An Evaluation of the Hard Ticks (Ixodidae) Infesting Cane Toads *Rhinella marina* (Bufonidae) in Northeastern Trinidad, W.I.

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ABSTRACT

The cane toad *Rhinella marina* provides a unique opportunity to investigate Neotropical ectoparasite communities, because individuals travel long distances and traverse multiple habitat types, each inhabited by different species of ectoparasite including ticks (Ixodidae). To evaluate the ticks parasitising *Rhinella marina* within northeastern Trinidad, individual toads were obtained from four agrarian sites with different environmental characteristics and proximity to urban or sylvan areas. Individuals were collected by hand and inspected for ticks within 5-10 minutes of capture. Parasitism by ticks occurred in 12 of the 39 toads collected. *R. marina* specimens were hosts to 12 species of ticks represented by five genera (*Amblyomma* spp., *Hamaphysalis* spp., *Hyalomma* spp., *Ixodes* spp., and *Rhipicephalis* spp.). Individuals sampled from sylvan environments exhibited the highest incidence of infestation with a low to moderate density of toads while individuals collected from urban environments showed lower incidences of infestation despite the greatest density of toads. 84% of all ticks collected were female and 43% of all ticks collected were immature. Individuals representing all instars were obtained for all five genera excluding *Amblyomma* spp., for which only adult specimens were observed. The data resulting from this study provides evidence that *R. marina* is infested by a moderate diversity of tick species in northeastern Trinidad which reinforces the utility of this species as a means to study ectoparasites within Neotropical environments.

Key words: Anura, Ixodidae, ectoparasite, host preference, instar

INTRODUCTION:

The cane toad *Rhinella marina* is a well-studied species in non-native areas due to their potential as an amplifying host for pathogens (Kelehear 2016). However, in Trinidad and Tobago, where they are native (Murphy *et al.* 2018), these toads are largely overlooked with the exception of a study by Burgon *et al.* (2012) and a few papers examining internal parasites (e.g. Ragoo and Omah-Maharaj 2003). Individual cane toads have been recorded travelling up to 55km per year (CABI 2018) making them a species of interest when it comes to the potential spread of disease agents over large areas. Previous studies describe adult cane toad habitats as lowland disturbed/urban areas, but eggs must be laid in shallow bodies of water existing further from preferred adult habitats, contributing to the need for locomotion across large distances (Zug and Zug 1979). This potential for dispersal across large areas may result in a higher probability of exposure to a greater diversity of ectoparasites (Clifford *et al.* 2014). Though several species of ticks are known to inhabit northern Trinidad (Basu and Charles 2017) a previous study reported only two species of ticks, *A. dissimili* and *A. rotundatum*, as ectoparasites of *R. marina* in Trinidad (Burgon *et al.* 2012).

Some of the species of ticks that are reported to inhabit northern Trinidad, including *Amblyomma ovale* and *Rhipicephalus microplus* can act as vectors for the spread of *Rickettsia* and *Ehrlichia* bacteria (Guerrero *et al.* 2010, Barbieri *et al.* 2015). Adults of the aforementioned species of ticks are typically associated with non-anuran preferred hosts (Basu and Charles 2017) and have yet to be reported as ectoparasites of cane toads in Trinidad. Host preference can be described as the host specialisation of a species of tick at a population dynamics level (McCoy *et al.* 2013). The concept of host preference is debated for adult ticks and is even more polarised in the conversations on the efficacy of host preference-based predictability in the younger stages of development (McCoy *et al.* 2013). Given a host-generalist based approach, the high probability of exposure to a variety of pathogens and interactions with various environments suggests that *R. marina* could serve as an appropriate indicator of which species of ticks (and associated disease agents) may be present within a particular environment (Kelehear 2016). The aims of this study are (1) to determine the burden of tick infestation on cane toads collected in four different areas in northeastern Trinidad to assess which species of ticks infest this common species of anuran; and (2) to assess whether additional species of tick are parasitising *R. marina* in northeastern Trinidad than have been reported previously.

MATERIALS AND METHODS:

Sample sites

Specimens of *Rhinella marina* were collected from one of four areas in the vicinity of Toco in northeastern Trinidad. All four sites were considered agrarian based on a proximity to human dwellings, but differed slightly in terms of habitat and the level of human activity. The perimeters of all sample areas were positioned at least 500 metres apart (Table 1).

Toad Collection and Ectoparasite Inspection

Each of the four sites were surveyed for individuals of *R. marina* on two separate occasions with each survey separated by four days to decrease the probability of recapture. Handling of toads and collection of ticks was permitted via a special game license granted through the Trinidad Wildlife Section of the Forestry Division. Samples were collected from 5 May to 1 June 2019 during the dry season. Toads were collected by baiting a collapsible hexagon trap with cat food as well as through capture by hand from 2000-2100h using flashlights. Captured toads were placed into plastic collection tubs and brought back to the field laboratory for measurements, photographs, and tick inspections. The snout to vent length of each individual was recorded using a caliper and photographs of each individual were taken using a Canon 80D camera, to minimize the risk of recapture. Tick inspections were performed visually. All ticks were removed from toads by hand using fine-tip tweezers and placed directly into a vial containing 70% ethanol. All toads were released after the removal of ticks at their site of capture. Ticks were examined under an Opti-TekScope (electrical microscope) to identify instars, sex, and species. Species identifications were performed through the use of published keys (Krantz and Walter 2009, Basu and Charles 2017, Keirans and Durden 1998) and verified by the Texas A&M Insect Collection (Voucher #744). The first two instars (larva and nymph) were referred to as immatures for comparison purposes with adult specimens within this paper.

Table 1. Sample site descriptions and GPS coordinates for nearest points between transect perimeters.

Site	Collection Area	Position of the Area	Characterisation of Area
Site 1	Grounds of Jammev Beach Resort	10.8264°N, -60.9291°W	Concrete paved areas, maintained gardens, and a nearby drainage ditch where toads were found spawning.
Site 2	Roadside to the northeast of Site 1	10.8314°N, -60.9291°W	More human-populated area, featuring homes interspersed between unmanaged lands.
Site 3	Roadside to the southeast of Site 1	10.8233°N, -60.9350°W	Bordered by dense vegetation on one side and a drop off into a valley with a creek bed on the other side. Mostly sylvan environment, determined by the observation of the greatest variety of species of amphibians, reptiles, and mammals were observed.
Site 4	Toco Beach	10.8346°N, -60.9219°W	Adjacent to a drainage ditch located between small restaurants directly south of Toco Beach-front.

RESULTS

In total, 39 individuals of *R. marina* were collected, 13 specimens from Jammev grounds, 11 specimens from Roadside SE, ten specimens from Roadside NE, and five specimens from Toco Beach. Of the 39 toads collected, 12 were infested with ticks. A total of 61 ticks were collected, representing five genera, 12 species, and all life stages except eggs (Figure 1). The genera collected include *Amblyomma* spp., *Haemaphysalis* spp., *Hyalloma* spp., *Ixodes* spp., and *Rhipicephalus* spp., all within the family Ixodidae. (Figure 2).

The mean density of ticks per cane toads examined as well as intensity of infestation (IOI) of toads carrying ticks were calculated per NIH guidelines (Agustin 2013). The mean density of ticks, calculated as number of ticks per cane toad, across all examined individuals of *R. marina* collected was 1.47. Mean density of individual sites ranges between 0.38 and 4.09. The intensity of tick infestation on cane toads, calculated as the number of ticks per infested individuals of *R. marina* collected, was 4.38 (Table 2). IOI of individual sites range between 1.5 and 9. Roadside SE yielded the highest number of ticks, while Roadside NE yielded the second highest number. followed by Jammev and Toco Beach (Table 2). The average ticks per host across all sites is 5.08 ticks per toad. This drops to 2.4 ticks per toad with the exclusion of the two outlying highly infested toads. These highly infested individuals were both collected from Roadside SE. Individually their infestations were comprised of 55% immature for the first specimen and 84.6% immature for the second specimen. 83% of the immature ticks on specimen one were *Ixodes* spp. and 37% of the immature for the second specimen were also *Ixodes* spp.. Across all infested toads, there were varied proportions of immature ticks relative to adults between each genus of tick (Figure 1). There were high proportions of females, especially amongst genera containing high levels of immature ticks (Figure 3).

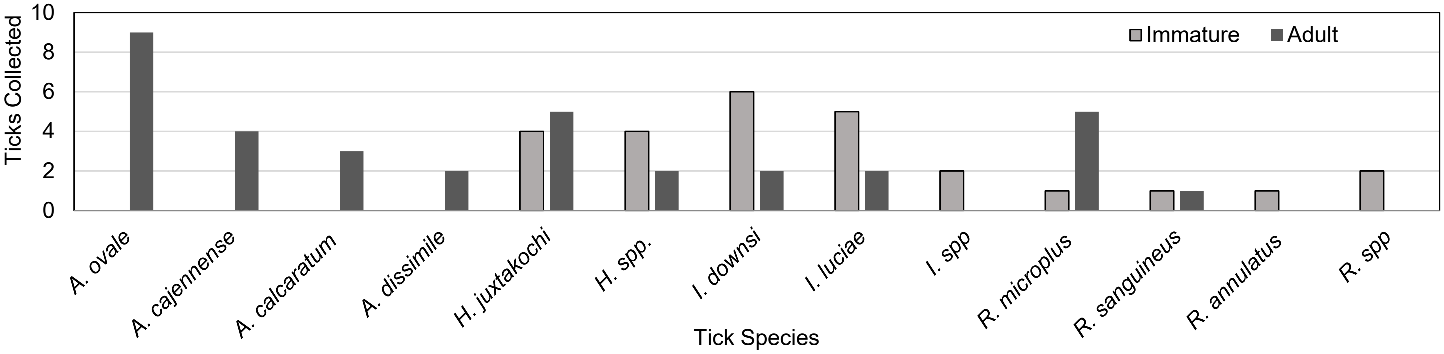

Fig. 1. Number of immature and adult individuals of each species of tick collected during the course of this study. Total number of ticks collected=61.

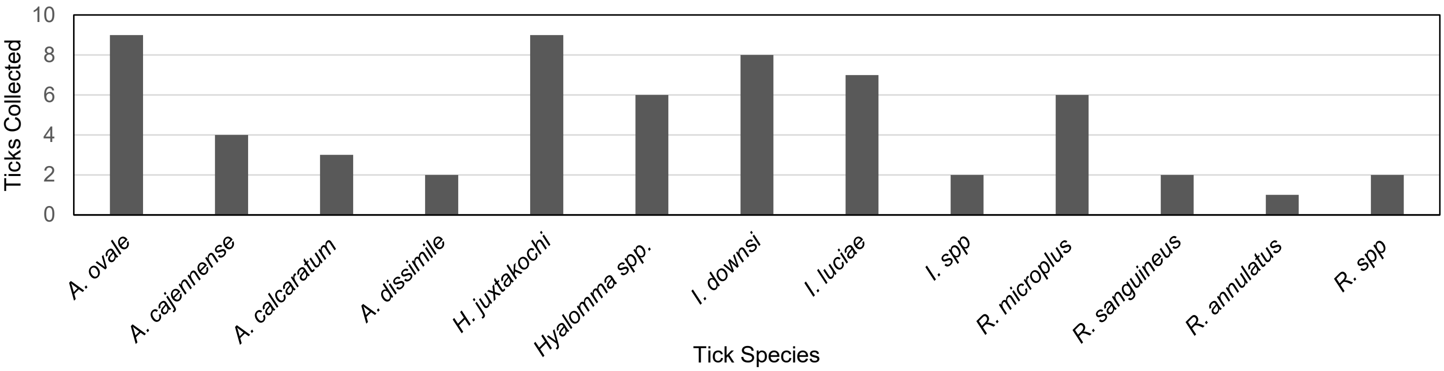

Fig. 2. Number of each species of tick collected from 12 cane toads during the course of this study. Total number of ticks collected=61.

Table 2. Summary descriptive statistics for the four collection sites.

Site	Number of Toads Sampled	Number of Toads Infested	Total Number of Ticks Found	Proportion of Ticks Collected per Site	Density of Infestation (+/- SE)	Mean Number of Ticks per Toad Infested (IOI) (+/- SE)
Jammev	13	1	5	8.20%	0.38 +/- 0.75	5 +/- 0
Roadside NE	10	4	6	9.84%	0.6 +/- 0.52	1.5 +/- 0.46
Roadside SE	11	5	46	75.41%	4.09 +/- 5.05	9.2 +/- 9.79
Beach	5	2	4	6.56%	0.8 +/- 1.14	2 +/- 1.96

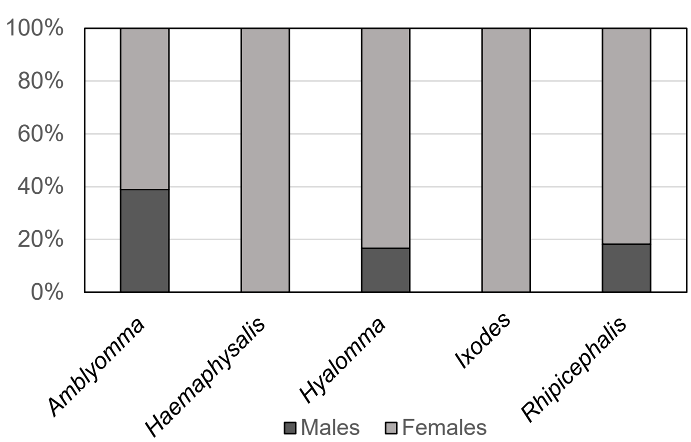

Fig. 3. Percentage of male and female individuals for each of the five genera of ticks collected during the study.

DISCUSSION

This study supports the idea that a large variety of tick species exist, parasitise cane toads, and reproduce perennially in northeastern Trinidad. Twelve species spanning five genera from the family Ixodidae were found parasitising cane toads, while previously only *A. dissimile* and *A. rotundatum* were reported as ectoparasites of this species in Trinidad (Burgon *et al.* 2012).

Effect of Site Variation on Ectoparasitic Burden

Roadside SE site contributed 75.41% of the ticks encountered in this study. This site represents the least disturbed of the four areas sampled, based upon visual observations made during collection times. Within the more urban areas sampled (Toco Beach and Jammev) the incidence of toads was higher than the less urban sites, while the incidence of ticks on those toads was lower. This provides support for the hypothesis that more ticks of a greater diversity exist in areas with a greater abundance of hosts (Meeüs *et al.* 2007). These areas are characterised by dense vegetation and access to water even though these are not requirements for either the tick or are associated with cane toad habitats (Page *et al.* 2008, Agustin 2013). Therefore, the question of why both ticks and toads are encountered in more sylvan areas, such as Roadside SE, exists.

These data may represent the higher infestation rate speculated to exist when travelling across a variety of environments (Clifford 2014). Roadside SE was along a path that toads cross between breeding grounds and preferred habitats, not where the toads would regularly come into contact with people, which would favour the spread of diseases to animals and not humans. If the incidence of infestation becomes great enough, however, a spillover event could affect human populations (Harrus and Beneth 2005); thus, continued monitoring of tick densities even in sylvatic populations of wildlife is recommended.

The variety of tick species collected on cane toads at a single time in the year, represents the potential for a variety of associated disease agents to exist within cane toad populations at any given time. It is unknown, however, if cane toads in northern Trinidad are competent intermediaries for tick to tick serial infections for the diseases associated with the ticks found in this study. If *R. marina* is capable of amplifying disease agents such as *Rickettsia* from *Haemaphysalis* spp. and *Amblyomma* spp.; Ehrlichiosis, Babesiosis, and Anaplasmosis bacteria from *Rhipicephalus* spp.; *Bartonellae* bacteria from *Ixodes* spp., or even Crimean-Congo Hemorrhagic Fever Virus from *Hyalomma* spp., then cane toads may represent a greater public health risk than they are currently considered (Hornak and Horváth 2012, Constable *et al.* 2017, Bayer 2019, Guerrero *et al.* 2010, Scott *et al.* 2017, Souza *et al.* 2018, Reis *et al.* 2011). Cane toads have been found to amplify Myxosporean parasites, *Myxidium* spp. in non-native areas (Hartigan *et al.* 2011), but investigations into their ability to amplify *Rickettsia, Ehrlichia*, or other bacteria are lacking. An essential step in considering the public health risk of any species is assessing their ability to be an amplifying host. Beyond that, is the presence of the disease agents in the environment (Rodricks 1994). Due to the increasingly globalised world, even in the case where the pathogens are not currently in the area of concern, continued monitoring is recommended.

Relative Abundance of Immature and Mature Ticks

Individuals of *Hyalomma* spp., *Ixodes* spp., and *Rhipicephalis* spp. collected in this study comprised >50% immatures while 100% of the individuals of *Amblyomma* spp collected were adult. All larval ticks were collected from toads in the Roadside SE site. This sample area was characterised by an abundance of potential amphibian and mammalian host dwellings, and thus, adult ticks are more likely to exist in the vicinity, leading to a greater probability of female ticks laying their eggs in this area (Aeschlimann *et al.* 1976). These eggs would then hatch, and larval ticks would be seeking their first blood meal. Since larvae cannot travel more than a few feet on their own (Meeüs 2007), it is probable that a ground dwelling animal such as a toad would be the optimal host whether the toad is the preferred host of the adult tick or not. Since immature ticks have been found in significantly greater densities in low lying vegetation, when sampled in other studies, it is logical to presume this is where their first blood meal will be derived (Aeschlimann *et al.* 1976, Mejlon and Jaenson 1997).

Due to high humidity and temperatures year-round in Trinidad, the probability of finding varying life stages of ticks was high (Bale *et al.* 2002). In multi-season areas, tick life cycles can be predicted and tracked throughout the year due to the rise and fall of temperatures dictating when life stages can progress (Krasnov and Matthee 2010, Cradock and Needham 2010). In more tropical areas such as Trinidad, it is likely that ticks collected throughout the year will vary in instar and thus vary in predictability of host-preferences for any given species of tick. In this study there were several genera represented at different life stages, thus no definitive conclusions on a single tick species causing the most significant burden for cane toads could be made.

Sex Distribution

Female ticks accounted for 83.6% of specimens (across all genera and life stage) and 100% of *Haemaphysalis* spp. and *Ixodes* spp. collected were female. The reason for this is unknown, but this phenomenon has been observed previously (Meeüs *et al.* 2007, Krasnov and Matthee 2010). Aeshlimann *et al.* (1976) hypothesized that male ticks may prefer hosts that have larger zones of existence thus causing lesser densities of males across regions through being passively carried by the host to locations away from where the tick emerged initially (Aeschlimann *et al.* 1976). Thirty one years later, Meeüs *et al.* reinforced this hypothesis in finding that fewer males have been repeatedly collected in small defined regions and suggesting that this may be due to their larger distribution patterns (Meeüs *et al.* 2007). However, these hypotheses have not been fully supported by empirical data and should be further researched. Another explanation suggested was based upon the observation that lab-bred ticks commonly yield more females than males in each clutch (Pinter *et al.* 2002). Furthermore, male lab-bred ticks have shown longer feeding durations than females, correlating with a longer interval of time between emerging or molting and host seeking (Pinter *et al.* 2002).

Recommendations

Due to proportions of immatures as well as the number of species found, it is likely that cane toads and potentially various other ground dwelling vertebrates such as rodents and lizards play a vital role in the life cycle of various tick species. However, because the majority of genera encountered were represented by different life stages, no definitive conclusion on a single tick species causing the greatest burden for cane toads can be made. The tick species collected are capable of vectoring several disease agents (Hornak and Horváth 2012, Constable *et al.* 2017, Bayer 2019, Guerrero *et al.* 2010, Scott *et al.* 2017, Souza *et al.* 2018, Reis *et al.* 2011), while the competence of the cane toad at amplifying these agents is unknown at this time. For this reason, research on cane toad amplification competence should be continued, alongside the monitoring of tick species and their associated agents.

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