



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

Anne Lichtenwalner^{1,2}, Ann Bryant¹, Cassie Miller¹ (presenting author), Sarah Paluso¹

¹School of Food and Agriculture and ²Cooperative Extension, University of Maine, Orono ME 04469



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



Gram stain of *C. psTB* from Singh et al, Journal of Animal Research: v.8 n.3, p. 497-500. June 2018



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/72310 9283892513011/



Internal Abscesses



A: Goat liverB: Lung with abscessC: Ribcage with pleural adhesions from abscessrupture

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)



Essential Oils as an Alternative Approach

- 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



Photo: Lichtenwalner/UMaine

- EOs in general contain monoterpene 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



Photo: Lichtenwalner/UMaine



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 37°C in air for 48 hours:
 - Treatments or controls added and incubated additional 72 hours



Photo: Lichtenwalner/UMaine

- At 120 hours, spectrophotometer reading at 600 NM
- Plated onto BAP and incubated in 20% CO2 at 37°C 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





Results – Controls and Treatments

Graph 2: Average broth spectrophotometer readings after 72 hours



Control and Treatment Groups



Results – Treatments

Graph 2: Average broth spectrophotometer readings after 72 hours





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



Photo: Lichtenwalner/UMaine



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!



THE UNIVERSITY OF TENNESSEE KNOXVILLE

https://www.tennessean.com/story/sports/college/ut/2016/02/22/16ut-coaches-hold-news-conference-tuesday/80779482/



Acknowledgements

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





References

Baird, G.J. and Fontaine, M.C. "Corynebacterium pseudotuberculosis and its Role in Ovine Caseous Lymphadenitis." *Science Direct,* Vol. 137, 179-210, 2007. Accessed 28 February 2018.

Food and Drug Administration. "Veterinary Feed Directive." *The Daily Journal of the United States Government.* 1 October 2015. Accessed 13 November 2018.

- Jiang et al. "Chemical composition and antimicrobial activity of the essential oil of Rosemary." *Science Direct,* Environmental Toxicology and Pharmacology Vol 32, Issue 1, Pg. 63-69, 2011. Accessed 30 October 2018.
- Lichtenwalner, A. "Farm-based control measures for caseous lymphadenitis in small ruminants: Offering a choice to the producer." *USDA Sustainable Agriculture Research & Education.* Final Report ONE12-164, 31 December 2013. Accessed 14 November 2018.
- Mehdi et al. "Carvacrol and human health: A comprehensive review." *Wiley Online Library.* Phytotherapy Research. 2018;32:1675-1687. Accessed 25 March 2019.
- Minozzi et al. "First insights in the genetics of Caseous lymphadenitis in goats." *Italian Journal of Animal Science*, Vol. 16, NO. 1, 31-38, 2017. Accessed 28 February 2018.
- Moreno et al. "Antioxidant and antimicrobial activities of rosemary extracts linked to their polyphenol composition." *Free Radical Research*, 2009. Accessed 20 March 2018.
- Oreiby, A.F. "Diagnosis of caseous lymphadenitis in sheep and goat." *Science Direct,* Small Ruminant Research, 123 (2015) 160-166, 2014. Accessed 7 March 2018.
- Swamy et al. "Antimicrobial Properties of Plant Essential Oils against Human Pathogens and Their
 Mode of Action: An Updated Review." *Agriculture & Environmental Science Database*, Evidence –
 Based Complementary and Alternative Medicine, New York, Vol. 2016. Accessed 30 October 2018.