Alternative Treatment for a Chronic Disease of Small Ruminants, Caseous Lymphadenitis (CL)

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What is CL and Why is it a Problem?

- CL is caused by the bacterium *Corynebacterium pseudotuberculosis* (C.psTB)
- Symptoms: dry, heavily encapsulated abscesses of the lymph nodes and internal organs (Baird and Fontaine, 2007)
- Very contagious among small ruminants (sheep and goats)
  - Rarely a zoonotic disease (BSL 2 organism)
  - Spread by direct inoculation or via respiratory route (abscesses in the lungs)
- Treatment of CL is difficult (Minozzi et al, 2017)
  - Common methods are not effective in the long term
  - Medications are very limited
- Sheep industry of Maine worth $10.1 million in 2017 (Lichtenwalner, 2013)
- 20% production losses in 2014 due to 43% of farms testing positive for CL (Lichtenwalner, 2013)
The Causative Organism

- Gram positive short rod
- Facultative intracellular parasite (Oreiby, 2014)
  - Can live within macrophages for up to 48 hours
- Contains multiple virulence factors (Baird and Fontaine, 2007)
  - Mycolic acid coat
    - Increases life span in the environment
    - Provides protection from host immune system
  - Phospholipase D
    - Increases vascular permeability of the host when released

Diagnosis

• External abscesses
  – Culture and do phenotypic identification
    • Gram stain, API, Biolog
    • MALDI-TOF (UNH)
  – Genomic identification

• Immune response of the animal: antibodies to Phospholipase D of C. psTB
  – Immune responses vary among animals
  – ELISA (several in use)
  – Synergistic Hemolysis Inhibition assay (UM)

• Absence of external abscesses makes diagnosis very difficult (Oreiby, 2014)

https://www.pinterest.com/pin/723109283892513011/
Internal Abscesses

A: Goat liver
B: Lung with abscess
C: Ribcage with pleural adhesions from abscess rupture

Photo by Lichtenwalner/UMaine
Treatment

- Surgical removal or draining and cleaning of abscesses
  - Temporary solution
- Antibiotics
  - Not typically strong enough to penetrate thick-walled abscesses
  - Come with many limitations/restrictions:
    FDA VFD – veterinary feed directive (FDA, 2015)
- Culling
  - Economic losses
- Injection of abscesses with formalin
  - Makes carcass unsuitable for human consumption (Washburn et al, 2009)
- **Ridding the environment of the bacteria**
  - **Up to 6 month life span in the environment** (Baird and Fontaine, 2007)
Recent studies of EOs as antimicrobial agents
  - Rosemary EO (REO) in particular

REO
  - Gram positive and negative bacteria, fungi (Swamy et al, 2016)
  - Meat preservation (Jiang et al, 2011; Moreno et al, 2009)

Antimicrobial mechanism
  - EOs in general contain monoterpenes compounds (carvacrol, thymol) (Mehdi et al, 2018)
    - Hydrophobic molecules that alter the cell membrane
    - Cause leakage of cellular materials such as ATP, ions, nucleic acids

Hypothesis: REO can prevent the growth of *C. psTB in vitro*
Methods 1: Culturing *C.psTB*

- **Challenges:**
  - BSL 2
  - Sticky surface of *C. psTB* colonies
- **Preliminary trials:**
  - Plate culture
  - Brain Heart Infusion (BHI) broth culture
- **Final method:**
  - Used cultures that were either ATCC or clinical isolates identified by MALDI-TOF
  - Grown in BHI broth on shaker for 48 hours, then treatments added and incubated for additional 72 hours
- **Gram stain** – confirmation of growth of *C. psTB*
Methods 2: REO Treatment

- Challenges:
  - REO causes hemolysis of blood agar plates (BAP)
  - REO is insoluble in water
- Preliminary trials:
  - Shifted to BHI broth culture
  - Added 1:1 Tween to REO and diluted mixture in BHI broth

Photo: Bryant/UMaine
Methods 3: Study Design

- Multiple controls
  - Blank (BHI broth only)
  - Tween in BHI broth
  - Tween:REO (1:1 v:v) in BHI broth
  - C. psTB in BHI broth
  - C. psTB with Tween in BHI broth

- Treatment groups
  - C. psTB with Tween:REO (1:1) in BHI broth
  - C. psTB with Tetracycline in BHI broth

Photo: Lichtenwalner/UMaine
Methods 3: Study Design

- Triplicate broth tubes of each treatment/control:
  - 21 total tubes
  - Experiment run twice
- Tubes with C. psTB incubated on a shaker at 37ºC in air for 48 hours:
  - Treatments or controls added and incubated additional 72 hours
  - At 120 hours, spectrophotometer reading at 600 NM
- Plated onto BAP and incubated in 20% CO2 at 37ºC for 48 hours
- After 48 hours, colonies gram stained for confirmation of C. psTB
- Spectrophotometer values compared using ANOVA followed by Tukey’s
Results – Controls

Graph 1: Average broth spectrophotometer readings after 72 hours

Control Groups

% Transmittance (mean ± SD: n=3)

- BHI
- BHI w/ Tween
- BHI w/ Tween & REO
- BHI w/ C. psTB
- BHI w/ C. psTB & Tween

Graph legend:

A: No significant difference
B: Difference significant at p < 0.05
C: Difference significant at p < 0.01
Results – Controls and Treatments

Graph 2: Average broth spectrophotometer readings after 72 hours

% Transmittance (mean ± SD: n=3)

Control and Treatment Groups
Results – Treatments

Graph 2: Average broth spectrophotometer readings after 72 hours

% Transmittance (mean ± SD: n=3)

Control and Treatment Groups

- BHI
- BHI w/ Tween
- BHI w/ Tween & REO
- BHI w/ C. psTB
- BHI w/ C. psTB & Tween
- BHI w/ C. psTB, Tween, & REO
- BHI w/ C. psTB, Tween, & tetracycline
Results – Plate Observations

- Cultures containing REO and tetracycline demonstrated no growth (tubes 1-9 and 16-21)
- Cultures containing no treatment or just tween demonstrated growth
  - White, pinpoint colonies consistent with C. psTB

Photo: Bryant/UMaine
Conclusions

• Tween enhances *C. psTB* growth *in vitro*.
  – Detergent activity prevents colony clumping?
• Rosemary essential oil hemolyzes RBCs *in vitro*.
  – Will it be safe for *in vivo* use?
• Rosemary essential oil prevented the growth of *C. psTB* in BHI broth and on blood agar plates.
  – Antimicrobial action or competitive exclusion?
• Rosemary essential oil may be effective against *C. psTB* on inanimate surfaces.
  – Wooden feeders common on farms; splinters act to inject *C. psTB* into animals
  – REO may penetrate wood and kill *C. psTB*
Future Directions

- Experiment with other solvents vs. Tween for *C. psTB*
- Evaluate other alternative treatments / disinfectants for *C. psTB*
- Investigate the mechanism of action of Rosemary EO
  - Destruction of essential nutritional components for *C. psTB*?
  - Direct bactericidal activity?
- Pinpoint the best method of administration
  - Environment vs. animal (oral, topical)
  - Treatment vs. prevention
  - Combination with other methods?

Photo: Lichtenwalner/UMaine
Future Directions

- Cassie Miller Future Direction: vet school in Tennessee!

Acknowledgements

• SARE
• University of Maine School of Food and Agriculture
• Maine Agricultural and Forest Experiment Station
References


