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Incidence of the Brown dog tick, *Rhipicephalus sanguineus* and its parasitoid, *Ixodiphagus hookeri* on dogs in South Texas

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ABSTRACT

The southern cattle fever tick, *Rhipicephalus microplus,* is a livestock pest worldwide, including South Texas, and can vector Babesia spp.; the causal agents of bovine babesiosis. Its congener, the brown dog tick, *Rhipicephalus sanguineus*, is also common worldwide and is frequently parasitized by a wasp, *Ixodiphagus hookeri*. To better understand the life history and host location cues of parasitic wasps of ticks, which supports the cattle fever tick eradication program for *R. microplus*, we examined the incidence of *R. sanguineus* and its parasitoid *I. hookeri*. Dogs in Hidalgo County, TX were observed (n=624) from Oct 2018 to April 2019. Our results show that the sampled dogs had very low levels of *R. sanguineus* (1.89%) with 219 nymphs, and no parasitoids were recovered. We also found that *R. sanguineus* nymph incidence is significantly higher on female dogs and puppies than other classes of dogs.

Additional index words: biological control, cattle fever ticks

Ticks (Super family: Ixodoidea; Phylum Arthropoda) are ectoparasites that can be vectors of severe and even life-threatening diseases in various animal groups including humans, livestock, wildlife and pets via transmission of pathogenic protozoa, bacteria, and viruses (Jongejan et al. 2007). The southern cattle fever tick, *Rhipicephalus* (=*Boophilus*) *microplus* Canestrini (Acari: Ixodidae), and bovine babesiosis transmitted by it, caused an annual loss to the U.S. livestock industry of $3 billion in the currency of today before they were eradicated from the U.S. (Graham and Hourrigan 1977). The tick eradication program in the permanent quarantine zone along the Texas-Mexico border needs novel strategies due to growing evidence of resistance to acaricides, invasion of pathogenic landscape-forming weed species such as carrizo cane, *Arundo donax* L., or other exotic grass species that enhance survival of cattle fever ticks (Perez de Leon et al. 2012, Esteve-Gassent et al. 2014), and the emerging role of white-tailed deer, *Odocoileus virginianus* (Zimmerman) and exotic nilgai antelope, *Boselaphus tragocamelus* (Pallas), as tick hosts (Foley et al. 2017). Nilgai antelope are competent hosts of *R. microplus* and have large home ranges, moving frequently between public lands set aside for wildlife conservation and private lands managed for cattle and/or wildlife. Because they are exotic animals, nilgai do not have a hunting season and are commonly harvested year-round in South Texas. Therefore, pesticides that have withdrawal periods for meat consumption from a hunter-harvest would not be suitable for treatment of cattle fever tick-infested nilgai. Biological control agents, such as parasitic wasps, especially the tick specialists, *Ixodiphagus* (Hymenoptera: Encyrtidae), may be useful in reducing populations of *R. microplus* on wildlife and support the cattle fever tick eradication program in the United States (Goolsby et al. 2016).

Rhipicephalus sanguineus (Latreille), the brown dog tick, is a congener of *R. microplus* and very common worldwide. *Rhipicephalus sanguineus* sensu latu is a complex of populations worldwide and is most often found on dogs, *Canis lupus familiaris* L. These ticks can cause skin damage, produce toxins, and predispose infected dogs to myiasis, the infection of fly larva in tissue, and dermatophytosis- the infection of the hair, skin, or nails- commonly known as ringworm. *Rhipicephalus sanguineus* also has similarities (e.g., questing behavior) to the southern cattle fever tick, *R. microplus*. Brown dog ticks have been found to occur in both urban and rural areas and are commonly attacked by parasitic wasps in these environments (Lopes et al. 2012, Bezerra Santos et al. 2017).

A brief history of tick parasitoid research shows that the parasitoids were first discovered in Texas, where *Hunterellus (=Ixodiphagus) hookeri* (Howard) was found parasitizing brown dog tick, *Rhipicephalus sanguineus* (Wood 1911). Bowman et al. (1986) documented that *Hunterellus texanus* (Howard) collected in Texas were specialized on *Dermacentor variabilis* (Say). Later, Mwangi et al. (1997) conducted a small-scale inoculative release of *I. hookeri* (Hymenoptera: Encyrtidae) and found reports of more than 49% parasitism of *Amblyomma variegatum* (Fabricius) nymphs on cattle in Kenya. Hu et al. (1998) reviewed the history of tick biological control programs including the intensive releases of *Ixodiphagus* parasitoids in Russia and the northeastern USA in the 1920s and 50s. Knipling and Steelman (2000) developed a conceptual model for area-wide biological control of *Ixodes scapularis* (Say), the vector of Lyme disease using augmentative releases of *I. hookeri*. Lopes et al. (2012) reported parasitism of *Rhipicephalus sanguineus* and *Amblyomma* spp. by *I. hookeri* and *I. texanus* in several geographic regions of Brazil with distinct climates. Collatz et al. (2011) reported unique host finding and oviposition behaviors of *I. hookeri* when collected and exposed on their original co-evolved tick host. They further speculated that even though this parasitoid may have a global distribution they may be specialized to attack their native hosts and have biological traits, such as a lengthy diapause, to adapt to their local climates. This may explain the apparent lack of success from introducing *Ixodiphagus* spp. against novel hosts in different climates. Collatz et al. (2011) found high levels of parasitism in Germany on *Ixodes ricinus* (Linnaeus) by *I. hookeri*. In this system, parasitoids oviposited into nymphs and emerged from engorged nymphs. Bezerra Santos et al. (2017) in Brazil found 30% parasitism of *R. sanguineus* by dissection and used molecular methods to identify the parasitoid as *I. hookeri*.

To better understand the methods for collection, rearing, and potential release of parasitoids for biological control of *R. microplus*, *R. sanguineus* is being studied as a model because of its phylogenetic similarity to *R. microplus* and because *R. sanguineus* is known to be parasitized by *Ixodiphagus hookeri* in Texas where tick parasitoids were first discovered (Wood 1911). Similar to *R. microplus*, *R. sanguineus* have four life stages, egg, larva, nymph, and adult (Takasu and Nakamura 2008, Wood 1911). Tick larvae exhibit a behavior known as "questing" in which they will climb vegetation (e.g., grasses) and wait for a passing host. The larvae attach and feed for 3-7 days until they detach and molt into the nymphal stage, and nymphs feed for an additional 4-8 days, detach from the host and molt to the adult stage (Takasu and Nakamura 2008, Wood 1911). All known *Ixodiphagus* spp. oviposit primarily into unfed nymphs and emerge from engorged nymphs (Hu and Hyland 1997, Takasu and Nakamura 2008, Collatz et al. 2011). Multiple parasitoids emerge from the mummified tick nymph, emergence rates ranging from days to three months after collection (J. Collatz, pers. comm.).

Even though *I. hookeri* was collected from *R. sanguineus* in Corpus Christi and Brownsville, TX in 1911, to the best of our knowledge there have been no subsequent collections, especially in Hidalgo Co. [Lower Rio Grande Valley, south Texas] which has a mix of urban, peri-urban and rural environments and where untreated dogs are common. This mix of environments and animal hosts may be ideal for the ecological studies of *R. sanguineus* and its parasitoid *I. hookeri*.

MATERIALS AND METHODS

Tick Collections: The study was conducted in the Rio Grande Valley in south Texas. *Rhipicephalus sanguineus* were collected by staff at the Palm Valley Animal Center located in Edinburg, Texas. Dogs brought in by animal control operators were collected from all areas in Hidalgo County including urban, peri-urban and rural areas. Tick collections were made three times a week from Oct 2018 until April of 2019. Data on dog age (puppies vs adults), sex (male vs female), and health of the dogs (with or without fleas) was recorded to estimate any possible correlations with tick parasitoid incidence. Ticks collected from each dog were placed in 50mL labeled plastic centrifuge tubes. Ticks were identified morphologically, and *R. sanguineus* were separated into smaller glass shell vials (Thomas Scientific clear N-51A borosilicate vials, Swedesboro, New Jersey) based on life stage of the tick. Fewer than ten Gulf Coast ticks, *Amblyomma maculatum* (Koch), were collected but were not part of this study. The vials were then closed with tightly fitted cotton plugs to prevent ticks from escaping but allowing for air exchange. Shell vials were placed in Styrofoam vial holders in a 38 liter aquarium with a saturated solution of potassium nitrate (Sigma, St. Louis, Missouri) to maintain relative humidity at 85% at 26°C to increase the longevity of the ticks and allow for parasitoid emergence. The ticks were examined weekly, and specimens were held for three months to allow for parasitoid emergence. Villarreal et al. (2018) documented the northern expansion of the tropical lineage of *R. sanguineus* into California and Arizona from Mexico. Therefore, it is likely that the *R. sanguineus* collected in this study in the Rio Grande Valley of South Texas is the tropical lineage. Specimens of the ticks collected in the study have been preserved for future molecular studies.

Data analysis. Due to the non-normal distribution of the data, we used a generalized regression analysis, with negative binomial model and maximum likelihood estimation. Season (Fall and Spring), health, sex and age were the individual variables used as predictors, and the model was run for the dependent variable, number of nymphs collected from the dogs. Since the number of ticks on dogs was too low to carry out any analyses, this was expressed as percentage incidence. All analyses were carried out using the statistical software JMP (SAS Institute, Inc. 2012. JMP 10.0. Carey, NC).

RESULTS

Over the seven-month period of collection, 1.89% (12/634) of dogs sampled were infested with *R. sanguineus*, with a total of 219 nymphs collected (0.345 nymphs per dog). No *I. hookeri* or any other species of parasitic wasps emerged from the tick specimens. Weekly examination of *R. sanguineus* found no signs of having been parasitized such as mummification of the tick or emergence holes. The statistical analyses revealed that both age and sex influenced incidence of tick infestation; females and puppies carried significantly more nymphs than their counterparts. Season and presence of fleas had no effect on presence of *R. sanguineus* nymphs. Full model of the analyses is represented in Table 1.

Table 1. Result of negative binomial model for *Rhipicephalus sanguineus* nymph incidence by season, health, sex and age of the dog host.

Term	Estimate	Std Error	Wald Chi Square	Prob > Chi Square
Intercept	-8.1269	2.0680	15.4431	<0.0001*
Season[Fall-Spring]	3.0480	1.7491	3.0367	0.0814
Health[Flea-Healthy]	1.9692	1.5506	1.6127	0.2041
Sex[Female-Male]	2.9623	1.4372	4.2483	0.0393*
Age[Adult-Puppy]	3.4237	1.3553	6.3812	0.0115*

*Values in rows with * indicate significant values.

DISCUSSION

We found a low incidence of both adults and nymphs of *R. sanguineus.* In fact, the numbers of adult ticks were too low to run any meaningful statistical tests. Although our analyses on nymphs revealed that female dogs and puppies significantly had more nymphs, we suspect that the clustering of nymphs on few dogs may have also affected the results, although the analyses have taken this into account. Regardless, to the best of our knowledge, the study is the first one to examine the incidence of *R. sanguineus* on dogs in the Rio Grande Valley, many of which were stray dogs. Ayodhya (2014) and Adhikari et al. (2013) found that there was a higher number of ticks (~50% more) on stray dogs as opposed to pet dogs. Data on the history of each dog in the study could not be determined; although, the number of *R. sanguineus* per dog seemed low to us, animal caretakers indicated this was a normal infestation level.

We found that health status of the dogs examined (the presence of fleas) did not affect tick incidence, although fleas were more common than tick infestations. This is similar to other studies; Kumsa and Mekonnen (2011) found the most prevalent flea species (*Ctenocephalides felis Bouché*) was on 82.9% of dogs sampled, but the most prevalent ticks (*Amblyomma* spp.) were only found on 3.5% of dogs. On the other hand, our data shows the correlation between age and sex and tick incidence. Puppies were more likely to be infested with *R. sanguineus* as compared to adult dogs, which concurs with Ayodhya (2014) who found 56.2% tick infestation on puppies and 43.8% on adults. The higher infestation levels on puppies may be explained by their developing immune systems, increased likelihood of remaining in one location, and proximity to other possibly infested puppies from the same litter (Mohamed et al. 2014). This could occur naturally or could be a result of how puppies are transported by animal control to the animal center, kept together in a small, confined space that would facilitate mass infestation. The increased amount of ticks found on female dogs could stem from the puppies as well; female dogs, especially mothers, are more likely to spend more time in close contact with their puppies, and the stationary behavior of feeding puppies also facilitates infestation (Mohammed et al. 2014).

Although *I. hookeri* has previously been collected (early 1900s) from *R. sanguineus* in Corpus Christi, Texas, and Brownsville, Texas, which is geographically close to where the current study was conducted, none were recovered from collected nymphs; unlike Bezerra Santos et al. (2017) who found approximately a 30% parasitism rate year-round in Brazil. One possibility for the absence of *I. hookeri* in nymphs collected in our study is because the dogs may have resided in or near an environment with heavy pesticide use.

More than 80% of land in Hidalgo County is used for agriculture which may have affected the prevalence of *I. hookeri*. Additionally, Hidalgo County has a mix of both conventional agriculture and urban areas with only 2% of the natural landscape remaining. *Ixodiphagus hookeri* may still exist in eastern Cameron County where natural environments are more common in the eastern portion, such as Laguna Atascosa National Wildlife Refuge (92,000 acres) which is 12.5 % of the county. Perhaps *I. hookeri* still persists in this environment and on other canine hosts such as the coyote, *Canis latrans* Say. Future studies are needed to determine if *I. hookeri* still exists in South Texas and the impact this parasitoid may have on tick populations.

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LITERATURE CITED

Adhikari, S., Mohanty, B., Panda, M.R., Sardar, K.K., Dehuri, M. 2013. Prevalence of tick infestation in dogs in and around Bhubaneswar. Veterinary World. 6: 982-985. Management of tick infestation in dogs.

Ayodhya, S. 2014. Management of tick infestation in dogs. Journal of Advanced Veterinary and Animal Research. 1: 145-147.

Bezerra Santos, M.A., Oliveira de Macedo, L., Barbosa de Souza, I., do Nascimento Ramos, C.A., Alves, L.C., Nascimento Ramos, R.A., Aparecida de Carvalho, G. 2017. Larvae of Ixodiphagus wasps (Hymenoptera: Encyrtidae) in *Rhipicephalus sanguineus sensu lato* ticks (Acari: Ixodidae) from Brazil. Ticks and Tick-borne Diseases. 8: 564-566.

Bowman, J.L., Logan, T. M., and Hair, J. A. 1986. Host suitability of *Ixodiphagous texanus* Howard on five species of hard ticks. Journal of Agriculture and Entomology. 3: 1-9.

Collatz, J.H., Selzer, P., Fuhrmann, A., Oehme, R.M., Mackenstedt, U., Kahl, O., and Steidle, J.L.M. 2011. A hidden beneficial: biology of the tick-wasp *Ixodiphagus hookeri* in Germany. Journal of Applied Entomology. 135: 351 –358.

Esteve-Gassent, M.D., Pérez de León A.A., Romero-Salas, D., Feria-Arroyo, T.P., Patino, R., Castro-Arellano, I., Gordillo-Pérez, G., Auclair, A., Goolsby, J.A., Rodriquez-Vivas R.I., and Estrada-Franco, J.G. 2014. Pathogenic landscape of transboundary zoonotic diseases in the Mexico–US border along the Rio Grande. Frontiers Public Health. 2: 1-23

Foley, A.M., Goolsby, J. A., Ortega-S. Jr., A., Ortega-S., J., Pérez de León, A., Singh, N. K., Schwartz, A., Ellis, D., Hewitt, D. G., and Campbell, T. A. 2017. Movement patterns of nilgai antelope in South Texas: implications for cattle fever tick management. Preventive Veterinary. Medicine. 146: 166-172.

Goolsby, J.A., Mays, D. T., Schuster, G. L., Kashefi, J., Smith, L., Amalin, D., Cruz-Flores, M., Racelis, A., and Pérez de León, A.A. 2016. Rationale for Classical Biological Control of Cattle Fever Ticks and Proposed Methods for Field Collection of Natural Enemies. Subtropical Agriculture and Environments. 66: 7 -15.

Graham, O., and Hourrigan, J. 1977. Eradication programs for arthropod parasites of livestock. Journal of Medical Entomology. 13: 629-658.

Hu, R. and Hyland, K. E. 1997. Prevalence and seasonal activity of the wasp parasitoid, Ixodiphagus hookeri (Hymenoptera: Encyrtidae) in its tick host, *Ixodes scapularis* (Acari: Ixodidae). Systematic & Applied Acarology. 2: 95-100.

Hu, R., Hyland, K.E., and Oliver, J.H. 1998. A review on the use of I*xodiphagus* wasps (Hymenoptera: Encyrtidae) as natural enemies for the control of ticks (Acari: Ixodidae). Systematic and Applied Acarology. 3: 19-28.

Jongejan, F., Nene, V., Fuente, J., Pain, A., and Willadsen, P. 2007. Advances in genomics of ticks and tickborne pathogens. Trends in Parasitology. 23: 391-396.

Knipling, E.F. and Steelman, C.D. 2000. Feasibility of Controlling *Ixodes scapularis* Ticks (Acari: Ixodidae), the Vector of Lyme Disease, by Parasitoid Augmentation. Journal of Medical Entomology. 37: 645-652.

Kumsa, B. and Mekonnen, S. 2011. Ixodid ticks, fleas and lice infesting dogs and cats in Hawassa, southern Ethiopia. The Onderstepoort Journal of Veterinary Research. 78. E1-E4.

Lopes, A.J.O., Nascimento-Junior, J.R.S., Silva, C.G., Prado, A.P., Labruna, M.B., and Costa-Junior, L.M. 2012. Parasitism by *Ixodiphagus* Wasps (Hymenoptera: Encyrtidae) in *Rhipicephalus sanguineus* and *Amblyomma* Ticks (Acari: Ixodidae) in Three Regions of Brazil. Journal of Economic Entomology. 105: 1979-1981.

Mohammed, K., Biu, A., Ahmed, M., and Charles, S. 2014. Prevalence and seasonal abundance of ticks on dogs and the role of *Rhipicephalus sanguineus* in transmitting *Babesia* species in Maidugiri, North-Eastern Nigeria. Veterinary World. 7: 119-124.

Mwangi, E.N., Hanssan, S.M., Kaaya, G.P., and Essuman, S. 1997. The impact of *Ixodiphagus hookeri*, a tick parasitoid, on *Amblyomma variegatum* (Acari: Ixodidae) in a field trial in Kenya. Experimental and Applied Acarology. 21: 117-126.

Perez de Leon, A.A., Teel, P.D., Auclair, A.N., Messenger, M.T., Guerrero, F., Schuster, G., and Miller, R.J. 2012. Integrated strategy for sustainable cattle fever tick eradication in U.S.A. is required to mitigate the impact of global change. Frontiers in Physiology. 3: 1-17.

Takasu, K. and Nakamura, S. 2008. Life history of the tick parasitoid *Ixodiphagus hookeri* (Hymenoptera: Encyrtidae) in Kenya. Biological Control. 46: 114-121.

Villarreal, Z., Stephenson, N., and Foley, J. 2018. Possible Northward Introgression of a Tropical Lineage of *Rhipicephalus sanguineus* Ticks at a Site of Emerging Rocky Mountain Spotted Fever. Journal of Parasitology. 104: 240-245.

Wood, H.P. 1911. Notes on the life history of a tick parasite, *Hunterellus hookeri* Howard. Journal of Economic Entomology. 4: 425-431.