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An Investigation into the *Amblyomma* Tick (Acari: Ixodidae) Infections of the Cane Toad (*Rhinella marina*) at Four Sites in Northern Trinidad

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ABSTRACT

Two species of tick are known to parasitize cane toads on Trinidad, *Amblyomma dissimile* and *Amblyomma rotundatum*. The focus of this study was to investigate differences in the levels of tick parasitism in cane toad populations at four sites in northern Trinidad representing different habitat types, the effect of physical host characteristics on tick loads and to determine the impact of ticks on cane toad health. Significant differences were found in tick loads between sites, possibly due to human disturbance and modification. Toad sex and condition index appear to be unrelated to tick load but wounds associated with previous tick attachment were found to be common and in two cases these wounds were seen to be secondarily infected by larva of a *Notochaeta* species (Diptera: Calliphoridae). Toad size had varying levels of significance dependent upon sample site. Ticks were found to preferentially attach to certain body regions of the host (dorsum and chin) thought to be due to exposure and submersion in water. Differences in attachment sites were also seen between tick instars, though tick sex showed no relationship. *A. dissimile* was the more common of the two tick species by a considerable amount.

Key words: *Rhinella marina, Amblyomma dissimile, Amblyomma rotundatum*, parasite impact, Trinidad.

INTRODUCTION

Rhinella marina [=*Chaunus* (*Bufo*) *marinus*] (Linnaeus 1758), also known as the cane, giant or marine toad, is native to tropical regions of the Americas (Burridge and Simmons 2003; Sutherst *et al*. 1995) including Trinidad and Tobago (Ragoo and Omah-Maharaj 2003). They are now common in many non-native habitats, like Australia (Sutherst *et al.* 1995), through deliberate introductions and are thought to be having a negative impact on native fauna (Lampo and DeLeo 1998; Urban *et al*. 2007).

Cane toads are parasitized by two species of tick on Trinidad: *Amblyomma dissimile* Koch, 1844 and *Amblyomma rotundatum* Koch, 1844 (Lampo and Bayliss 1996b). The use of these ticks, along with other parasites such as the nematode lungworm *Rhabdias pseudosphaerocephala* (Kelehear *et al.* 2011), to control cane toad numbers in non-native regions is currently being considered (Lampo and Bayliss 1996a; Smith *et al*. 2008). However, introducing exotic ticks into new regions is of high concern due to the possible spread of tick-borne disease (González-Acuña *et al*. 2005). As *A. dissimile* has been implicated as a vector for the rickettsial disease *Ehrlicia* (=*Cowdria) ruminatum* of ruminants (Jongejan 1992), and the cane toad as a reservoir for the spirochete *Leptospira interrogans* which can cause leptospirosis on Trinidad and Grenada (Oliver *et al*. 1993), detailed information on the infection, transmission and impacts of ticks on toads must be known before any action is taken.

A lot of work has been carried out on mainland South America (Lampo and Bayliss 1996b) but published data on the parasitic tick infections of native cane toad populations in Trinidad is lacking. The aims of this project were to address the following questions: are there different rates of tick infection on cane toads at different locations; do the tick infections vary with the sex or size of the host; do tick loads have an impact on the health of cane toads; and finally, are tick loads clustered or evenly distributed across the host's body and does this vary between the three tick instars or according to the different species of tick?

METHODS

Sample sites

Four sample sites were used in this study, chosen for abundant cane toad populations and differing habitat:

1. Lopinot Stream: Natural mountain stream bordered by rainforest on one side and a recreational lawn and cemetery on the other (10.692575°N, 61.32175°W).
2. Las Cuevas: On the northern coast, largely undisturbed area. This site has a brackish river, rainforest and a sandy beach located close to the sea (within 100 m) (10.777894°N, 61.400562°W).
3. Simla: This site is in the Northern Range surrounded by rainforest with a maintained lawn and several man-made ponds (10.691868°N, 61.289282°W).
4. University of the West Indies Trinidad Campus (UWI): In the urban area of St. Augustine, this site is highly modified and maintained with grass fields, drainage ditches and a large artificial pond (10.63914°N, 61.395854°W).

Toad collection and inspection for ticks

Main investigation

Each site was sampled four times with 6-8 days between visits. On each trip, 10 toads were captured (first encountered) by hand between 1900-2100 h. These toads were taken to UWI, assigned a number and their snout to vent length (SVL; to the nearest 0.1cm) and weight (g) measured. The toads were sexed using the morphological characters in Narayan *et al*. (2008) (dorsal skin texture and colour, cream dorsal margin stripe and vocal sac openings) and photographs of the head, dorsal and ventral surfaces were taken to provide a record for recapture identification; toe-clipping was considered ethically unsound following May (2004). As reinfection rates and natural tick turnaround was unknown, all data from recaptured toads were excluded from the analysis.

Toads were examined for ticks (Fig. 1) and the number found on each of five predefined body regions (head, chin, legs, dorsum and venter) recorded. All ticks were removed and preserved in 95% ethanol and any sores/wounds/ulcers thought to be associated with previous tick attachment were also noted. Toads were returned to their original sample site within 24 hours.

Tick instar (1-larva; 2-nymph; 3-adult), sex (adults only) and species (adults only) were identified using a binocular microscope and published keys (Keirans and Durden 1998; Robinson 1926). No male *A. rotundatum* were expected as this species is regarded as parthenogenetic, although three males have been reported (Labruna *et al*. 2005).

Distribution of ticks on the host body

In order to investigate differences in attachment site between tick sexes, species and instars, clearly infected toads were collected at Lopinot, Las Cuevas and Simla (UWI was excluded due to low infection levels). These toads were captured after the study described above due to the non-random nature of their sampling. The final sample comprised nine parasitized toads per site. These were treated the same as the toads in the above study except the ticks removed from each body region were preserved separately.

Fig. 1. Image showing an engorged female tick (circled in red) attached to the chin of a cane toad.

Condition index (measure of health)

The condition index (CI) can be used as an indicator of the toad's health and was compared to tick load to investigate any correlation between the two factors. Although CI cannot be fully accounted for by tick parasitism, it has been previously used to investigate their impact on host health (Smith *et al*. 2008). CI was measured using the following equation from Eggert and Guyetant (2003):

$$CI = \frac{\text{weight (g)}}{SVL^3 (\text{cm})} \times 100$$

Population estimates

Host population size may help explain any differences in tick infection rates between sites. For this, photographs were used to identify toad recaptures. Population estimates were made using the Schnabel mark recapture equation (Sutherland 1996):

$$N = \frac{\sum(M_t C_t)}{\sum R_t + 1}$$

C_t= Total number of individuals caught in sample t, R_t= number of individuals already marked (recaptures) when caught in sample t , M_t= number of marked animals in the population just before the t^{th} sample is taken.

RESULTS

Sample size

For each site 40 toads were sampled for the main investigation (160 in total). However, with the removal of recaptures the final sample sizes for each site were: Lopinot - 35; Las Cuevas - 37; Simla - 28; UWI - 36 (a total of 136). All three tick instars as well as adults of both species (*A. dissimile* and *A. rotundatum*) were found (species was only able to be determined in adults, instar 3); no male *A. rotundatum* were seen as expected. In total, 878 ticks were recovered: 445 were instar 1; 274 instar 2; 93 male *A. dissimile*; 49 female *A. dissimile*; 17 *A. rotundatum* (female).

For the *distribution of ticks on the host body* aspect of the study, nine toads were sampled from each of the three sites used, 27 in total (none of which were recaptures). From these toads, 293 ticks were removed: 122 were instar 1; 98 instar 2; 44 male *A. dissimile*; 24 female *A. dissimile*; five *A. rotundatum* (female).

Effect of site on tick load

Mean tick loads differed between sites (Fig. 2), especially Lopinot (7.97 ± 1.2 [standard error, SE] ticks per toad; n=35) and UWI (2.61 ± 1.5 [SE] ticks per toad; n=36) which showed the highest and lowest mean tick loads respectively. Both showed a large variation; as well as higher mean infections. Lopinot had a few individuals which were highly parasitized and UWI had the highest proportion of individuals with no ticks (55%). Las Cuevas had 6.95 ± 0.9 [SE] ticks per toad (n=37) and Simla 4.00 ± 0.9 [SE] ticks per toad (n=28). Both showed small variation suggesting some level of consistency in tick loads.

A Log (x+1) transformation was used on the data, chosen as the observational data (tick loads) contained zero values (Zar 1999), and a one way ANOVA (*aov*) performed. This showed that tick load was significantly affected by sample site (*aov*: $F_{(3,132)}$=19.15, p<0.00001). A factor reduction process was then used to see which sites differed significantly. The boxplot (Fig. 2) indicated that most of the variance was captured within UWI, with this site excluded, and this time using a square root transformation, a significant difference was again seen (*aov*: $F_{(2,96)}$=8.426, p<0.00001). Excluding the next lowest, Simla, and just looking at Lopinot and Las Cuevas, we found no significant effect (*aov*: $F_{(1,70)}$=0.41, p>1). A comparison of Simla and UWI (using the log function) revealed a reduced but still significant effect (*aov*: $F_{(1,62)}$=6.448, p= 0.05). Therefore all sites, except Lopinot and Las Cuevas, show a significant difference in tick load.

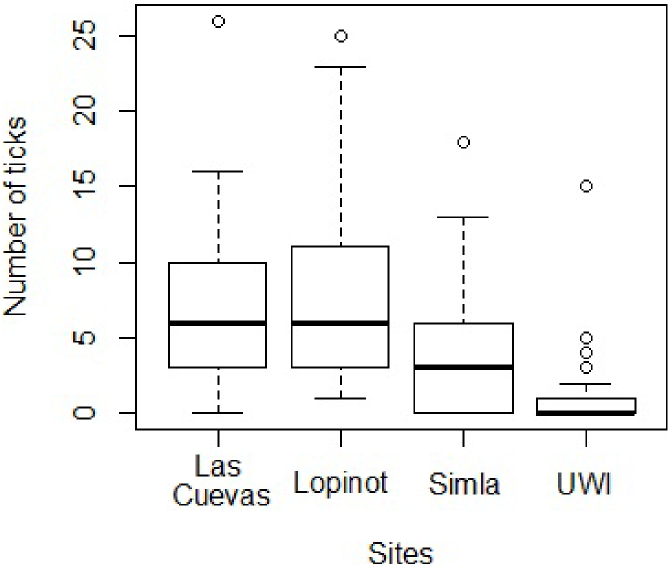

Fig. 2. Box and whisker plot showing the tick loads found at the four sample sites. One UWI sample (U075, tick n=52) has been omitted due to its outlying effect compressing the rest of the graph.

Large differences in toad population size were seen between the sites. Las Cuevas had the highest population (150), then UWI (120), Lopinot (100) and finally Simla (46). Recaptures were uncommon at all sites except Simla, the only site to show recaptures on all samples after the first. No minimum recapture number was indicated as necessary for this method; however, due to low recapture levels a dedicated population estimate may provide more insight and support. The accuracy of the recapture data is considered good with doubt about identifications in less than 3% of the sample. Although no definitive work on the uniqueness/consistency in the external features of cane toads could be found, if the toad's sex, markings and morphometric data (SVL and weight) corresponded to one captured on a previous sample at that site, it was marked as a recapture with confidence.

Relationships between the sex, weight and SVL of toads and tick loads

No significant difference was seen in the mean tick loads between male and female toads when using a t test (t=-1.77, df=134, P=0.078). The data also showed no significant relationships between toad weights and tick loads across the whole sample (General Linear Model [GLM]: $F_{(1, 135)}$=0.02, P=0.889), but did show a significant positive relationship at both Lopinot (GLM: $F_{(1, 34)}$=4.95, P=0.033) and Las Cuevas (GLM: $F_{(1, 36)}$=5.23, P=0.028). Likewise, SVL showed no relationship with tick loads across the whole sample (GLM: $F_{(1, 135)}$=0.05, P=0.826), but did have a significant positive relationship with tick load at Lopinot (GLM: $F_{(1, 34)}$=5.49, P=0.025) and Las Cuevas (GLM: $F_{(1, 36)}$=5.39, P=0.026).

Relationship between tick load and CI

CI appeared to be lower in infected males (CI: 0.0111 ± 0.0002 [SE]; n=17) compared to uninfected males (CI: 0.0115 ± 0.0004[SE]; n=67), whereas infected females appeared to have a higher CI (CI: 0.0118 ± 0.0003 [SE]; n=36) than uninfected females (CI: 0.0116 ± 0.0004 [SE]; n=16). However, these differences in CI were not significant in either males (GLM: $F_{(1, 83)}$=0.13, P=0.718; n=84), females (GLM: $F_{(1, 51)}$=0.00, P=0.980; n=52) or the population as a whole (GLM: $F_{(1, 135)}$=0.37, P=0.547; CI: 0.0114 ± 0.0001[SE]; n=136). Although CI showed no significant relationship to tick load, 29% of the sampled toads had open wounds or sores/ulcers associated with previous tick attachment sites; in two of these cases toads had been secondarily infected by Dipteran larvae which were living under the host's skin.

Distribution of ticks on the host body

The distribution of ticks on the host body was found to be non-random and highly dependent upon body region (GLM: $F_{(1,143)}=6.25$, P=0.002). Overall a higher than expected percentage of ticks were found on the chin (651% of expected value (EV) [based on tick load, percentage body area of region and random distribution]) and dorsum (138%[EV]), and a lower than expected percentage on the legs (57%[EV]) and venter (11%[EV]). Head results varied by site but overall were higher than expected (194%[EV]).

The distribution of instars on the host was not entirely random. Significant differences were seen in instar attachment to both the dorsal (GLM: $F_{(2, 45)}=3.29$, P=0.047) and chin (GLM: $F_{(2, 42)}=3.43$, P=0.034) regions. Instar 1 was found to have a greater attachment on both the chin and dorsal surfaces, instar 2 (n=98) had a higher than expected attachment on just the dorsal surface and instar 3 (n=73) showed a significant lack of attachment to the dorsal region. Instar distribution on the head (GLM: $F_{(2, 17)}=1.00$, P=0.391), venter (GLM: $F_{(2, 17)}=1.00$, P=0.391) and legs (GLM: $F_{(2, 21)}=1.74$, P=0.203) was found to be not significantly different to random.

Within the sampled *A. dissimile,* no significant difference in attachment site was seen between sexes (GLM: $F_{(4, 52)}=0.18$, P=0.945). Both sexes of *A. dissimile* were found on just 37% of the toads with only 57% of female ticks found on the same regions as males and 37% of males found on the same regions as females; however, this reduced sample was too low (n=45) to determine any significant relationships.

DISCUSSION

Effect of site on tick load

The significant differences seen in tick loads between the four sites studied may be due to the level of human modification at each site as disturbance levels can have a serious impact on tick larval populations (Stein *et al*. 2008). Although all four sites are visited regularly by humans, both Las Cuevas and Lopinot are used for recreational purposes and are not highly modified. Simla, which shows lower tick levels, can be considered highly disturbed due to being an active research centre with year-round occupation. UWI is located in an urbanised area and is maintained to high standards for human aesthetics. This means that the vegetation required by ticks (Bermudez *et al*. 2010) to avoid desiccation, predation and excessive moisture (Stein *et al*. 2008) is less available, potentially making the habitat at UWI less suitable. The possibility of alternative hosts at sites of lower infection levels (UWI) was not investigated in this study as cane toads (along with iguanas) are considered the studied tick's primary host (Lampo *et al*. 1998). However, both *A. dissimile* and *A. rotundatum* are known to infect amphibians and reptiles other than cane toads (Lampo *et al*. 1998; Keirans and Oliver 1993) and juvenile instars have been found on mammals and birds, though this is rare (Robinson 1926). Though not considered significant, the effect of alternative hosts may warrant further investigation.

An explanation for the major differences in estimated cane toad populations between sites is offered by Hagman and Shine (2006) who found that open spawning sites with gradually sloping muddy banks were better for cane toad reproduction; this would make Lopinot and Las Cuevas the better spawning sites explaining their higher populations. However, cane toads are known to be highly opportunistic in spawning, utilising many locations such as drainage ditches (Murphy 1997), making this explanation less likely. When looking at Las Cuevas, Lopinot and Simla, tick parasitism may be, at least partly, population dependent as the greater populations of Lopinot and Las Cuevas had higher mean tick loads than Simla. This does not hold for UWI which had the second highest population estimate but the lowest mean infection load. However, as discussed above, the habitat at UWI was possibly not ideal for tick survival and transmission.

Effect of toad sex, weight and SVL on tick loads

The lack of a significant difference in tick loads between male and female cane toads concurs with research conducted by Lampo and Bayliss (1996b). However, a preliminary study (Crocker 2009) did find a significant difference in the tick loads of male and female cane toads (higher for males), so this perhaps requires further study.

Lampo and Bayliss (1996b) found a positive relationship between toad size and tick load up to a toad SVL of 140 mm, declining thereafter. This was only partially found in this study. Only two out of four study sites showed a significant positive relationship between toad size and tick load despite both of these increasing the available surface area for attachment on the host. The data for SVL and weight are conflicting in this study with half the sample sites showing a positive correlation with tick load and half no correlation, indicating the need for further study.

Effects of tick load on CI and other aspects of toad health

No significant relationship was seen between toad CI and tick load in the sampled population. Infestations were possibly not high enough, as the highest mean tick load (Lopinot) was only 7.97 ± 1.2 and the highest infestation 105 ticks. However, these are comparable to tick loads found by Lampo and Bayliss (1996b) in northern Venezuela and northern Brazil (an average of 2.48 ticks per toad and as many as 146 ticks on a single toad) who did find a significant decrease in toad condition index with increasing tick load. This shows a need to more extensively sample the cane toad population on Trinidad. If the results presented here are confirmed by future work, it may indicate a difference between the mainland South American and the Trinidadian cane toad population's response to tick parasitism.

Although the CI appeared unrelated to tick load, other factors were noted during this study. Open wounds or sores/ulcers associated with tick attachment sites, which are known to attract parasites such as screwworms (Fig. 3) or become secondarily infected by bacterial pathogens (Keirans and Durden 1998), were found to be common (29% of the sample). In two cases during this study, toads were found to have *Notochaeta* maggots (Diptera: Calliphoridae) underneath their skin having used what appeared to be previous tick attachment sites to gain access to the host's body. This has been seen in other anurans (Bolek and Janovy 2004) but no examples of it occurring in cane toads could be found.

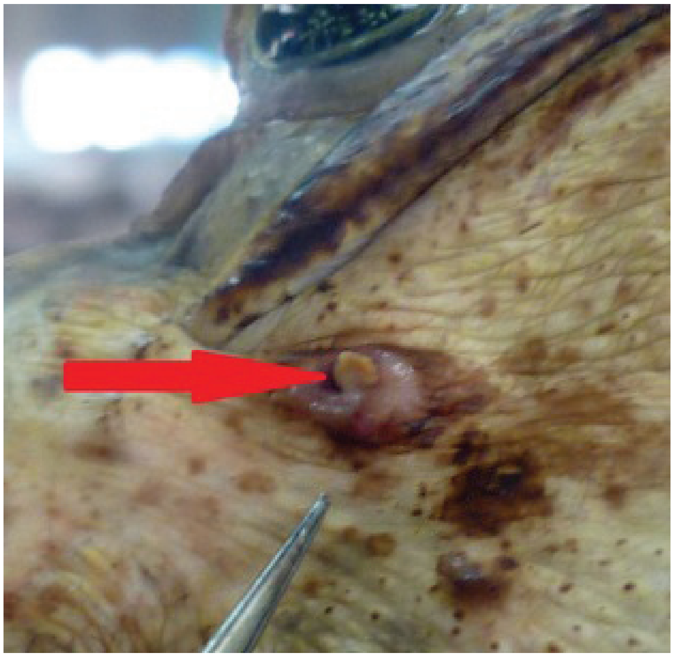

Fig. 3. The red arrow points to an example of the wounds caused by previous tick attachment; the cream/yellow object in the centre of the wound is a maggot emerging from under the host's skin; *Notochaeta* sp. (Diptera: Calliphoridae).

This study found no evidence to support the use of ticks to control cane toad numbers. However, as Smith *et al*. (2008) found data suggesting ticks' role in controlling *Chaunus schneideri* numbers, clearly more research must be done and it is these secondary infections we think should be the focus of this research.

Distribution of ticks on the host body

Tick distribution on the host was found to be highly aggregated on certain body regions with clustering on the dorsum, chin and (to a lesser extent) the head, with fewer seen on the legs and venter. This is similar to an *A. rotundatum* distribution found by Pontes *et al*. (2009) on snakes with greater attachment to the head and dorsum compared to the venter. The distribution seen in these two studies is possibly due to the fact that both snakes and cane toads drag their ventral surface on the ground as they move, preventing attachment as ticks tend to select areas of high feeding efficiency and minimal disturbance (Andrews and Petney 1981). However, Lampo and Bayliss (1996b) found no preference for tick attachment site on cane toads. An alternative explanation for the distribution seen in this study may be that tick attachment is not based on disturbance, but on host behaviour. Cane toads spend a lot of time partially submerged in the water and, as ticks are terrestrial parasites not adapted for an aquatic lifestyle (Pontes *et al*. 2009), this may explain their distribution. This is supported by Oliver *et al.* (1993) who found that when attached to submerged regions of the southern toad (*Bufo terrestris*), ticks would migrate across the host body to non-submerged locations. As this study was conducted during the rainy season when more frequent, and deeper, pools of water are available, this may help explain the observed avoidance of frequently submerged attachment sites by ticks.

There was a significant difference in attachment between tick instars on both the chin and dorsal surfaces. The reason for this is unknown as all seem to require the same thing, a blood meal. One possible explanation is the thickness of the cane toad's skin in relation to the length of the instars' mouthparts. Schwinger, Zanger and Greven (2001) found a significant difference between the cane toads' mean ventral, dorsal and lateral skin thickness (greatest on the dorsal, then lateral skin and thinnest on the venter) and Brown and Knapp (1980) found that instar 2 *Amblyomma americium* mouthparts penetrated 2.5x deeper than instar 1; though not reported, it is likely there is a further increase between instars 2 and 3. Also on warty regions, skin thickness will vary between thicker and thinner regions. This is unlikely to explain the variation seen here as specimens of all instars were found on all body regions and instars 1 and 2 had proportionally higher attachment rates than instar 3 on the dorsal surface, despite the greater skin thickness. Clearly other factors must be influencing tick instar distribution.

Instar size and disturbance/exposure to damage may help explain the distribution of ticks on the host. Instar 1 ticks (the most common instar found) from the same clutch are known to aggregate on a single host (Lampo and Bayliss 1996b) which may explain their clustered distribution. Little research has been done in this area and experimental work could be carried out to investigate the differences in attachment sites seen in this study and whether or not this represents a real difference in instar attachment site preference.

No significant differences were found in male and female *A. dissimile* attachment, most likely due to the fact that ticks mate whilst on the host (Jongejan and Uilenberg 2004) and both must avoid disturbance; females must feed to engorgement for egg production whereas males persist on the host in order to mate with multiple females (Drew and Samuel 1989; Jongejan and Uilenberg 2004).

A. dissimile was found to be a more common ectoparasite of cane toads at the four sites studied than *A. rotundatum*. The reason for this is unknown as they have a sympatric distribution, though *A. dissimile* is known to favour higher temperatures (Pontes *et al*. 2009), and both are a common parasite of the cane toad (Burridge and Simmons 2003). This could be studied further, including the effect of interspecific tick competition and ecological factors such as temperature, humidity and pH.

Tick specimens collected from this research have been added to the Hunterian Museum Collection, University of Glasgow (GLAHM: Entry Number 858) and a proportion sent to the University of the West Indies Zoology Museum (UWIZM), St. Augustine, Trinidad.

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