Evaluation of entomopathogenic nematode viability during winter?

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Introduction. Nilgai antelope *Boselaphus tragocamelus* (Pallas) are implicated in the long-range dispersal and cycling of southern cattle fever ticks, *Rhipicephalus microplus* Canestrini (Acari: Ixodidae) in the environment, especially in Cameron and Willacy Counties. Treatment methods for nilgai are needed to support the Cattle Fever Tick Eradication Program. Remotely activated sprayers developed for application of entomopathogenic nematodes *Steinernema riobrave* (Cabanillas, Poinar, and Raulston) entomopathogenic nematodes (Nemasys-R, BASF) at fence crossings, but treatment methods are needed in areas without fence crossing. Treatment of nilgai at water troughs may be an alternate location for application of nematodes. In this study, we observed the use of concrete water troughs and man-made ponds by nilgai and deer at ranches in Cameron Co. At one ranch concrete troughs at Russell Ranch were closed on 3 sides with wire fencing to force animals to drink at short end of trough. At this end we placed a sonic sensor facing up to activate the nilgai nematode sprayer. At the Buena Vista Ranch troughs were left open on all sides for comparison of wildlife use. At both ranches we also recorded water use by nilgai and deer at manmade ponds. Both nilgai and deer accessed water at both the enclosed and open water troughs at about the same rate. They also continued accessing water at ponds on both ranches. Overall, there was no delay or adjustment period for deer, but nilgai took up to 2 months to be accustomed to the enclosed troughs. Acclimation and or use of water troughs did not appear to be related to temperature or rainfall. Treatment of wildlife at water troughs may be a viable option in areas without fence crossings for deployment of remotely activated entomopathogenic nematode sprayers.

Cattle Fever Tick Impacts

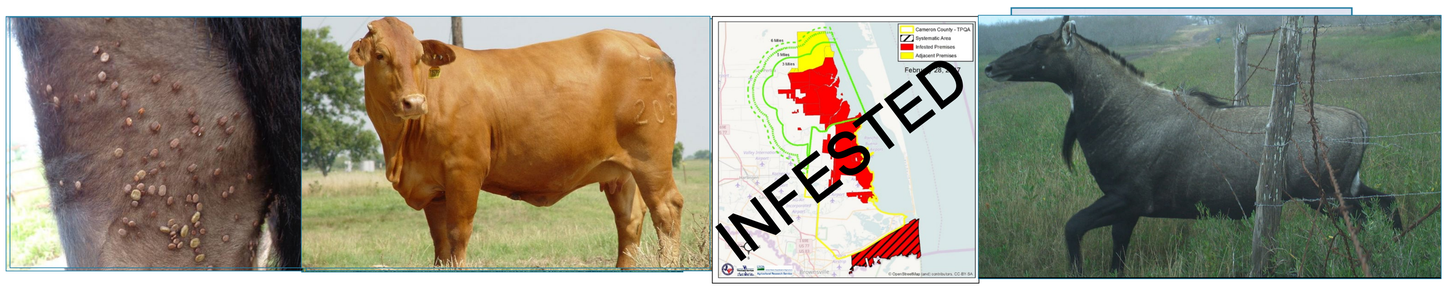


Fig 1. The southern cattle fever tick (CFT), *Rhipicephalus microplus* has several important hosts including domestic cattle, whitetail deer, and Indian nilgai antelope. Wildlife are implicated in the spread of cattle fever ticks into Cameron and Willacy Counties which impacts ranching and hunting activities. Parts of both counties have temporary quarantines which restricts movement of livestock and wildlife. An eradication program by USDA-APHIS and the Texas Animal Health Commission is in progress.

Remotely Operated Nilgai Sprayer

Fig 2. Nilgai antelope *Boselaphus tragocamelus* (Pallas) are implicated in the long-range dispersal and cycling of southern cattle fever ticks, *Rhipicephalus microplus* Canestrini (Acari: Ixodidae) in the environment, especially in Cameron and Willacy Counties. Treatment methods for nilgai are needed to support the Cattle Fever Tick Eradication Program. Remotely activated sprayers developed for application of entomopathogenic nematodes at fence crossings were tested in the summer of 2019 at private ranches in Cameron Co., TX. Sprayers are activated by ultra-sonic sensors as nilgai transit through fence crossings. Nilgai are treated directly with *Steinernema riobrave* (Cabanillas, Poinar, and Raulston) entomopathogenic nematodes (Nemasys-R, BASF) and passively as they contact wetted foliage and soil.

Materials and Methods

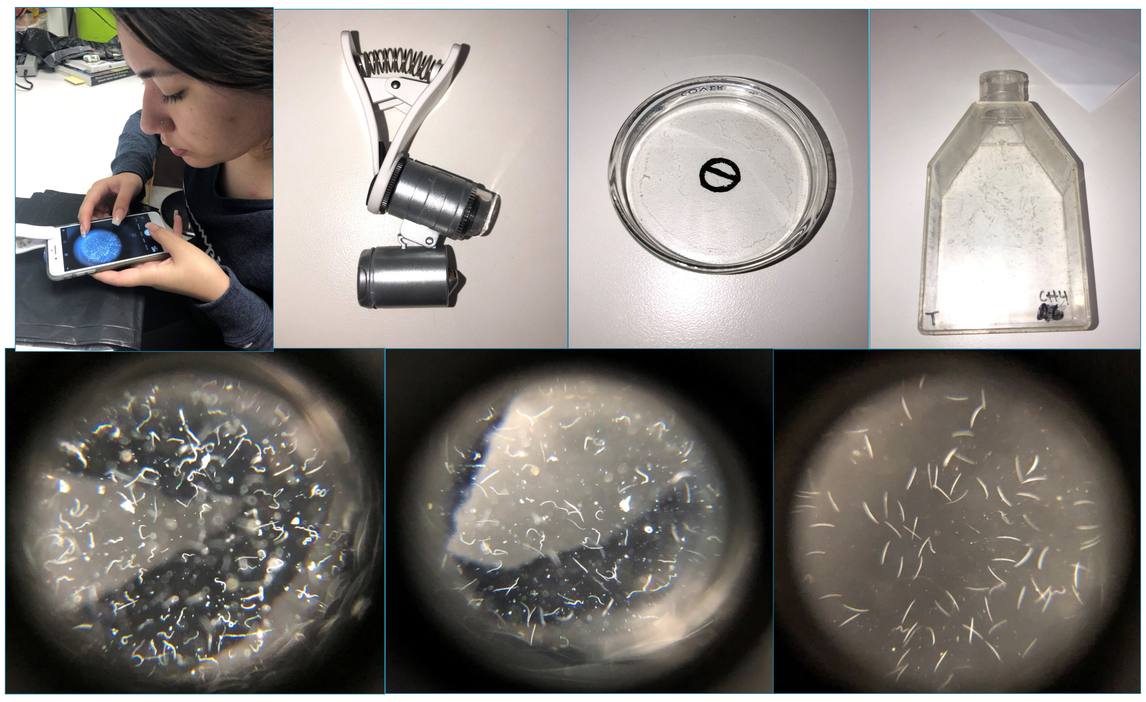
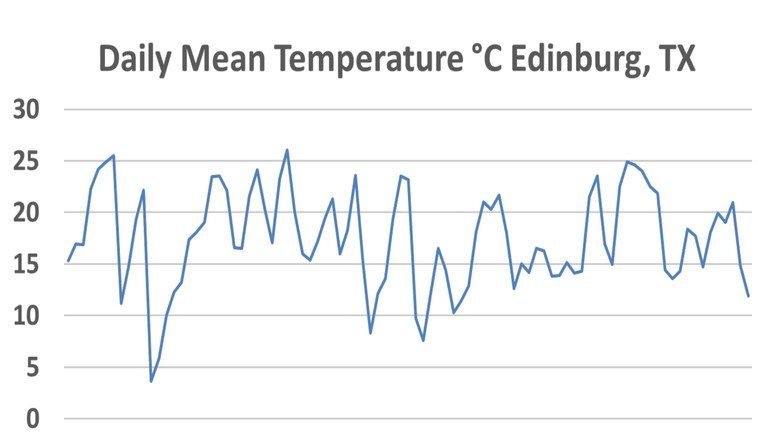
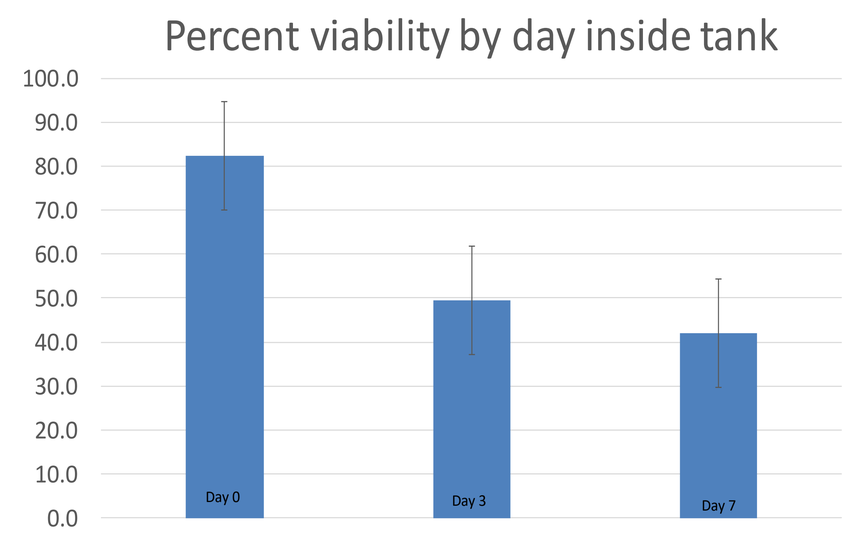


Fig. 3. (clockwise) Author taking pictures of infective *Steinernema riobrave* nematodes with iPhone 8s at the USDA-ARS Cattle Fever Tick Research Laboratory, Moore Airbase, Edinburg, TX. 20X Magnifier that clips on lens of camera phone. Petri dish with 0.5 cm circle used for sampling nematodes. Sampling flask. Pictures of nematodes in solution as seen within the 0.5 cm circle. The curved and/or moving nematodes are counted as live, and the rod straight counted as dead.

Hypothesis, Nematode viability in water is similar under cool conditions to warm summer temperatures

Results





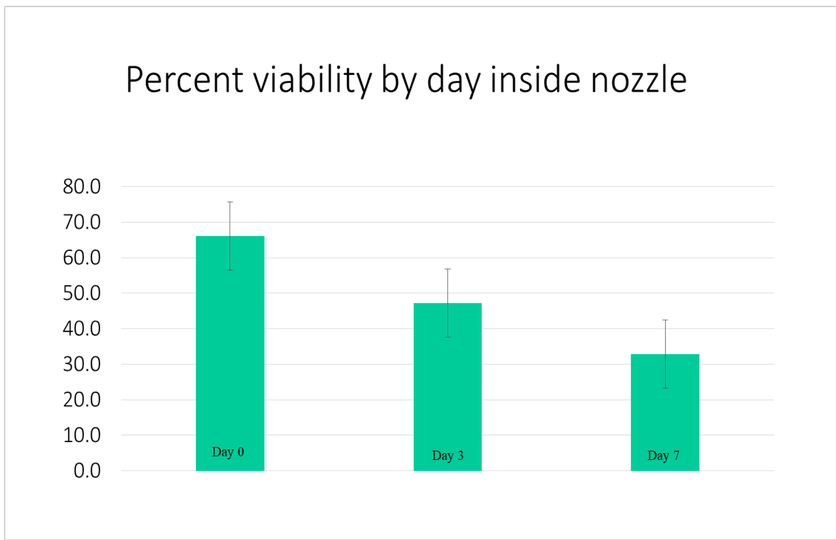


Fig. 4. (top to bottom) Average daily temperatures in Edinburg, TX from Nov 2019 to Jan 2020. Percent viability of nematodes on Day 0 when nematodes are mixed in water solution, followed by counts on Day 3, and 7. Samples and counts were made in the tank and at the nozzle head.

Discussion

Viability of the entomopathogenic nematode, *Steinernema riobrave* as formulated in the Nemasys-R gel matrix slowly declined during 1 week in the remotely operated sprayer, under cool, winter conditions. Water temperatures averaged 16 ºC during the study. Viability at 7 days, although lower was still adequate for application of against cattle fever ticks on nilgai. The nematodes used in these studies had been in cold storage at 5 °C for 6 to 8 months. It likely that viability would be higher from nematodes that had been stored for less than 3 months, which is the stated shelf-life of the Nemasys-R product. Viabliity of the nematodes during winter temperatures was similar to studies previously conducted during the summer months (Goolsby et al. 2019). This research should be duplicated for each batch of nematodes received from the supplier.

Goolsby, J.A., Cantu, D., A. Vasquez, A. E. Racelis, C. Hoffman, J. Hinojosa, C. Reed, D. Bonilla, D. Ellis, Adalberto Perez de Leon. 2019. Development of a remotely activated field sprayer and evaluation of temperature and aeration on the longevity of *Steinernema riobrave* entomopathogenic nematodes for treatment of cattle fever tick infested nilgai. Subtropical Agriculture and Environments 70:1-5.

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