Monitored by PCR, PCV, TP, and blood smear

Results

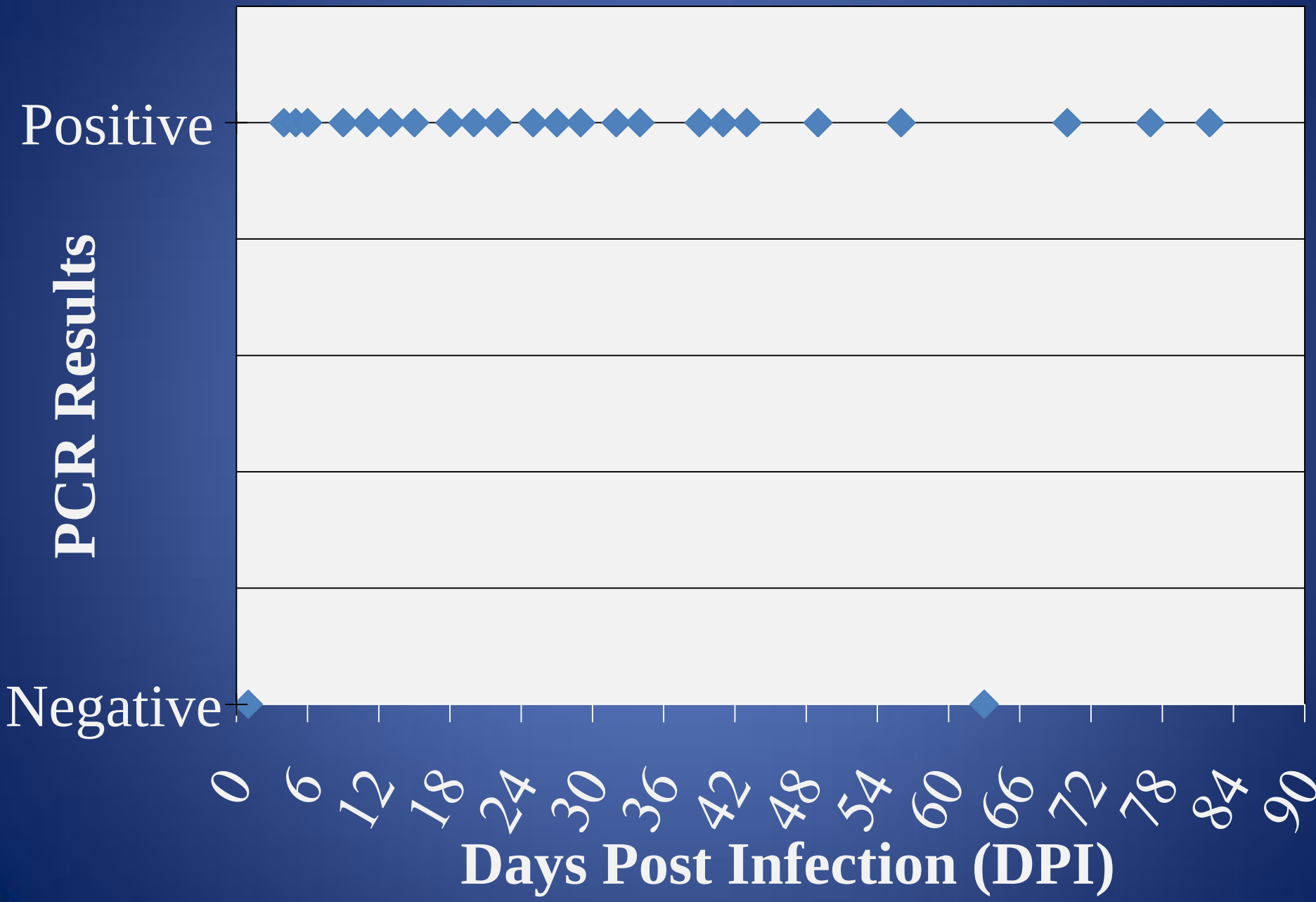
- All llamas were positive at least one time during treatment and one month after treatment



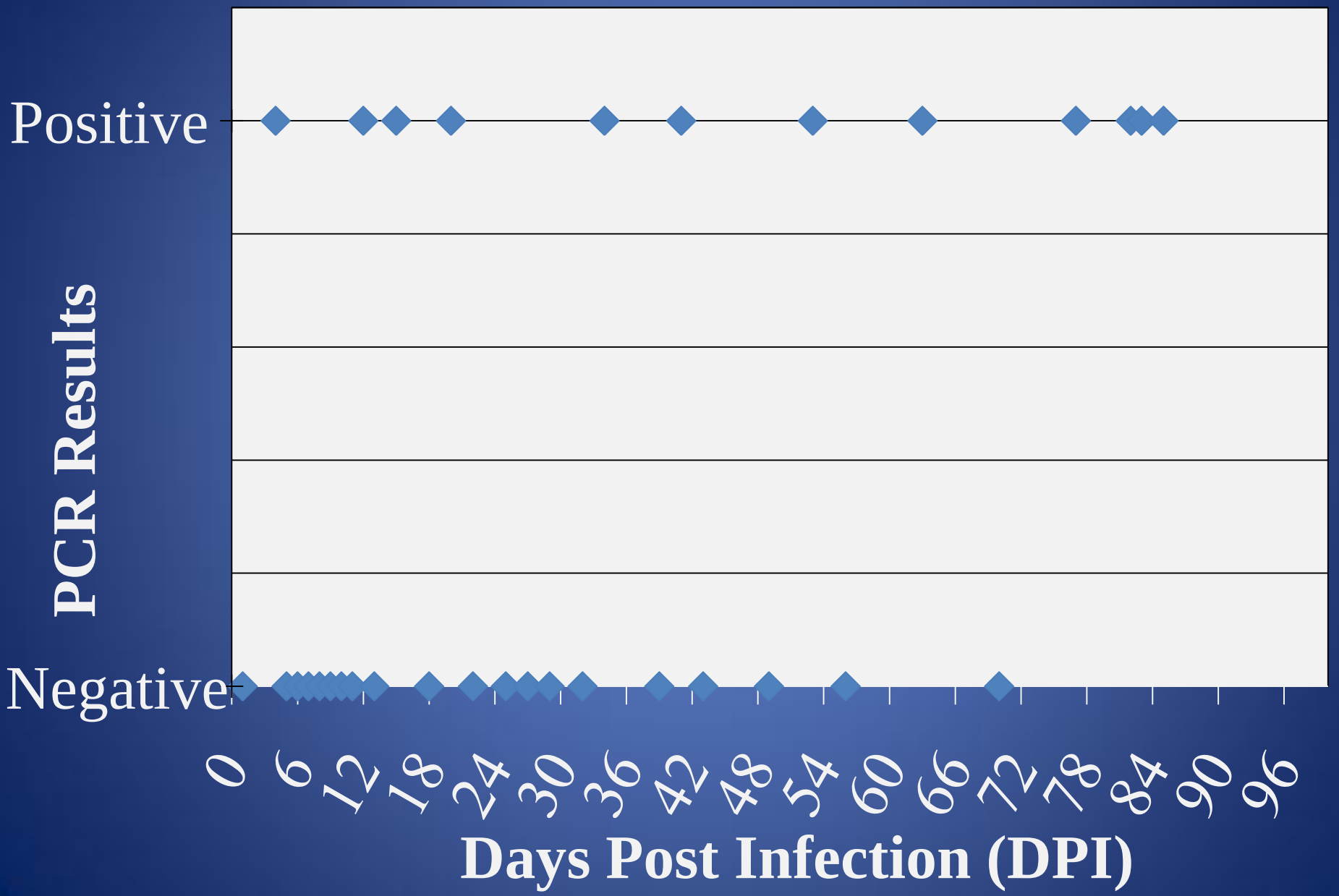

Llama 1 - Becker
Positive
Negative
PCR Results
0
6
12
18
24
30
36
42
48
54
60
66
72
78
84
90
Days Post Infection (DPI)


Llama 3 - Chestnut
Positive
Negative
PCR Results
0
6
12
18
24
30
36
42
48
54
60
66
72
78
84
90
Days Post Infection (DPI)

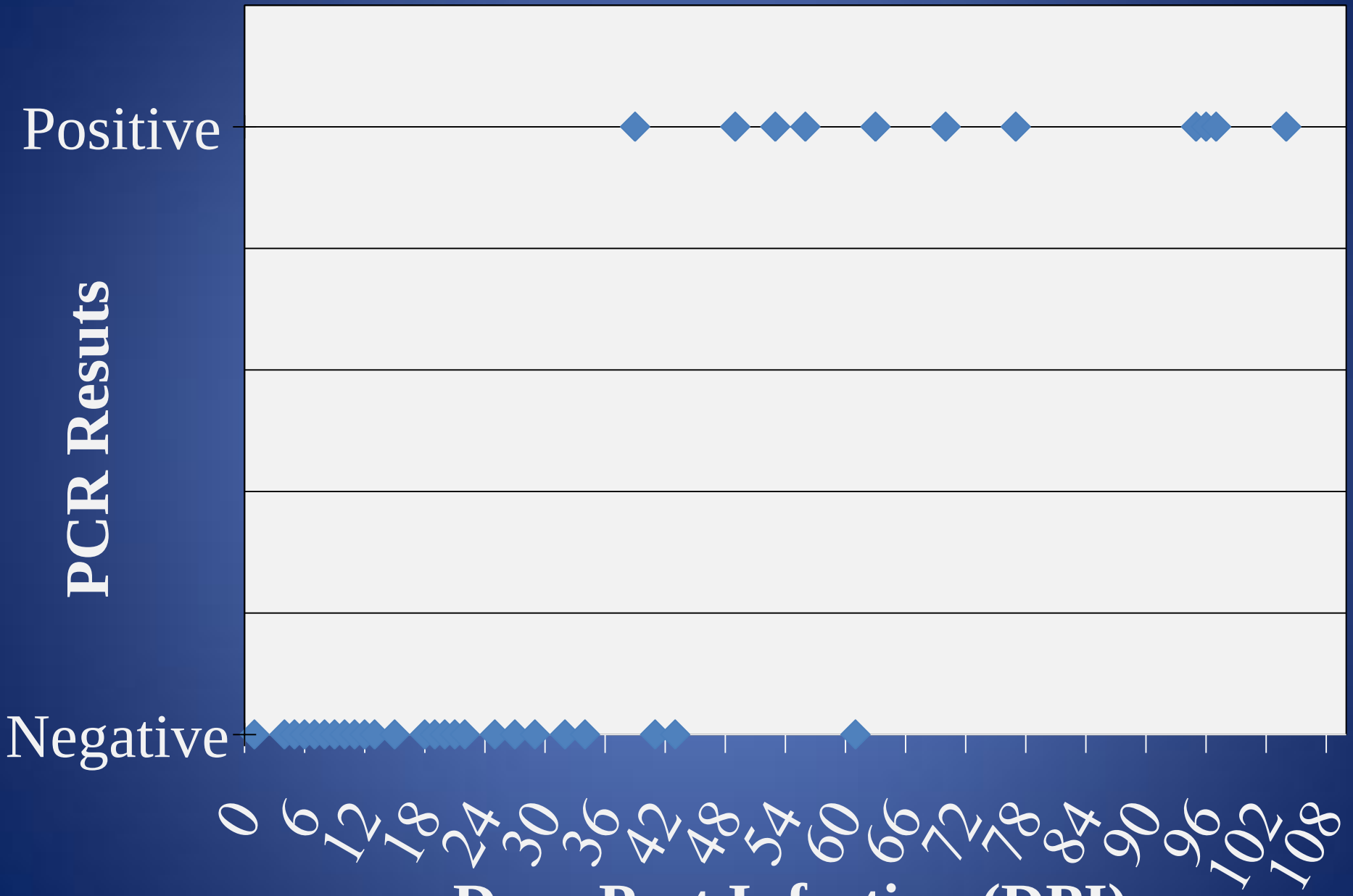
Llama 4 - Mouse



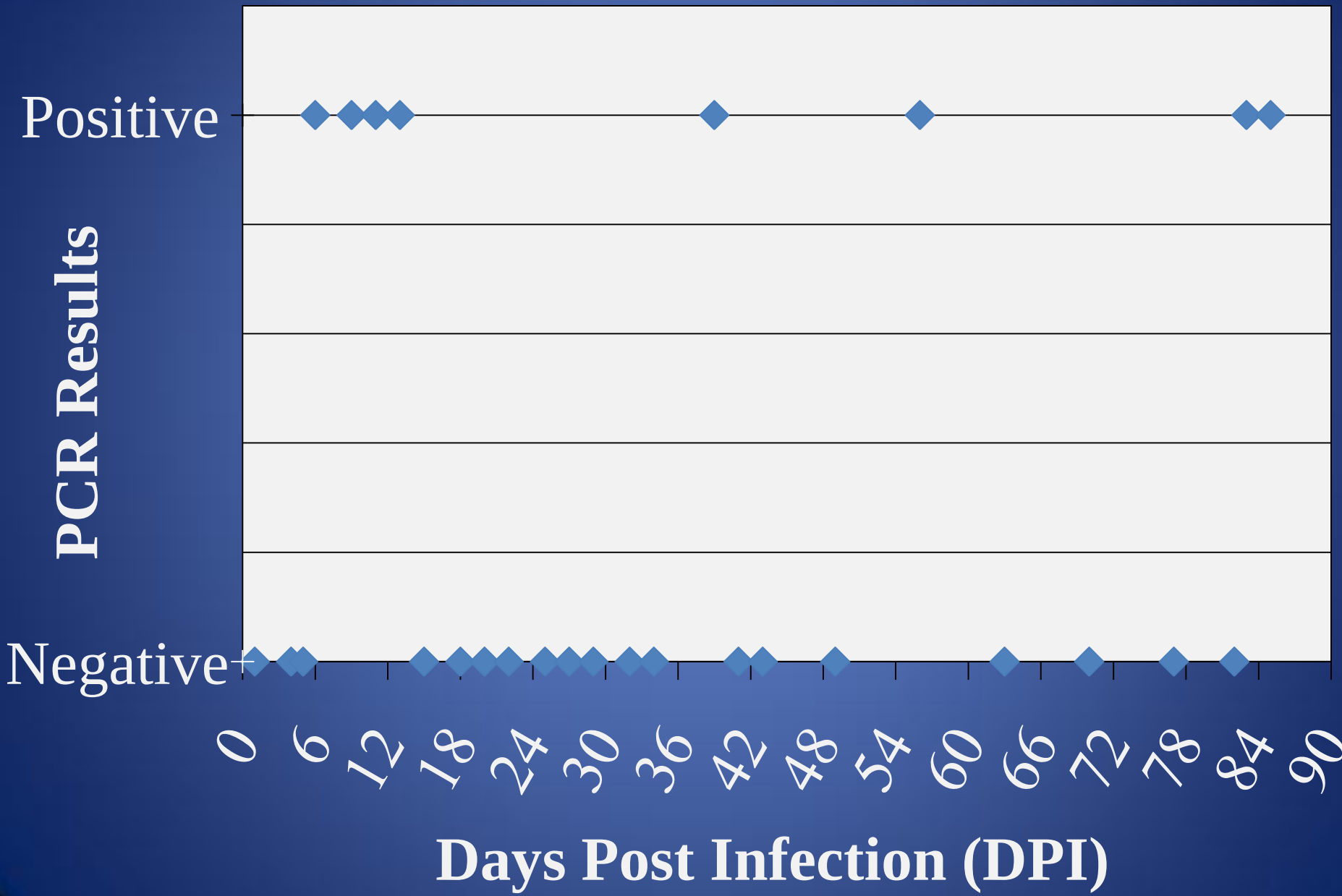
Llama 5 - Randy

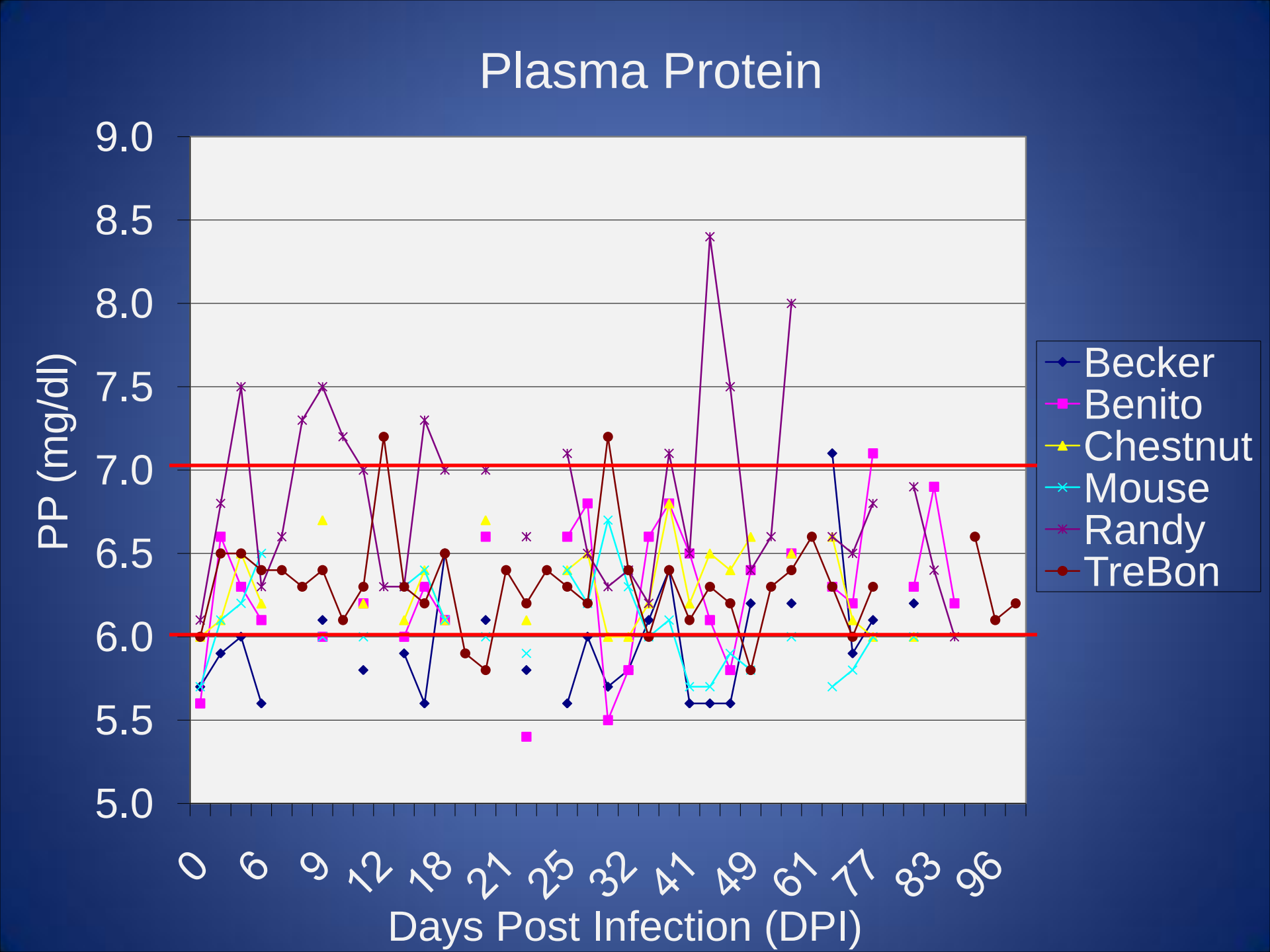


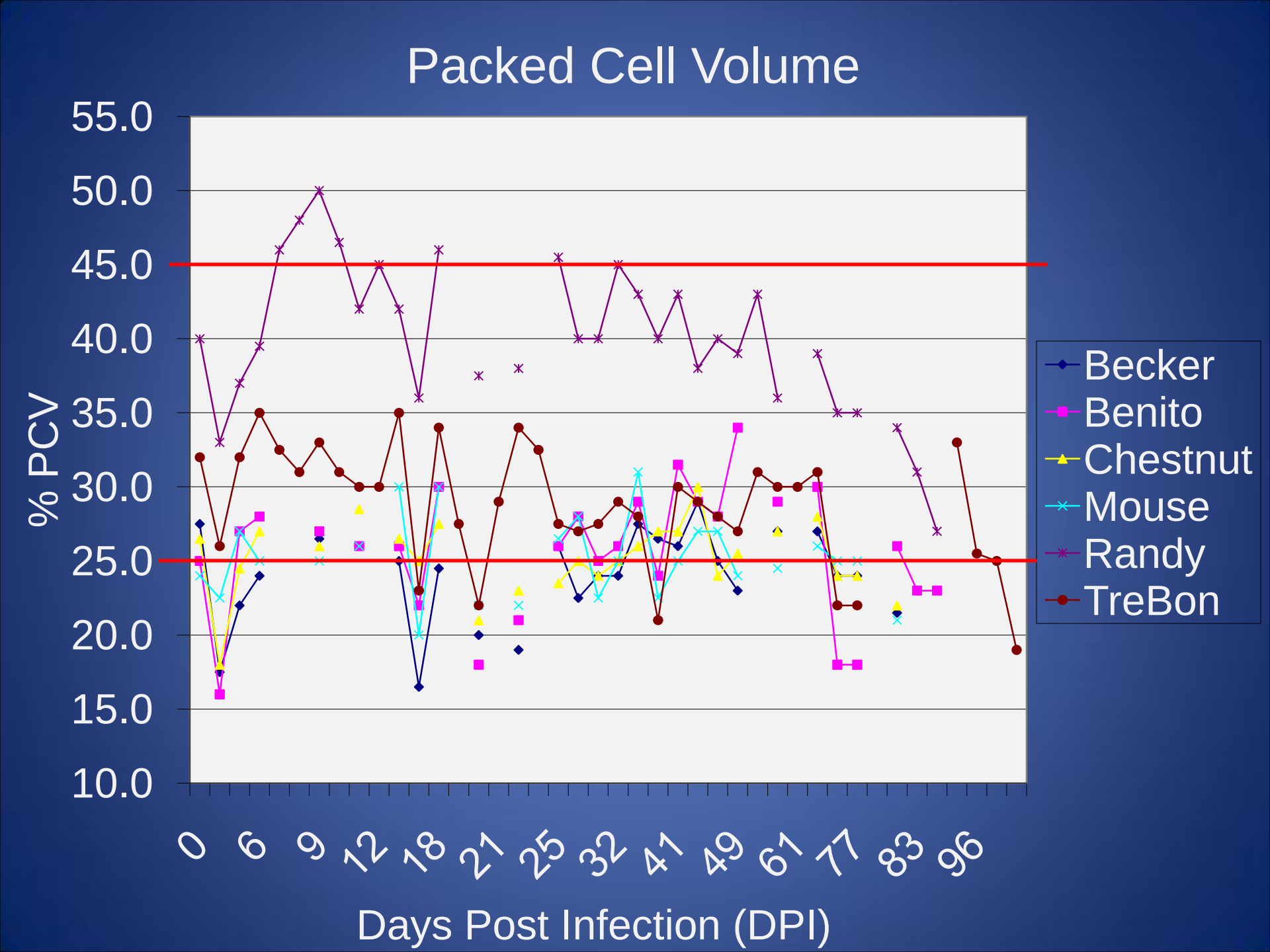
Llama 6 - TreBon



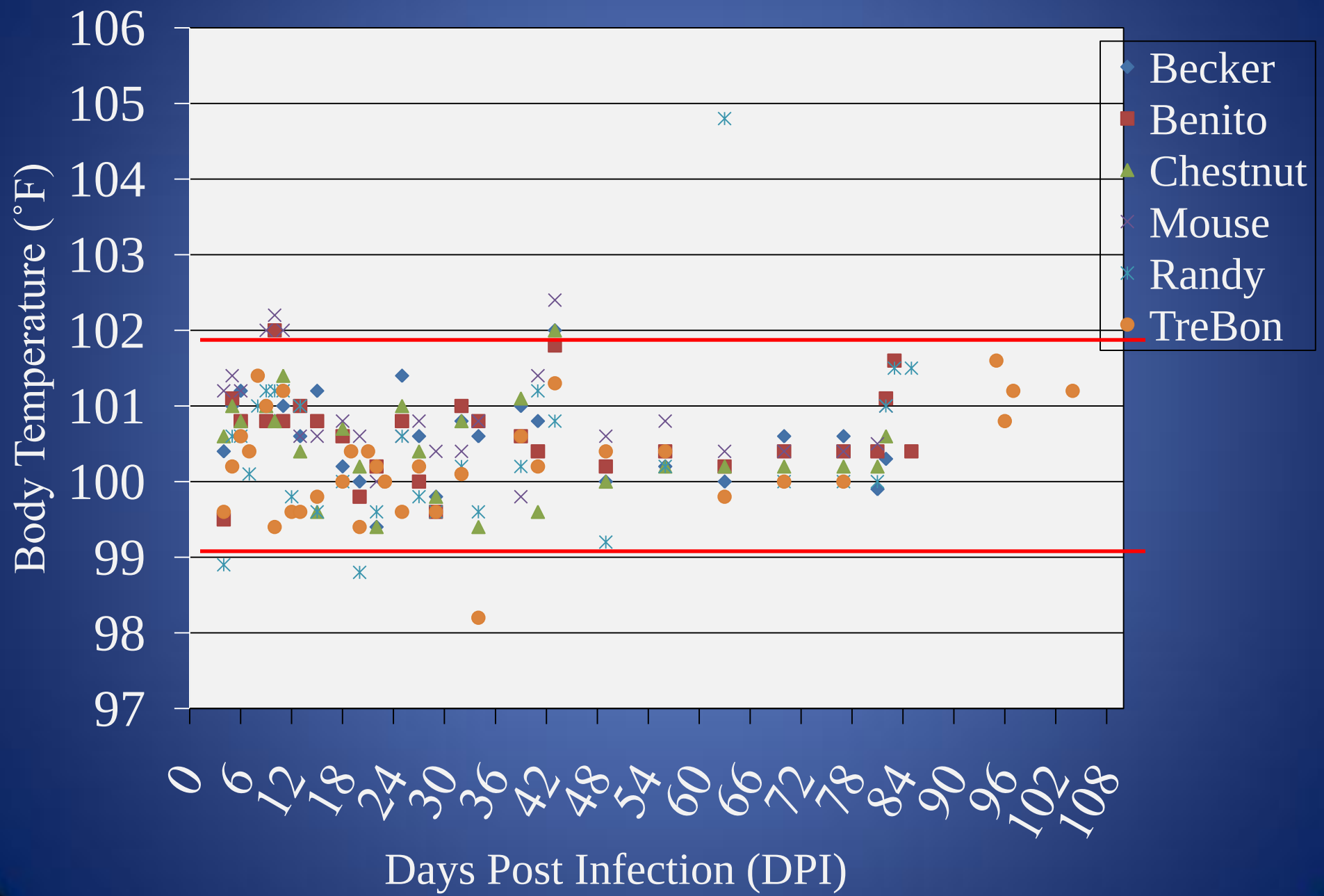
Llama 2 - Benito




Plasma Protein
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
PP (mg/dl)
0
6
9
12
18
21
25
32
41
49
61
77
83
96
Days Post Infection (DPI)
Becker
Benito
Chestnut
Mouse
Randy
TreBon


Packed Cell Volume
55.0
50.0
45.0
40.0
35.0
30.0
25.0
20.0
15.0
10.0
% PCV
0
6
9
12
18
21
25
32
41
49
61
77
83
96
Days Post Infection (DPI)
Becker
Benito
Chestnut
Mouse
Randy
TreBon

Body Temperature



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 artemisin on camelids
- Further studies on artemisin
  - Use different dosage
  - Longer amount of time
  - Different administration
- Keep looking for another possible treatment

Acknowledgments

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Questions?

References

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