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The Effect of Season on *in vitro* and *in vivo* Exsheathment Efficacy of Stored Infective *Haemonchus contortus* Larvae

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Introduction

Small ruminant farmers have a constant battle with infections of gastrointestinal nematodes (GIN) within their grazing sheep and goats. *Haemonchus contortus*, more commonly called the barber pole worm, is the most pathogenic GIN parasite that feeds on blood, eventually causing anemia, weight loss, and even death in susceptible animals (Veríssimo et al., 2012). The infective third stage (L3) of *H. contortus* have a protective sheath that must be shed within the rumen of the sheep or goat in order for the larvae to continue development into adults within the abomasum, the fourth chamber of the small ruminant stomach. The shedding of the sheath is called 'exsheathment' and is a critical part in the life cycle of *H. contortus*. Exsheathment initiates infection and without this process, infection would not occur. During infection, the process of exsheathment is host specific as well as site specific, ensuring that GIN will exsheath in the correct host (Johnstone, 1998). *Haemonchus contortus* have become increasingly resistant to the chemical dewormers (anthelmintics) commonly used to control infections. Increasing anthelmintic resistance in these GIN creates a need for alternative methods of GIN control. Plant secondary compounds have demonstrated anti-parasitic activity and are currently the focus of many studies. Stored L3 are commonly utilized in *in vitro* and *in vivo* assays of plant secondary compounds. Although researchers typically do not use L3 that have been stored longer than three months, the effect of season on the viability and exsheathment of stored L3 has yet to be examined. Studying the possible effects of seasonality on stored *H. contortus* larvae can provide vital information for creating a sustainable solution to the problems caused by GIN infections.

Figure 1. *Haemonchus contortus* larva

Objective

The objective of this study is to determine the effect of season on *in vitro* and *in vivo* exsheathment efficacy of stored *H. contortus* L3.

Methods

Animals:

- Two male twin rams were selected for each season of this study.
- Four rumen fistulated ewes were used for the *in vivo* exsheathment assays.

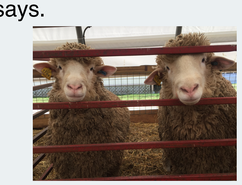


Figure 2. Donor Rams

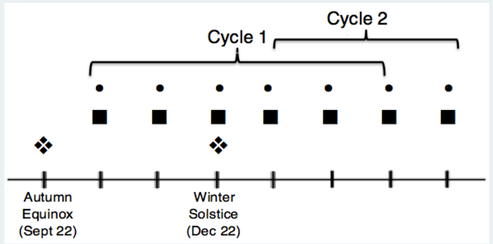
Figure 3. Fistulated Ewes

Methods

Experimental Design:

- Four donor rams were infected with 10,000 *H. contortus* L3 on the autumn equinox (fall, n=2) and on the winter solstice (winter, n=2).
- Fecal samples were collected after the infection matured (one month).
- Feces were cultured to L3 stage and were harvested and stored for five months at 4° C.
- Each month, *in vitro* and *in vivo* assays were performed to assess viability and ability to exsheath.
- Assays will be conducted all four seasons, but only fall and winter are examined here.

Cycles



Key
- • = *in vivo* assay
- ■ = *in vitro* assay
- ❖ = rams infected

Fecal Egg Count:

- The fecal samples collected from each ram were analyzed to determine a 'fecal egg count' using the modified McMaster technique (Whitlock et al., 1948)
- For each ram, a 2 gram sample was combined with concentrated salt solution (Fecasol, 1.2, Vetoquinol USA,Inc., Ft. Worth, TX) to release the eggs into an aqueous solution to be observed under the microscope. Eggs were counted to determine the number of eggs per gram of feces for each ram. Fecal egg counts were taken that week.

Fecal Culture:

- After the infection reached maturity (~ 4 weeks), enough fecal matter was collected to obtain >100,000 L3 needed for the six months of assays.
- The collected feces was used to make cultures using established Baermann procedures (Zajac et al., 2006).
- Each sample rested on top of 'cheese' cloth and incubated over water for two weeks. Cultures were flooded with water to collect L3. Collected larvae were stored in 4° C until assay.

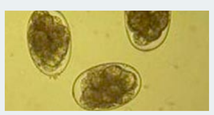


Figure 4. *H. contortus* eggs

Exsheathment assays:

- *in vitro*: Each month, four falcon tubes for each donor ram containing approximately 2,000 L3 were created. Three were bubbled with CO_2 for fifteen minutes and incubated for eighteen hours at 37° C. Percent viability and exsheathment were calculated: % viability=total live/total, % exsheathment=live exsheathed/total live
- *in vivo*: Each month, one capsule from each donor ram containing approximately 2,000 L3 was placed into the rumen of four fistulated ewes for eight hours. Percent viability and exsheathment were calculated as shown above.

Figure 5. Capsules

Results

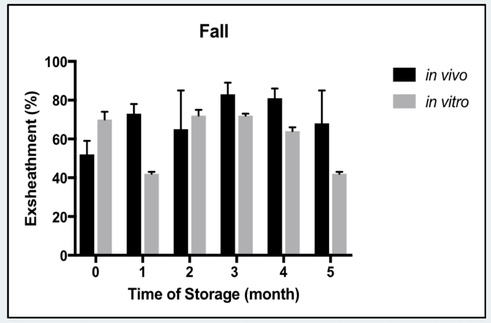


Figure 6. Percent exsheathment of *H. contortus* larvae during the fall cycle in both *in vivo* and *in vitro* assays. Exsheathment was determined by averaging each replicate used in *in vivo* and each replicate in *in vitro*. Exsheathment was determined on a monthly basis and is expressed as a percentage±SEM.

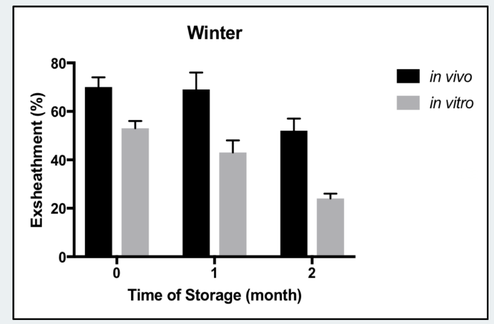


Figure 7. Percent exsheathment of *H. contortus* larvae during the winter cycle in both *in vivo* and *in vitro* assays. Exsheathment was determined the same way as the fall cycle. Exsheathment was determined on a monthly basis and is expressed as a percentage±SEM.

Discussion and Conclusion

The average exsheathment over six months for the *in vivo* and *in vitro* monthly assays of fall infection was 70 ± 4 (mean ± SEM) and 60 ± 4 respectively. The average exsheathment over three months for the *in vivo* and *in vitro* monthly assays of the winter infection was 63 ± 4 and 43 ± 4 respectively. Sampling is ongoing. Statistical results pending.

Acknowledgments

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References

Johnstone, C., 1998. Parasites and Parasitic Diseases of Domestic Animals

Verissimo, C.J. et al., 2012. Multidrug and multispecies resistance in sheep flocks from São Paulo state, Brazil. Vet. Parasitol. 187 (1–2), 209–216.

Whitlock, H.V., 1948. Some modifications of the McMaster Helminth egg-counting technique and apparatus. J. Counc. Sci. Res. 21, 177–180.

Zajac, A., Conboy, G. 2006. Veterinary Clinical Parasitology. Seventh Edition. Victoria, Australia:Blackwell Publishing. 12-13.