Efficacy of chlortetracycline to protect against clinical anaplasmosis in transiently immunosuppressed cattle

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ABSTRACT

Bovine anaplasmosis is a tick-transmitted disease that costs the United States cattle industry an estimated $300 million annually. The agent of anaplasmosis is *Anaplasma marginale*, a pathogen transmitted to cattle by ticks or contaminated fomites. The only FDA-approved drug to control active anaplasmosis and to help limit economic loss is chlortetracycline (CTC). The effectiveness of CTC treatment to control anaplasmosis has been documented in immunocompetent animals, but the usefulness of CTC during periods of transient immunosuppression is unclear. Due to intensive tetracycline usage, the efficacy of CTC to control active anaplasmosis caused by contemporary *A. marginale* strains may be reduced, such that upon immunosuppression, cattle are not protected from recrudescent clinical anaplasmosis. The objective of this study was to determine the efficacy of CTC to control active anaplasmosis in transiently immunosuppressed cattle infected with a historic or contemporary *A. marginale* strain. We first generated infected animals by inoculating 18 Holstein calves with the historic Virginia or the contemporary KS2 strain. Animals were monitored for signs of clinical anaplasmosis (packed cell volume, temperature, and bacteremia) during acute disease. Upon transition to persistent infection, calves will be immunosuppressed using dexamethasone. The efficacy of CTC to protect transiently immunosuppressed calves will be determined by evaluating groups of calves: immunosuppressed with CTC treatment, immunosuppressed without CTC treatment, and not immunosuppressed with CTC treatment. The results of this study will provide evidence of the effectiveness of CTC to control active anaplasmosis in cattle during transient immunosuppression.

INTRODUCTION

- Tetracycline antimicrobials are the most commonly used antimicrobial class in cattle production.
- Chlortetracycline (CTC) is the only FDA-approved antimicrobial indicated for the control of active anaplasmosis in cattle.
- No fully USDA-licensed anaplasmosis vaccine is available.
- Cattle experience periods of immunosuppression naturally during harsh weather conditions, estrus, and calving.
- Transmission of *A. marginale* among cattle can occur via a biological tick vector, or mechanically by blood-contaminated fomites such as needles, ear taggers, and dehorners (**Fig 1**).
- Historic *A. marginale* strains are strains isolated at least 20 years ago that have not been under similar cattle management selective pressures as *A. marginale* strains actively circulating today.
- Contemporary *A. marginale* strains are strains actively circulating in cattle and that have been under continuous selection pressures associated with common production management practices.

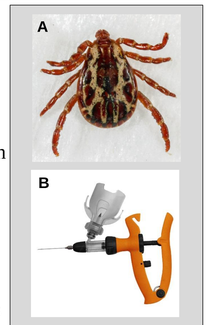


Figure 1. Routes of *A. marginale* transmission. A) *Dermacentor* spp. ticks are natural biological vectors. **B)** Injection gun needles are examples of mechanical transmission vectors.

METHODS

❖ PHASE I: Generating persistently infected calves

- Eighteen, ~10 month old Holstein calves were inoculated with either the **Virginia** strain or **KS2** strain (**Fig 2**).
- Blood samples were collected from calves 2-3 times per week to monitor *A. marginale* infection and clinical parameters.
- Blood was collected via the jugular vein into collection tubes containing EDTA, lithium heparin, or no anticoagulant (**Fig 3A**).

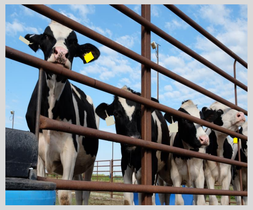


Figure 2. Holstein calves at the fence line. Eighteen ~10 month old Holstein calves were challenged with either the historic Virginia or the contemporary KS2 strain of *A. marginale*.

❖ PHASE II: Chlortetracycline treatment and immunosuppression

- CTC-treated study groups will be provided CTC-medicated feed at 0.5 mg/lb/day daily, beginning 10 days prior to immunosuppression and continuing for 30 days upon immunosuppression (**Fig 4**).
- Immunosuppression study groups will be treated with 0.5mg/kg dexamethasone (IM or IV) for 3 consecutive days, and then every other day for up to 4 more injections week (**Fig 4**).
- Non-immunosuppressed and non-CTC-treated study groups are included as controls (**Fig 4**).

❖ ANALYTICAL METHODS

- DNA extraction and real-time PCR: DNA was extracted from whole blood and real-time PCR was used to detect and quantify *A. marginale*.
- Packed cell volume (PCV) determination: The percent of erythrocytes was determined from whole blood samples to evaluate for anemia.
- Percent parasitized erythrocytes (PPE) determination: PPE was evaluated from thin blood smears to monitor *A. marginale* infection.
- Competitive ELISA (cELISA): Calf serostatus will be evaluated using an *A. marginale*-specific commercial cELISA.
- CTC plasma concentration: Plasma samples will be submitted to determine CTC blood plasma levels in CTC-treated calves.
- Complete blood cell count (CBC): CBC will be conducted to monitor calf blood cell counts during immunosuppression.

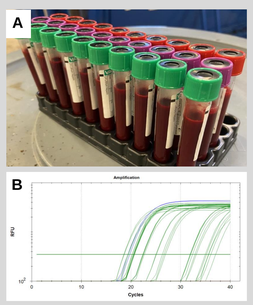


Figure 3. Blood samples collected to evaluate *A. marginale* infection. A) Blood samples collected into tubes with EDTA, lithium heparin, or no anticoagulant. **B) A**mplification curve from Msp5 real-time PCR assay used to detect and quantify *A. marginale* infection.

STUDY TIMELINE

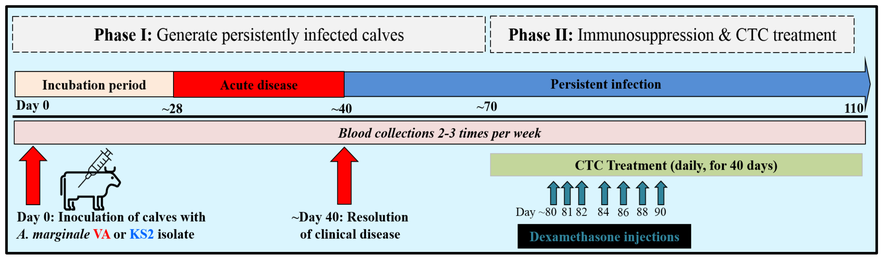
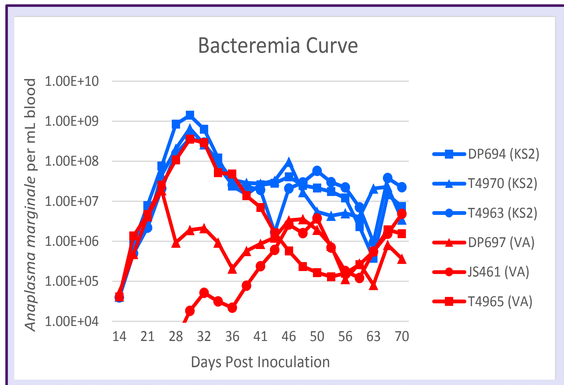


Figure 4. Study timeline. The study timeline begins at Day 0 (inoculation) and concludes ~30 days initial post-immunosuppression (~Day 110). In Phase I of the study, cattle will be challenged with either the historic VA strain or contemporary KS2 strain of *A. marginale* and monitored for signs of clinical disease during initial acute infection. Upon fully recovering from clinical disease, Phase II of the study will commence and groups of calves persistently infected calves with the VA or KS2 strain will either be: i) immunosuppressed and treated with CTC, ii) immunosuppressed but not treated with CTC, or, iii) not immunosuppressed and treated with CTC, and all calves will be monitored for signs of recrudescent clinical anaplasmosis.

RESULTS



- All calves challenged with *A. marginale* developed a detectable level of bacteremia.
- Bacteremia peaked for most calves around 30 days post-inoculation.
- Bacteremia was never cleared from any calf, and all calves remain persistently-infected with low bacteremia levels.
- The KS2 strain maintained higher levels of bacteremia than the VA strain.

Figure 5. Real-time PCR-determined bacteremia values. Representative bacteremia curves from three calves infected with either the historic VA or contemporary KS2 strain of *A. marginale*. Bacteremia was determined using quantitative real-time PCR assay targeting a portion of the single-copy gene *msp5*.

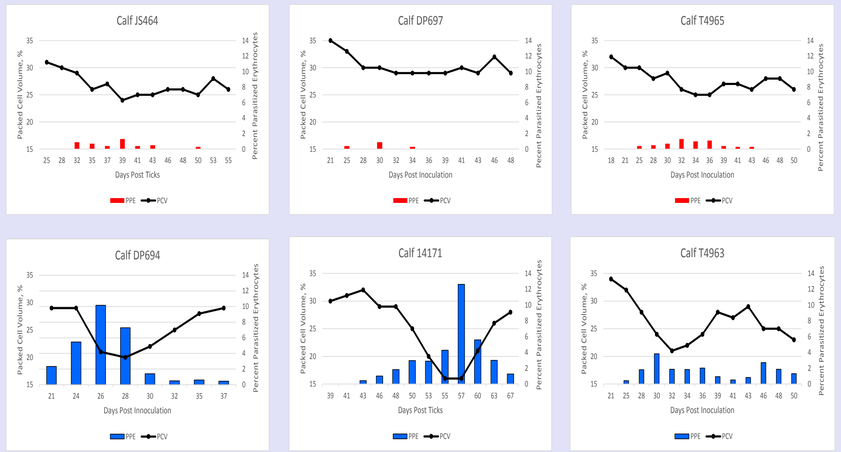


Figure 6. Percent parasitized erythrocytes (PPE) and packed cell volume (PCV) during acute anaplasmosis. Representative PPE (bars) and PCV (line) curves from three calves infected with either the historic VA (Calf IDs: JS464, DP697, T4965) or contemporary KS2 (Calf IDs: DP694, 14171, T4963) strain of *A. marginale*. PPE was determined by evaluating the number of *A. marginale*-infected red blood cells (RBCs) from thin blood smears (a minimum of 200 RBCs evaluated in duplicate). PCV was determined by evaluating the percent of packed red blood cells from a blood sample after microhematocrit centrifugation.

- **All *A. marginale*-challenged calves developed signs of clinical anaplasmosis.**
- **Calves infected with the KS2 strain exhibited a higher average PPEs.**
- **Although all calves experienced a drop in their PCV, calves infected with KS2 experienced lower PCV nadirs compared to calves infected with VA.**
- **2/9 calves infected with KS2 required rescue treatment with oxytetracycline during acute anaplasmosis. No calves infected with VA required oxytetracycline rescue treatment.**
- **After acute anaplasmosis, all calves remained infected and their PCV values returned to pre-infection (or near pre-infection) levels.**

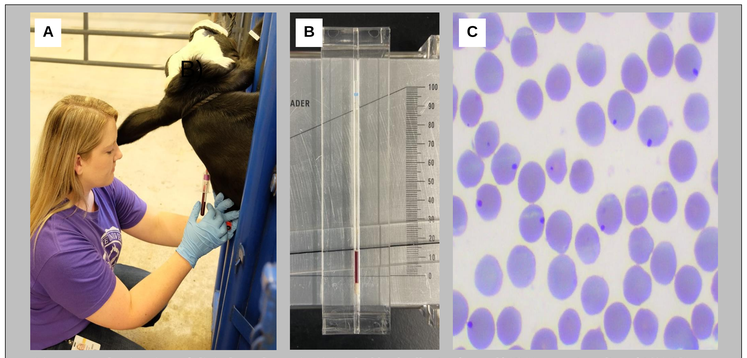


Figure 7. Evaluating signs of clinical anaplasmosis. A) Jugular blood collection from calf. B) Hematocrit reading of 16% PCV (severe anemia). C) Erythrocytes infected *with A. marginale* (basophilic-staining bodies at margin of red blood cells).

CONTINUED RESEARCH

❖ Study Phase II: Immunosuppression and CTC treatment (*in progress*).

- Study Phase II design and anticipated results presented in **Fig 8**.
- CTC treatment: Study groups will receive CTC-medicated feed or unmedicated feed daily for 40 days, beginning 10 days prior to first dexamethasone injection.
- Immunosuppression: For the respective study groups, calves will be immunosuppressed with dexamethasone (0.5 mg/kg) by IV injection for 3 consecutive days, and then by intramuscular injection every other day for up to 4 more injections.
- All animals will be monitored for recrudescent signs of clinical anaplasmosis.

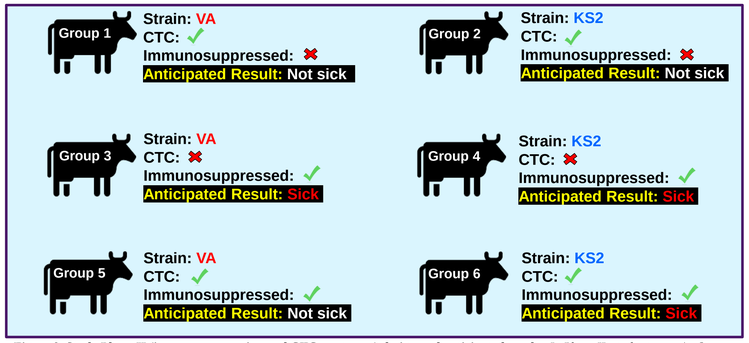


Figure 8. Study Phase II (immunosuppression and CTC treatment) design and anticipated results. In Phase II, study groups (n=3 calves/group) will vary by *A. marginale* strain (VA vs KS2), CTC treatment (treated vs untreated), and immunosuppression (immunosuppressed vs not immunosuppressed). Anticipated results of whether animals will develop recrudescent anaplasmosis (sick) or remain well (not sick) are also indicated.

SUMMARY

- Tetracycline antimicrobials are the most commonly used antimicrobials in cattle production to control disease-causing agents such as *A. marginale*.
- The intensive use of tetracycline antimicrobials over the past half century may have selected for less susceptible strains of *A. marginale*.
- CTC is the only antimicrobial FDA-approved to control active anaplasmosis in cattle.
- The immune system of immunocompetent cattle can normally prevent recrudescent anaplasmosis; however, cattle may experience periods of transient immunosuppression throughout a normal production season.
- If calves in Group 6 develop recrudescent clinical anaplasmosis upon transient immunosuppression, this will call into question the efficacy of CTC at the approved dosage to control anaplasmosis caused by contemporary *A. marginale* strains.
- If CTC is no longer effective at controlling active anaplasmosis at the approved dosage, the approved dosage may need to be re-evaluated, as providing an antimicrobial at a non-effective dose is costly for producers and is not a judicious use of a medically important antimicrobial.

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