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Prevalence of infection by the protozoan *Hemolivia mariae* in ticks

Abstract This paper considers the prevalence of natural infections of the protozoan *Hemolivia mariae*, in its hosts the Australian sleepy lizard*, Tiliqua rugosa*, and the ixodid tick species, *Amblyomma limbatum*. We investigate whether the protozoan may be influencing the tick population in the field, by comparing the observed prevalence of infection in ticks with the prevalence expected from known transmission dynamics. The prevalence of infection in nymphs was similar to the expected prevalence, but the observed prevalence in adults was higher than expected. These results provide no evidence for infection-induced mortality in ticks. We also found that tick loads on infected and uninfected lizards were not significantly different and, overall, infected lizards were as likely to be tick-infested as uninfected lizards. However, infected lizards were less likely to be found carrying female ticks. On balance, the evidence did not strongly support the hypothesis that ticks avoid feeding on infected lizards. We use known parameters of *H. mariae* transmission to estimate the rate of tick ingestion that may be required to sustain the observed prevalences in the field.

Introduction

This paper considers the prevalence of natural infections of the protozoan *Hemolivia mariae*, a blood parasite of the Australian sleepy lizard (*Tiliqua rugosa)*, and gut parasite of the ixodid tick species, *Amblyomma limbatum* (Smallridge and Paperna 1997). A previous study found little variation in the prevalence of *H. mariae* in a population of lizards over a number of years, but suggested that infection was associated with relatively poor body condition in male lizards (Smallridge and Bull 2000). In this study, we investigate whether the protozoan may have an impact on the tick population in the field. The tick host, *A. limbatum*, has four life-stages – egg, larva, nymph and adult. Larvae and nymphs feed and then detach to moult off-host, so adults must have located a new host three times. After mating, females engorge and detach to lay their eggs before dying. Males do not engorge, but remain attached, mating with other females that may attach to the same host. *Hemolivia mariae* is ingested by ticks when they feed on blood from infected lizards. In the tick, the parasite reproduces sexually to produce a star-shaped oocyst, and then asexually to develop oval cysts (Smallridge and Paperna 2000a, b). In laboratory-fed nymphs, this process takes at least 3 weeks, and is not usually completed until after the tick has detached from its host (Smallridge and Bull 1999). Parasite cysts remain within the ticks during moulting and subsequent feedings, so that ticks fed on infected lizards as larvae are still infected as unfed adults even if their nymphal host was not infected (Smallridge and Bull 1999). Similarly, infections accumulate such that ticks fed as larvae and as nymphs on infected lizards have heavier parasite loads than those fed just once on an infected host (Smallridge and Bull 1999). The contents of the guts of heavily infected ticks are almost completely taken up with parasite cysts (C.J. Smallridge, personal observation). The parasite is transmitted to the vertebrate host when lizards ingest infected ticks (Smallridge and Bull 1999).

This paper reports the prevalence of *H. mariae* infection in lizards and their attached nymph and adult ticks in the field. We investigated whether infection with *H. mariae* could influence tick fitness. If *H. mariae* induces mortality in ticks, the prevalence of infection in the later tick stages should be lower than expected from accumulation dynamics. Alternatively, if *H. mariae* reduces tick fitness, natural selection should result in

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ticks avoiding infected lizards. In this case, observed tick loads should be lower on infected lizards than on uninfected lizards and overall infection frequencies in ticks should be lower than expected.

Materials and methods

This study area was in semi-arid chenopod shrubland near Mt. Mary, South Australia (33°56′S, 139°26′E) where *A. limbatum* infest sleepy lizards. Most lizard activity occurs in spring and early summer (September–December) (Bull et al. 1991). In that period in 1998, lizards were sampled along a 15-km gravel road transect.

Each lizard was individually marked by toe-clipping, sexed, weighed, and measured (snout–vent length; SVL). The number of each life-stage of attached ticks was recorded, and a smear was made on a microscope slide from lizard blood taken either from the caudal vein or from a toe-nail clip. Any attached adult and nymph stage *A. limbatum* ticks were pulled from the lizard with forceps, and placed individually into labelled, ventilated tubes. Larvae were not collected. The lizard was released at the point of capture.

Ticks were maintained at 4 °C for up to 1 week. Adult ticks survived these conditions, but a proportion of the collected nymphs died before they could be dissected. The surviving ticks were dissected in the laboratory by removing the mouthparts with a scalpel and pressing the gut contents into a drop of water on a slide. The tick-gut preparations were examined under a microscope (×400) for the presence of parasite stages (Smallridge and Paperna 1997). The air-dried blood smears from lizards were fixed in absolute methanol for 5 min and then stained in Giemsa (pH 7.0) for 15 min. Then, 100 fields were examined under the microscope (×1000) and the presence or absence of *H. mariae* recorded (Smallridge 1998).

The prevalences of *H. mariae* infection in lizards or ticks (the proportion of individuals found infected; Margolis et al. 1982) were compared among subgroups of lizards, or among subgroups of ticks, using contingency tables (*G*-tests; Sokal and Rohlf 1973). William's correction (G_{adj}) was used where the sample size was less than 200 (Krebs 1989). To compare mean tick numbers on infected and un-infected lizards, non-parametric Mann-Whitney *U*-tests were used because the distribution of the number of ticks per lizard was not normal.

Results

A total of 185 different lizards was captured in 1998. Since ticks were removed during sampling, the data from any lizards that were captured more than once were not included in the analyses. Four captured lizards were small and immature with body mass <350 g and SVL <25 cm. None of these were infected with *H. mariae*, and only one of them had ticks attached. A previous study has shown that immature lizards are significantly less likely to be host to ticks or to be infected with *H. mariae* (Smallridge and Bull 1999). These lizards were omitted from further analyses. Of the 181 adult lizards, 93 were females, 87 were males and the sex of 1 lizard was indeterminate. There were 0–95 *A. limbatum* ticks found on each lizard. The distribution of ticks on lizards was highly skewed, with 25.4% of lizards carrying no ticks, 40.4% carrying 1 or 2 ticks and only 4.4% of lizards carrying more than 20 ticks.

Prevalence of *H. mariae* and tick infestation in the lizard population

Overall, 23 (12.7%) of the 181 adult lizards were infected with *H. mariae*. Of these 23 infected lizards, 16 (69.6%) were also infested with ticks, while 119 of the 158 (75.3%) uninfected lizards were infested with ticks. These proportions were not significantly different ($G_{\text{adj}} = 0.3372$; $df = 1$, $P > 0.05$).

Prevalence of *H. mariae* in the tick population

Nymphs

Of the 211 *A. limbatum* nymphs on the lizards in the survey, 104 were examined microscopically, and 15 (14.4%) were found to be infected with parasite cysts.

There was no difference between the proportion of infected nymphs found on infected (33.3%, $n = 3$) and uninfected hosts (13.9%, $n = 101$; $G = 0.70$, $df = 1$, $P > 0.05$).

Of the 104 nymphal ticks dissected, 19 had not yet started feeding and 1 of these (5.3%) was found infected. Of the 85 engorging nymphs, 14 (16.5%) were found infected. These proportions were not significantly different ($G = 1.907$, $df = 1$, $P > 0.05$). Thus the prevalence of infection in the nymphs was independent of the engorgement status of the tick.

Adults

Of the 318 adult *A. limbatum* examined, 129 (40.6%) were found to be infected. This was significantly higher than the proportion of nymphs (14.4%) found infected ($G = 26.45$, $df = 1$, $P < 0.005$).

Of the adult ticks examined, 30 of 61 females (49.2%), and 99 of 257 males (38.5%) were found infected. These proportions were not significantly different ($G = 2.29$, $df = 1$, $P > 0.05$). The proportion of infected males was significantly higher on infected lizards (78.4%) than on uninfected lizards (31.8%; $G = 28.76$, $df = 1$, $P < 0.005$), but the same was not true for females ($G = 0.392$ $df = 1$, $P > 0.05$).

Of the 61 female ticks found, 15 were less than 5% engorged and 8 of these (53.3%) were found infected. Of the 46 more fully engorged females, 22 (47.8%) were found infected. These proportions were not significantly different ($G = 0.135$, $df = 1$, $P > 0.05$). Thus the prevalence of infection in the females was independent of the engorgement status of the tick.

Tick infestation of infected and uninfected lizards

Overall, the mean tick load for hosts without *H. mariae* (4.9 ± 0.97, $n = 158$) was not significantly different from that for hosts with *H. mariae* (2.4 ± 0.80, $n = 23$; Mann-Whitney *U*-test, $z = 1.091$, $P = 0.275$).

Five of the 23 infected adult lizards (21.7%) were host to nymphs while 38 of the 158 (24.1%) uninfected lizards were host to nymphs. These proportions were not significantly different ($G = 0.060$, $df = 1$, $P > 0.05$). The proportions of infected lizards found with no nymphs, one nymph, two to ten nymphs and more than ten nymphs (Table 1) were also not significantly different ($G = 2.434$, $df = 3$, $P > 0.05$).

Of the 23 infected hosts, 14 (60.9%) had males attached, while 93 (58.9%) of the 158 uninfected hosts had males attached. These proportions were not significantly different ($G_{\text{adj}} = 0.0335$, $df = 1$, $P < 0.05$). However, a significantly lower proportion of infected hosts (2 of the 23 lizards, 8.7%) had females attached compared with uninfected hosts (43 of 158 lizards, 27.2%; $G_{\text{adj}} = 4.421$, $df = 1$, $P < 0.05$).

Discussion

This study found that, for the cyst stage of *H. mariae*, infection prevalence in nymphs and females was independent of the engorgement status of the tick. This observation confirms previous laboratory studies that showed that the reproductive process of the parasite in the tick did not reach the cyst stage until after detachment from the host (Smallridge and Bull 1999). The most likely explanation for the observation that, among male ticks, infections were more prevalent in those attached to infected hosts compared with those attached to uninfected hosts is their lengthy period of attachment, which extends beyond the time required for *H. mariae* to complete its two reproductive phases.

The aim of this study was to determine the prevalence of *H. mariae*-infection in lizards and their attached nymph and adult ticks in the field and examine the data for indications of an impact of *H. mariae* on the ticks. Our first hypothesis was that if *H. mariae* induces mortality in ticks, the prevalence of infection in the later tick stages would be lower than expected from accumulation dynamics. If larvae were exposed to hosts at random, then since 12.7% of hosts were infected, 12.7% of larvae should feed on infected hosts. Assuming a 78% transmission rate from infected hosts to larvae (Smallridge and Bull 1999) and no transovarial transmission (Smallridge 1998), the expected prevalence of *H. mariae* in unfed nymphs should be 10%. Infection-induced mortality would reduce this even further. In this study, the observed infection prevalence in attached nymphs (14.4%) was slightly higher ($\chi^2 = 2.261$, $df = 1$, $P > 0.05$). A similar prediction could be made for the infection prevalence in adult ticks. Assuming 10% of unfed nymphs waiting for a host were already infected, 12.7% of the remaining 90% of uninfected nymphs may be expected to attach to and feed on an infected host. Hence an additional 11% of the ticks should be infected by the time they moult into adults, resulting in a total expected prevalence of 21% in the unfed adult stage. Some additional adults, particularly males, may become infected whilst still attached to their third host, so potentially a maximum of an additional 12.7% of the remaining 79% of uninfected ticks (10%) may become infected as adults. Therefore, up to 31% of adult feeding ticks could be expected to be infected. The overall observed prevalence of infection in adult attached ticks was almost 10% higher than the expected outcome ($\chi^2 = 10.723$, $df = 1$, $P < 0.005$). This result is contrary to the hypothesis, which predicted lower infection frequencies in the later tick stages, and therefore provides no support for the idea that *H. mariae* induces mortality in infected ticks. A possible explanation for the higher than expected prevalence of infection in adult ticks is that the prevalence of infection detected in the lizards was underestimated because lizards that were very lightly infected were scored as uninfected.

Our second hypothesis was that the ticks may have evolved a mechanism to avoid feeding on infected lizards. If this were the case, infection frequencies would be lower than expected in all tick stages and observed tick loads would be lower on infected lizards than on uninfected lizards. Infection frequencies in nymphs and adult ticks were not lower than expected from the prevalence of infection in the lizard population and known transmission dynamics. Overall, tick loads on infected and uninfected lizards were also not significantly different, and infected lizards were as likely to be tick-infested as uninfected lizards. The only exception was that infected lizards were less likely to carry female ticks. On balance, the evidence did not strongly support the hypothesis that ticks avoid feeding on infected lizards.

Little is known about the natural transmission dynamics of this blood parasite in the field. Transmission of *H. mariae* from the tick to the lizard has been demonstrated in the laboratory through consumption of infected ticks by a lizard (Smallridge and Bull 1999). In the wild, sleepy lizards feed mainly on plant material rather than invertebrates (Brown 1983; Dubas and Bull 1991) and they also do not groom (C.J. Smallridge, personal observation). Although transmission by ingestion of ticks may be very rare, it is hard to determine its frequency from direct observations in the field. Other

Table 1 The total number of lizards found in four categories of nymph load, and the number (and proportion, %) of those in each category that were infected

Nymph load	Number of lizards in each nymph load category	
	Total	Infected lizards (%)
0	138	18 (13.0)
1	18	4 (22.2)
2–10	19	1 (5.3)
>10	6	0 (0.0)

parameters of transmission dynamics can be used to estimate the rate of tick ingestion that may be required to sustain the observed prevalences. In the laboratory, infections in lizard blood can persist for up to 2 years (Smallridge 1998). Thus, an estimated 6.4% of the lizards should become infected annually to sustain a 12.7% prevalence. In the laboratory, 46% of lizards fed with a potentially infective nymph developed a detectable parasitaemia in the blood (Smallridge and Bull 1999). The variation in susceptibility among lizards may be because lizards vary in their resistance to infection, or because the infective stages observed in the tick may vary in their viability. If 14% of nymphs are infected and the transmission efficiency from eating an infected tick is 46%, then for 6.4% of the lizards to become infected, each lizard must ingest on average about one nymph per year. The actual required rate of field tick ingestion may be even lower when the potential contribution of adult ticks is also taken into account. This suggests that even though ticks may not be commonly ingested by lizards, only a low frequency of tick ingestion is needed to sustain *H. mariae* in the prevalence currently found in the lizard population.

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