

# 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

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## ABSTRACT

A remotely activated field sprayer was developed for application of the entomopathogenic nematode, *Steinernema riobrave* (Cabanillas, Poinar, and Raulston) for eradication of the southern cattle fever tick, *Rhipicephalus microplus* (Canestrini) infesting free-ranging nilgai (*Boselaphus tragocamelus*) in South Texas. The battery powered sprayer is activated by sonic sensors that detect movement of nilgai through fence crossings. An onboard computer operates the sprayer pump and aerator that oxygenates the solution of nematodes. Several types of aeration, agitation, and cooling were tested to prolong the viability of the nematodes in water. Continuous aeration extended the longevity of *S. riobrave* to more than two weeks as compared to cooling or intermittent agitation. The potential use of nematodes pathogenic to *R. microplus* dispensed by the remotely activated sprayer to infested nilgai, and potentially white-tailed deer (*Odocoileus virginianus*), as part of integrated cattle fever tick eradication efforts is discussed.

*Additional index words:* wildlife disease vector control, livestock entomology, biological control, pathogenic landscape

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The southern cattle fever tick (SCFT), *Rhipicephalus* (= *Boophilus*) *microplus* Canestrini (Acari: Ixodidae) has a wide geographic distribution, spanning tropical and subtropical regions between parallels 32°N latitude and 35°S latitude (Goolsby et al., 2016). It causes huge economic losses to milk and meat production, both directly (reduced weight gain and milk production, anaemia, and, mortality) and indirectly by transmitting various disease-causing pathogens such as *Babesia* spp. *Anaplasma* spp. (Grisi et al., 2014; Pérez

de León et al., 2012). Southern cattle fever tick and bovine babesiosis are estimated to have caused losses to the U.S. livestock industry close to \$3 billion annually in today's currency before they were eradicated from the U.S. (Graham and Hourrigan, 1977; USDA, Texas Cattle Fever). An eradication program based on continuous surveillance by mounted inspectors and the use of acaricides has been implemented in the U.S. along the Texas-Mexico border to manage periodic outbreaks. However, novel strategies are needed for

the continued detection, suppression and eradication of SCFT in the permanent quarantine zone (PQZ) due to the emerging role of 1) exotic nilgai antelope, *Boselaphus tragocamelus* (Pallas) and white-tailed deer, *Odocoileus virginianus* (Zimmerman) as SCFT hosts; 2) growing evidence of acaricide resistance; and 3) the invasion of pathogenic landscape-forming species such as the giant reed, *Arundo donax*, and other exotic plant species that contribute to suitable habitat for the SCFT (Perez de León et al., 2012; Esteve-Gassent et al., 2016; Foley et al., 2017; Singh et al., 2018a).

Nilgai are competent hosts of *R. microplus* with large home ranges and are known to move frequently between public lands set aside for wildlife conservation and private lands managed for cattle and/or wildlife (Foley et al., 2017; Olafson et al., 2018). Because they are exotic animals, nilgai do not have a regulated hunting season and are commonly harvested year-round in South Texas. Therefore, pesticides are not suitable for treatment of cattle fever tick-infested nilgai because they have withdrawal periods that must be followed before the meat can be consumed from a hunter-harvested animal. Currently, there are no viable treatment options to manage SCFT infestations in nilgai other than culling.

To meet the need for an effective treatment of nilgai, a remotely operated field sprayer was developed for use in field settings where the SCFT is present. The sprayers were designed to treat nilgai with a water solution of entomopathogenic nematodes as they pass through established fence crossings. Entomopathogenic nematodes in the genera *Steinernema* and *Heterorhabditis*, including *Steinernema riobrave* Cabanillas, Poinar, and Raulston have been commercialized as biopesticides and are suitable for treatment of tick-infested nilgai (Goolsby et al., 2018, Singh et al. 2018, a,b). These nematode species only affect arthropods and have no impact on non-target vertebrates or plants (Grewal, Ehlers and Shapiro-Ilan, 2005, Shapiro-Ilan et al., 2006). Additionally, *S. riobrave* is native to South Texas including the areas inhabited by SCFT-infested nilgai (Cabanillas et al. 1994). Entomopathogenic nematodes kill ticks and other arthropods with the aid of mutualistic bacteria; *Xenorhabdus* and *Photorhabdus* spp. bacteria are symbiotically associated with *Steinernema* and *Heterorhabditis* spp., respectively (Gaugler and Kaya 1990). Infective juveniles, the only free-living stage, enter hosts through natural openings (mouth, anus, and spiracles), or occasionally the cuticle, and release bacterial symbionts into the hemocoel, which are primarily responsible for killing the host. Therefore, the solution containing nematodes must make direct contact with SCFT on the body of nilgai for this treatment to be effective.

Herein, we discuss the design of the nilgai sprayer and methods to increase the longevity of *S. riobrave* in the sprayer. Longevity of *S. riobrave* in water can be limited which influences the time at which the sprayer needs to be serviced to add fresh entomopathogenic nematodes. This study was conducted to examine the

effects high ambient summer temperatures, aeration, agitation, and cooling of the water solution on longevity of *S. riobrave* in the field sprayers. The potential use of nematodes pathogenic to *R. microplus* dispensed by the remotely activated sprayer to tick-infested nilgai, and potentially white-tailed deer (*Odocoileus virginianus*), as part of integrated cattle fever tick eradication efforts is discussed.

## MATERIALS AND METHODS

The remotely operated nilgai sprayer was developed initially at USDA-ARS, College Station, TX and modified at USDA-APHIS, Edinburg, TX to be more compact and portable. The nilgai sprayers were designed to dispense an aqueous solution of entomopathogenic nematodes to control cattle fever ticks (Fig 1.). A sprayer is activated by two ultra-sonic sensors that are placed on either side of nilgai fence crossings. Ultra-sonic sensors detect movement of large animals at up to 1m away from the sensor. Once activated the control module powers the pump to spray 180 mls of the solution through an 8m, 1 cm tubing to 3 spray nozzles. Most of the 8m hose extended from the sprayer to the fence crossing is covered by the shade of vegetation and water temperature in the hose remains cool, thus nematode viability is similar to the samples taken from the sprayer tank. The sprayer is powered by a deep-cycle 12-volt battery. Inside the tank is an aerator that constantly oxygenates and mixes the aqueous solution of nematodes. The computer that operates the sensor, spray pump, and aerator is fully programmable. The standard program calls for the sensor to operate 24 hours a day, which can activate the pump to dispense the aqueous solution. Nilgai sprayers were fabricated from parts accessible on the World Wide Web (Table 1). All electronics are wired directly to the control box and battery via conduits. The cost per sprayer is approximately \$1200 USD per unit including parts and labor to assemble.

Evaluation of nematode longevity was conducted at the USDA-ARS Cattle Fever Tick Research Lab at Moore Air Base in Edinburg, TX under outdoor conditions from June to August 2018. Four treatments were evaluated to determine the longevity of *S. riobrave* as follows: Treatment 1, nematodes were held in water without agitation, aeration or chilling (Control); Treatment 2 (Control + Agitator), nematodes were agitated for 3 minutes every 30 minutes with a recirculating pump; Treatment 3 (Agitator + Nano-chiller), nematodes were agitated as in treatment two and chilled (reduced water temperature 5°C) with a thermoelectric water-cooling device (Nano-chiller, Nova Tec products, San Rafael, CA); and Treatment 4 (Aerator only), nematodes were continuously aerated using a bubble ring aerator (Table 1).

All tanks were filled with 26 L of water along with 3.05 g of *S. riobrave* (Nemasys-R, BASF, Inc Florham Park, NJ, USA) resulting in a solution with



**Figure 1.** Remotely operated nilgai sprayer

approximately 5000 infective juvenile nematodes per ml. Water temperature samples inside the tank, was recorded hourly during work hours to record nematode viability using a thermometer. Air temperature inside the tank was measured constantly using a HOBO 2x External Temperature Data Logger, (ONSET, 470 MacArthur Blvd., Bourne, MA 02532).

**Counting Nematode Methodology.** A 30 mL aliquot of nematode solution was taken every hour between 8 am and 4 pm, Monday - Friday. The nematodes were then observed under a dissecting microscope at 35X and recorded as dead or alive. If the nematode were pin straight, they were counted as dead. If the infective juvenile nematode was moving, curled, in an “S” position or its tip curved, it was counted as alive. All

**Table 1.** Parts list for remotely activated nilgai sprayer.

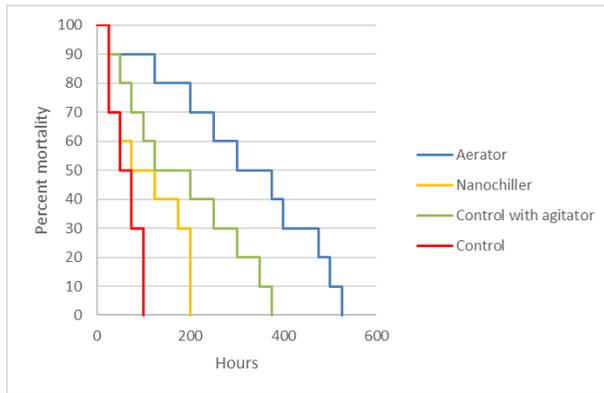
ITEM	SOURCE
<b>Sprayer tank</b>	
57L tank INFD15-19	Plastic Water Tanks <a href="https://www.plasticwatertanks.com">https://www.plasticwatertanks.com</a>
Submersible Pump C1C/S/PV 12V 20L	U.S. Solar Pumps <a href="https://www.ussolarpumps.com">https://www.ussolarpumps.com</a>
12 Volt Battery SRM 24	Interstate Battery <a href="https://interstatebatteries.com">https://interstatebatteries.com</a>
Electronics Enclosure HW-J10080SCHQR	Automation Direct <a href="https://www.automationdirect.com">https://www.automationdirect.com</a>
Aerator 14213	Frabill <a href="https://www.frabill.com">https://www.frabill.com</a>
<b>Controller</b>	
CO-01DD1-D Click PLC	Automation Direct
UK1C-EN-OE Sensor	Automation Direct
CD12M-OB-070-C1 field connector	Automation Direct
7000-12481-0000000 panel connector	Automation Direct
781-1C-12D relay SPDT	Automation Direct
781-1C-SKT socket relay	Automation Direct
PSP24-PC12-1 power supply	Automation Direct
KN-F10-10 fuse holder	Automation Direct
external switch red button	Automation Direct
KN10J12 jumpers	Automation Direct
3pin metal Amphenol cannon connectors	Automation Direct
fuses	Automation Direct
DIN terminal block	Automation Direct
On-off toggle switch	Automation Direct
<b>Hoses and nozzles</b>	
2 - ½ tee (22252-312-500NY)	Wylie <a href="https://www.wyliedirect.com">https://www.wyliedirect.com</a>
1 - ½ elbow (22251-311-500NY)	Wylie
3 - spray caps (1114441-1-CELR)	Wylie
3 - spray tips (8005-VS)	Wylie
25 feet - ½ hose (2-200-050)	Wylie
Quick connect from sprayer to hose	United States Plastic Corporation <a href="https://www.usplastic.com">https://www.usplastic.com</a>
1 - ½ inch NPT HFC 12 Series polypropylene coupling body - shut off (60685)	United States Plastic Corporation

nematodes within a 0.78 cm<sup>2</sup> circle were scored. The average number of nematodes found within the circle was approximately 30 individuals. Percent viability was calculated as the number of live nematodes divided by the total number counted. Once a treatment reached <5% nematode viability, the tests were terminated. Data was analyzed using SYSTAT 13.0 (SYSTAT, 2009).

## RESULTS

Analysis of variance on log transformed data indicated a strong effect of treatment on the rates of nematode mortality (indicated by slopes) ( $F_{(3,19)} = 59.4, P = <0.001$ ). Post hoc analysis indicated that nematodes in the aerator treatment had the lowest rate of mortality (transformed  $M = -6.870, SD = 0.57$ ), as compared to the other treatments. The nano-chiller (Trt 3) had a negative effect on survivorship of nematodes as compared to treatments 2 and 4, decreasing their lifespan by almost 7 days. Aeration (Trt 4) had the greatest effect on nematode longevity with surviving up to 532 hours, approximately 23 days. The shortest time being 486 hours, approximately 14 days (Fig 2). The mean

water temperatures ranged from 33°C in treatments 1,2, and 4 and 31°C in treatment 3 the nano chiller. Mean air temperatures in the tank for treatment were 40°C for treatments 1 & 2, 36°C for the nano-chiller treatment 3 and 39°C for treatment 4 with continuous aeration.



**Figure 2.** Survivorship of entomopathogenic nematodes in water solution inside nilgai sprayer tanks.

## DISCUSSION

The sprayer developed for this study represents a novel device for treatment of nilgai and other wildlife at fence crossings or other points of congregation for control of cattle fever ticks or possibly other ectoparasites. Using ‘off the shelf’ inexpensive electronic components, this simple device allows for treatment of nilgai under natural conditions in the environment when infested with SCFT. Currently, the sprayer relies on treatment of nilgai passing through fence crossings, but it could be adapted for treatment at other locations such as feeders, food plots, or water troughs. Sprayers will need to be maintained every 1-2 weeks to add more water, nematodes, and charged batteries. The labor cost for operation is high, but considering the benefits of treating SCFT infested cattle fever ticks on nilgai and the overall benefits of eradicating these outbreak populations in South Texas, the total costs may be economically worthwhile.

Use of entomopathogenic nematodes allows for treatment of cattle fever ticks in remote areas. Entomopathogenic nematodes, including *S. riobrave* are unique in that they are non-toxic to vertebrates. This provides the safety factor necessary for field use. Further, *S. riobrave* is native to the environment proposed for treatment including environmentally sensitive wildlife refuges. This species is commercially produced and used for a wide variety of pests, which makes it readily available should the use of *S. riobrave* and sprayer be expanded.

One of the key factors limiting the use of entomopathogenic nematodes in the nilgai sprayer device was longevity of the infective juveniles. If longevity

was too short, the water solution of nematodes needed to be changed frequently, thus increasing labor costs. The addition of the bubble aerator proved to be the most significant factor in prolonging the longevity of *S. riobrave* infective juveniles. Further testing is needed to determine if shorter periods of aeration are sufficient to maintain viability so that battery life can be extended, and labor reduced. We had anticipated that chilling the water solution would extend the longevity of *S. riobrave*, but in fact we found the opposite. The overall results indicated that *S. riobrave* could survive long periods (3 weeks) at high water temperatures (40°C) if the water was sufficiently aerated. It’s not surprising an organism native to the subtropical climate of the Lower Rio Grande Valley of Texas would have high heat tolerance.

The remotely operated nilgai sprayer is a unique device that has the potential for treatment of nilgai in their natural environment. We believe this is the first device of its kind and has been observed to treat nilgai at fence crossings (Fig. 1). Considering the frequent interaction of livestock and wildlife, the sprayer may have additional applications. Additional field testing is needed to determine the efficacy of these alternative strategies for integrated cattle fever tick eradication.

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