Parasitol Res (1999) 85: 858–863

ORIGINAL PAPER

Catherine. J. Smallridge · C. Michael Bull

Transmission of the blood parasite *Hemolivia mariae* between its lizard and tick hosts

Received: 12 April 1999 / Accepted: 6 May 1999

Abstract The haemogregarine *Hemolivia mariae* is found in the erythrocytes of a natural population of the lizard *Tiliqua rugosa*. It infects two tick species, *Amblyomma limbatum* and *Aponomma hydrosauri*, which parasitise lizards. In laboratory experiments, engorged *Amb. limbatum* nymphs from infected lizards transmitted the haemogregarine to uninfected lizards significantly more often than engorged *Ap. hydrosauri* nymphs. Dissections of larvae and nymphs of both species fed on the same infected hosts showed that *Amb. limbatum* ticks were significantly more likely to become infected than *Ap. hydrosauri* ticks. In *Amb. limbatum*, oval cysts containing parasite stages thought to be infective to the lizard host had developed within 15 days of engorged nymphs detaching from an infected host. The chance of *Ap. hydrosauri* becoming infected and the intensity of infection in *Amb. limbatum* increased when ticks were fed on infected hosts as larvae and as nymphs compared with those fed on an infected host only as a nymph. This suggests that infections can accumulate over the tick life stages. Since the two tick species have broadly parapatric distributions, the boundary between the tick species may have implications for the distribution of *H. mariae*.

Introduction

Persistence of a parasite species in a natural host population depends on successful transmission between individual hosts. For microparasite species with indirect life cycles involving a vertebrate host and blood-feeding invertebrate hosts, a number of factors may affect the success of transmission. These include the feeding interval and probability of survival of the invertebrate host, the incubation period of the parasite, the probability of the invertebrate host becoming infective after feeding on an infected host (vector competence) (Dye 1992) and the frequency of vectoring events (Thrall et al. 1995). These factors combine to influence the distribution and abundance of the parasite in the host population.

This study examined some of these factors and how they may influence the dynamics of a newly identified haemogregarine parasite *Hemolivia mariae* recorded from natural populations of the Australian skink *Tiliqua rugosa* (Smallridge and Paperna 1997). The haemogregarine has an indirect life cycle involving an invertebrate host tick species, either *Amblyomma limbatum* or *Aponomma hydrosauri*, and a vertebrate host, the sleepy lizard *T. rugosa* (Smallridge 1998). The two tick species have parapatric distributions across eastern South Australia (Bull 1995; Bull et al. 1981; Smyth 1973) so differences in their ability to transmit the haemogregarine may affect the field distribution of the blood parasite in natural populations of its lizard host. *H. mariae* develops in the gut epithelium and lumen of ticks that feed on infected lizards, undergoing a primary (sexual) reproductive phase producing large stellate oocysts (Smallridge and Paperna 1997). Progeny from the oocysts undergo a secondary (asexual) reproductive phase to produce oval cysts containing parasite stages thought to be infective to the vertebrate host (Smallridge and Paperna 1997). The aim of this study was to examine the relative efficiency of each tick species in transmitting the haemogregarine to the lizards, and the probability of each tick species becoming infected with *H. mariae* after feeding on infected lizards. Timing of the primary and secondary reproductive phases for the parasite was also examined in *Amb. limbatum* nymphs fed on infected lizards.

C.J. Smallridge · C.M. Bull
Flinders University of South Australia,
School of Biological Sciences,
GPO Box 2100, Adelaide,
South Australia, 5001, Australia
e-mail: Michael.Bull@flinders.edu.au, Fax: +618 8201 3015

Materials and methods

Animal maintenance

All maintenance, blood sampling and tick attachment procedures were approved by the Flinders University Animal Welfare Committee. Lizards (*T. rugosa*) originated from near Mt Mary, South Australia (33°55′S, 139°15′E) and were maintained at 24 °C in individual glass tanks (30 × 75 × 35 cm) with a 12:12 h photoperiod and a heat source for basking for 8 h each day. They were fed diced vegetables with egg or meat twice a week. Infection with *H. mariae* was detected from mono layer smears of lizard blood, taken either by clipping a toe nail or by using a syringe and aseptic techniques to sample the caudal vein just behind the cloaca. Smears were fixed for 5 min in 100% methanol and stained for 15 min with Giemsa. Approximately 10^4 erythrocytes (100 fields each of about 100 cells) from the smear were examined at ×1000 magnification. Infection intensity was estimated from the number of erythrocytes containing the microparasite.

Laboratory tick stocks also originated from Mt Mary, and were maintained by feeding on uninfected lizards. Unfed ticks attached to hosts when they were confined overnight in calico bags with a lizard. The lizard had its mouth taped shut to prevent incidental consumption of the ticks. A metal grid with holes was placed just above the floor of the tanks of tick-infested lizards. As ticks detached, they fell through the grid and were collected each day from the floor below. Tick stocks were maintained during non-feeding periods in ventilated vials at 85% relative humidity and 25 °C.

Tick to lizard transmission

Four transmission experiments were carried out in spring or summer between 1993 and 1997. In each case, lizards had been maintained tick free for at least 3 months before the experiment. This is longer than the pre patent period for most haemogregarines (Mackerras 1962; Petit et al. 1990, Svahn 1975).

One or two engorged tick nymphs (15–30 mg) were fed to each lizard. In each experiment, some lizards were fed *Amb. limbatum* nymphs while others were fed *Ap. hydrosauri* nymphs. The ticks were usually eaten voluntarily after being dropped in front of a lizard. A few lizards which did not voluntarily eat the ticks were gently force-fed by prising open the jaws and dropping the tick into the mouth. Half of the lizards were fed engorged nymphs that had fed on infected hosts. Those infected host lizards had between 0.01% and 1.0% of their red blood cells infected. Engorged nymphs from uninfected lizards were fed to the other lizards as controls.

In all of the experiments, 13 lizards were fed *Amb. limbatum* ticks from infected lizards, 13 were fed *Amb. limbatum* ticks from uninfected lizards, 11 lizards were fed *Ap. hydrosauri* ticks from infected lizards, and 11 lizards were fed *Ap. hydrosauri* ticks from uninfected lizards. In each group, two lizards were each fed two nymphs. All other lizards were fed one nymph. Blood was sampled weekly from each lizard for up to 12 weeks after the ticks were ingested. In cases where transmission was detected, the observed prepatent period (the time before parasites were detected in the blood) was recorded in weeks.

Lizard to tick transmission

The probability of larval infection

The probability of larvae of each species becoming infected with *H. mariae* was compared by simultaneously feeding larvae of both species on the same infected lizards. Larval ticks were attached to 12 different host lizards in four trials between December 1996 and May 1997. Seven of the 12 lizards were used in two separate trials resulting in a total of 19 cases of tick attachments. All of the lizards had a parasitaemia of at least 0.1% infected red blood cells throughout each trial. In separate trials, larvae of a tick species had hatched from different clutches. One hundred larvae of each species were exposed to each lizard, although not all of the ticks attached. Engorged larvae were collected and stored as they detached 2–4 weeks later.

Three or more weeks after detachment, 3–20 of each tick species from each host were dissected. Similar numbers of each species were dissected each week. The anterior quarter was cut with a scalpel from each tick on a microscope slide and the intestinal diverticulae were pulled into a drop of water with fine forceps. Coverslips were applied to the dissections and the slides were examined using an Olympus CH-2 microscope. *H. mariae* cysts in infected tick dissections could usually be observed under the microscope at ×100 magnification. However, if no infection was detected initially at this magnification, at least 50 fields were examined for cysts at ×400 magnification. No attempt was made to quantify how heavily the ticks were infected. A paired *t*-test was used to compare the arcsine-transformed percentage of infected ticks of each species from each lizard.

Probability of nymphal infection

Experiments were conducted to examine the influence of both the larval and the nymphal host on the level of infection in each tick species after nymphal feeding. In November 1996, 20–40 unfed nymphs of each species, which had previously fed as larvae on uninfected lizards, were attached to each of six infected lizards. Five of those lizards had over 0.1% infected red blood cells, while one lizard had only 0.01% infected red blood cells. In February and May 1997, this experiment was repeated with four infected nymphal host lizards and two other feeding regimes were also tested. Ticks that had been fed as larvae on infected host lizards, were fed as nymphs either on one of three infected hosts, or on one of four uninfected hosts. The infected host lizards used in 1997 each had a parasitaemia of at least 0.1% infected red blood cells, and had not been used in the 1996 trial.

Between 3–13 randomly chosen individual ticks of each species from each nymphal host lizard were dissected 1 month or more after detachment of the engorged nymphs from the host. Those nymphs had usually moulted to adults, and the sex was recorded before the tick was dissected. Spermatid-containing vas deferens of male ticks were removed during dissection before the gut preparations were examined microscopically. Tick infection intensity was recorded as "light" when less than 20 *H. mariae* cysts were found after searching 50 fields at ×400 magnification, "heavy" when hundreds of cysts could be seen in each field at ×100 magnification, and when intact diverticulae of the gut were filled with cysts, and "moderate" for infections between those two extremes.

In analyses comparing percentage infection, percentage data were arcsine transformed and normality of these transformed data was confirmed. To compare proportions of ticks with different levels of infection intensity, contingency chi-squared tests were used, except in cases where cell values were low, where we used *G*-tests with Williams' correction for continuity (G_{adj}) (Krebs 1989).

Infection of Amb. limbatum nymphs over time

Twenty-nine *Amb. limbatum* nymphs which had fed as larvae on uninfected hosts and as nymphs on one of six infected lizards (with parasitaemia between 0.01–0.84%) were dissected either as engorged nymphs (5–20 days after detachment) or as moulted adults (more than 30 days after detachment). All of the nymphs had engorged and detached within 6–20 days of attachment. For each tick dissected, the number of arms of each oocyst was recorded, and the presence or absence of parasite cysts was noted.

Results

Tick to lizard transmission

None of the 24 control lizards that ate nymphs that had engorged only on uninfected lizards became infected. Six

of the 13 lizards (46.2%) that ingested one or more *Amb. limbatum* nymphs that had engorged on infected hosts became infected. The two lizards that ingested two nymphs became infested, but so did four lizards that had ingested only one nymph. Although the experiment did not specifically test the effect of tick age, infective ticks were eaten between 6–12 weeks after first attaching to infected hosts. Infective ticks had fed on hosts with parasitaemia between 0.01% and 1.0% of blood cells infected. The prepatent period was between 4–9 weeks (mean 6.3 weeks).

One of the 11 lizards (9.1%) that ingested *Ap. hydrosauri* nymphs that had engorged on infected hosts became infected. That lizard had eaten one tick, 8 weeks after it first fed on a host with a parasitaemia of 0.02%. The infection appeared 7 weeks after ingestion. The two lizards that ate two nymphs remained uninfected. A chi-squared analysis showed that ingestion of *Amb. limbatum* from infected lizards resulted in significantly more host infections than ingestion of *Ap. hydrosauri* from infected lizards ($\chi^2 = 3.96$, $df = 1$, $P < 0.05$).

Lizard to tick transmission

Observations on the tick dissections indicated a major difference in the colour of the gut contents of engorged ticks of the two species, suggesting that the ticks feed in different ways. *Amb. limbatum* guts were usually dark red, indicative of a meal rich in erythrocyte-derived material, whereas *Ap. hydrosauri* intestinal contents were a pale creamy colour, suggesting a meal derived from other sources of host tissue.

Probability of larval infection

Infections were detected in 124 (77.5%) of 160 *Amb. limbatum* larvae fed on infected hosts, but only 25 (12.3%) of 204 *Ap. hydrosauri* ticks fed on infected hosts (Table 1). The mean rate of infection with *H. mariae* was significantly higher for *Amb. limbatum* than for *Ap. hydrosauri* when larvae engorged on the same host individuals ($t_{18} = 5.90$, $P < 0.001$).

Probability of nymphal infection

There were ten cases where ticks of both species were fed as larvae on uninfected hosts, and as nymphs on infected hosts (Table 2). A paired *t*-test of the arcsine-transformed percentage of infected ticks per lizard from these cases showed that *Amb. limbatum* were more susceptible than *Ap. hydrosauri* to infection by *H. mariae* when fed as nymphs on the same lizards ($t_9 = 23.4$, $P < 0.001$). Similarly, *Amb. limbatum* nymphs were more susceptible than *Ap. hydrosauri* nymphs to infection by *H. mariae* both in the four cases where ticks were fed as larvae on infected hosts and as nymphs on uninfected hosts ($t_3 = 4.75$, $P = 0.018$), and in the three cases where ticks were fed as larvae and as nymphs on infected hosts ($t_2 = 12.2$, $P = 0.007$).

Table 1 Proportion of *Amblyomma limbatum* and *Aponomma hydrosauri* ticks found infected after feeding as larvae on infected lizard hosts (*n* number of ticks dissected)

Experiment	Host	*Amblyomma limbatum*		*Aponomma hydrosauri*	
		n	Percent infected	*n*	Percent infected
December 1996	22	5	100	10	20
	45	5	100	10	0
	57	5	100	10	20
	27	5	100	12	33
	60	5	100	10	20
	16	5	100	14	14
	18	9	100	15	33
	19	8	100	11	18
January 1997	71	18	44	20	5
	72	10	70	20	20
March 1997	27	4	50	8	0
	57	10	90	3	0
	60	10	100	6	17
	16	11	27	10	0
	18	10	80	5	0
	19	10	90	10	0
May 1997	42	10	100	10	0
	28	10	100	10	0
	72	10	100	10	0
		160	78	204	12

The overall percentages of infected and uninfected ticks in each of the three feeding treatments were compared for each tick species with 3×2 *G*-tests of independence. *Amb. limbatum* did not show differences among the three treatments ($G_{adj} = 2.16$, $df = 2$, n.s.), but *Ap. hydrosauri* did ($G_{adj} = 15.3$, $df = 2$, $P < 0.05$). Pairwise comparisons of treatments for *Ap. hydrosauri* showed a significantly lower percent infection when fed on infected hosts only as nymphs than when fed on infected hosts only as larvae ($G_{adj} = 7.60$, $df = 1$, $P < 0.05$) or when fed on both infected larval and infected nymphal hosts ($G_{adj} = 14.57$, $df = 1$, $P < 0.05$). The latter two treatments did not produce significantly different percentages of infected *Ap. hydrosauri* nymphs ($G_{adj} = 1.49$, $df = 1$, $P > 0.05$).

Comparisons between the sexes within each species (Table 3) indicated that infection frequency was not significantly different for male and female *Amb. limbatum* ($\chi^2 = 2.18$, $df = 1$, $P > 0.05$), or for male and female *Ap. hydrosauri* ($\chi^2 = 0.53$, $df = 1$, $P > 0.05$). Infected males of both species dissected 2 or more months after detaching had fully developed vas deferens containing many mature spermatids.

The proportion of ticks found in each of the three categories of infection intensity (light, moderate or heavy) is given in Table 4. A 2×2 contingency χ^2-test showed significantly more heavy than other levels of

Table 2 Proportion of adult *Amb. limbatum* and *Ap. hydrosauri* found infected after feeding on infected lizard hosts either as larvae or as nymphs or as both larva and nymph (*n* number of ticks dissected)

Treatment	Host	*Amblyomma limbatum*		*Aponomma hydrosauri*	
		n	Percent infected	*n*	Percent infected
Larval host uninfected, nymphal host infected	1	8	100	4	0
	2	9	100	4	0
	3	5	100	3	0
	4	4	100	3	0
	5	5	100	4	0
	6	6	100	3	0
	7	6	100	11	9
	8	6	100	10	0
	9	10	100	5	0
	10	10	80	7	0
Group total		69	97	54	2
Larval host infected, nymphal host uninfected	11	10	100	10	10
	12	13	100	10	30
	13	10	100	10	10
	14	10	60	10	20
Group total		43	91	40	13
Both larval and nymphal hosts infected	15	10	100	10	10
	16	10	100	10	40
	17	5	100	10	40
Group total		25	100	30	30
Overall total		137	96	124	14

infection in *Amb. limbatum* than in *Ap. hydrosauri* ($\chi^2 = 8.17$, $df = 1$, $P < 0.005$). In *Amb. limbatum*, a significantly higher proportion of infected ticks had heavy-intensity infections when fed twice on infected hosts than when fed only once on infected hosts ($\chi^2 = 7.04$, $df = 1$, $P < 0.01$). For *Ap. hydrosauri*, the higher incidence of moderate infection in ticks fed on two infected hosts than in ticks fed on only one infected host was not statistically significant ($G_{adj} = 2.10$, $df = 1$, $P > 0.05$).

Table 3 The proportion of adult *Amb. limbatum* and *Ap. hydrosauri* ticks found infected after feeding either as a larva or as a nymph, or as both larva and nymph on infected hosts. The data include only those ticks that had moulted prior to dissection

Tick sex	*Amblyomma limbatum*		*Aponomma hydrosauri*	
	n	Percent infected	*n*	Percent infected
Male	55	96.4	49	8.2
Female	60	93.3	74	17.6

Infection of Amb. limbatum nymphs over time

Star-shaped oocysts were present in all 12 *Amb. limbatum* nymphs dissected from 5 to 10 days after detachment. One of the six nymphs dissected at 15 days appeared to be uninfected. Four of the five remaining nymphs in this group contained oocysts and cysts, while one nymph contained only oocysts. Ten of the 11 nymphs dissected 20 or more days after detachment contained both cysts and oocysts, the remaining nymph, dissected 48 days post-detachment, contained only parasite cysts. Overall, a total of 896 oocysts with two to five arms were counted. Of these, 59.9% had three arms, while 32.4% had four arms.

Table 4 The percentage of infected *Am. limbatum* and *Ap. hydrosauri* ticks in each of three categories of infection intensity (light, moderate and heavy) after feeding as nymphs. Number of infected hosts = 1 for ticks fed either as a larva or as a nymph on an infected host, and 2 for ticks fed both as a larva and as a nymph on infected hosts. Infection intensity is defined in the text

Number of infected hosts	*Amblyomma limbatum*				*Aponomma hydrosauri*			
	n	Percent ticks			*n*	Percent ticks		
		Light	Moderate	Heavy		Light	Moderate	Heavy
1	84	2.4	70.2	27.4	8	87.5	12.5	–
2	25	–	44.0	56.0	9	55.6	44.4	–
Total	109	1.8	44.2	34.0	17	70.6	29.4	–

Discussion

The combined results of these experiments explain aspects of the dynamics of this natural host-parasite interaction between *Hemolivia mariae* and its lizard and tick hosts. The results show that both tick species can transmit the microparasite *H. mariae*. Serial dissections of *Amb. limbatum* nymphs that had engorged on infected hosts showed that stellate parasite oocysts were present from day 5 post-detachment, and parasite cysts were present from day 15 under laboratory conditions. Ticks which had fed on infected lizards were infective if ingested by another lizard within 6–12 weeks. Although this study did not test for the effect of tick age on parasite infectivity in lizards, it is probable that parasite cysts are the infective stage for this parasite species.

The transmission experiments involving *Ap. hydrosauri* resulted in fewer successful transmissions than those involving *Amb. limbatum*. Dissections of ticks that had fed on infected hosts showed infection can develop in both tick species, whether they feed as larvae or as nymphs on infected lizards hosts. There was, however, a significant difference in susceptibility to infection by *H. mariae* between tick species. In every trial, *Ap. hydrosauri* was infected significantly less often than *Amb. limbatum* that fed on the same host lizards. Furthermore, among those ticks that did become infected, infection in *Ap. hydrosauri* individuals was significantly less heavy. This may explain why *Ap. hydrosauri* transmitted the infection to lizards less efficiently than *Amb. limbatum*.

The reason for the differential susceptibility between the two tick species is unknown, but the observed difference in the apparent composition of the meal ingested by each species may play a part. Blood meal constituents are known to vary between tick species (Coons et al. 1986). For most ticks, whole blood is essential for normal development and reproductive activity (Sonenshine 1991). However, some species can feed almost entirely on tissue fluid, leucocytes and lymph or plasma exudates (Kemp et al. 1982). The creamy-coloured meal observed in engorged *Ap. hydrosauri* ticks suggests that this species may be a lymph-feeder, and would thus ingest relatively few of the host red blood cells containing *H. mariae* parasites. Alternatively, *Ap. hydrosauri* may have a more effective internal defence or barrier to prevent *H. mariae* development in the gut. Several insect species, and at least one tick species (Zung et al. 1989), have a peritrophic membrane in the midgut lumen adjacent to epithelial cells, which is thought to inhibit the penetration of some ingested pathogens (Sonenshine 1991). It is not known whether *Ap. hydrosauri* also has this membrane.

For *Amb. limbatum*, there was no significant difference in the percentage of nymphs found to be infected after feeding as a larva or as a nymph on an infected host. Both larvae and nymphs of this species were highly susceptible to infection. For *Ap. hydrosauri*, more nymphs were found infected after feeding as larvae on infected hosts than after feeding as nymphs on infected hosts. This is surprising, since engorged nymphs are at least an order of magnitude heavier than engorged larvae (Chilton 1989), and therefore would have ingested more host tissue and thus potentially more parasites. An explanation may lie in differences in feeding techniques of the different life stages. Tick larvae are thought to take in mostly lysed tissue and a low volume of intact erythrocytes compared with the later life stages (Coons et al. 1986, Sauer and Hair 1972). Larval *Ap. hydrosauri* may ingest lysates, including parasites that have been released from lysed erythrocytes. Those parasites outside intact blood cells may effectively infect ticks even if the blood cells themselves are not ingested. Nymphs feeding mainly on lymph or blood plasma may be less at risk.

In this study, even the relatively small amount of host tissue ingested by larvae was sufficient to initiate a tick infection when the host had a parasitaemia as low as 0.1% infected red blood cells. Feeding on infected hosts at both larval and nymphal stages significantly increased the percentage of ticks that became infected (*Ap. hydrosauri*) or the intensity of infection (*Amb. limbatum*) suggesting a cumulative effect of infection. If a tick became infected at the larval stage, it remained infected until adulthood, even if, in the interim, it fed as a nymph on an uninfected host.

Whatever the mechanism promoting the difference in susceptibility between the two tick species, it is likely to have a profound influence on the distribution of *H. mariae* in natural populations. *Ap. hydrosauri* larvae and nymphs were less likely than *Amb. limbatum* to develop an infection, and less efficient at transmitting the parasite to new lizard hosts. Since these two tick species have broadly parapatric distributions (Bull 1991), we predict a lower prevalence of the microparasite in areas dominated by *Ap. hydrosauri* than in areas dominated by *Amb. limbatum*. The tick species boundary may represent a limit to the distribution of *H. mariae*. The prevalence and intensity of the micro parasite in the field will be affected by other factors important in parasite transmission, such as the relative fitness and survival rate of infected and uninfected ticks and the frequency of vectoring events in the field. Observations in this study from dissections of unfed adult male ticks indicated that both infected and uninfected ticks were able to develop mature spermatids, but apart from this result we have as yet no indication whether *H. mariae* influences the fitness or survival of ticks that it infects. It is not known how frequently lizards find and consume ticks in the field, although lizards willingly eat ticks in the laboratory, and one study has recorded ixodid ticks in the diet of *T. rugosa* (Brown 1983). These areas need further investigation to understand the transmission dynamics of this newly identified haemogregarine species.

Acknowledgements This research was supported by funds from the Australian Research Council and from the School of Biological

Sciences, Flinders University, South Australia. We thank Leah Correll and Cheryl Greaves for care of the lizards in the Flinders University Animal Care Unit, and Prof. I Paperna for providing important insights into the life history of *Hemolivia*.

References

Brown GW (1983) Comparative feeding ecology of south-eastern Australian scincids. PhD thesis La Trobe University, Bundoora

Bull CM (1991) Ecology of parapatric distributions. Annu Rev Ecol Syst 22: 19–36

Bull CM (1995) Population ecology of the sleepy lizard *Tiliqua rugosa*, at Mt Mary, South Australia. Aust J Ecol. 20: 393–402

Bull CM, Sharrad RD, Petney TN (1981) Parapatric boundaries between Australian reptile ticks. Proc Ecol Soc Aust 11: 95–107

Chilton NB (1989) Lifecycle adaptations and their implications in the distribution of two parapatric species of tick. PhD thesis, Flinders University of South Australia, Adelaide

Coons LB, Rosell-Davis R, Tarnowski BI (1986) Bloodmeal digestion in ticks. In: Sauer JR, Hair JA (eds) Morphology, physiology and behavioural biology of ticks. Wiley, Chichester, pp 248–279

Dye C (1992) The analysis of parasite transmission by bloodsucking insects. Annu Rev Entomol 37: 1–19

Kemp DH, Stone BF, Binnington KC (1982) Tick attachment and feeding: role of the mouthparts, feeding apparatus, salivary gland secretions, and the host response. In: Obenchain FD, Galun R (eds) Physiology of ticks. Pergamon, Oxford, pp 119–168

Krebs CJ (1989) Ecological methodology. Harper Collins, New York

Mackerras MJ (1962) The life history of a Hepatozoon (Sporozoa Adeleidea) of varanid lizards in Australia. Aust J Zool 10: 35–44

Petit G, Landau I, Baccam D, Lainson R (1990) Description et cycle biologique d'*Hemolivia stellata* n. g., n. sp., Hémogrégarine de crapauds Brésiliens. Ann Parasit Hum Comp 65: 3–15

Sauer J, Hair J (1972) The quantity of blood ingested by the lone star tick (Acarina: Ixodidae). Ann Entomol Soc Am 65: 1065–1068

Smallridge CJ (1998) Studies on a haemogregarine parasite of the Australian skink, *Tiliqua rugosa*. PhD thesis, Flinders University of South Australia, Adelaide

Smallridge CJ, Paperna I (1997) The tick transmitted haemogregarinid of the Australian sleepy lizard, *Tiliqua rugosa* belongs to the genus *Hemolivia*. Parasite 4: 359–363

Smyth M (1973) The distribution of three species of reptile ticks, *Aponomma hydrosauri* (Denny), *Amblyomma albolimbatum* Neumann, and *Amb. limbatum* Neumann. I. Distribution and hosts. Aust J Zool 21: 91–101

Sonenshine DE (1991) Biology of ticks, vol 1. Oxford University Press, Oxford

Svahn K (1975) Blood parasites of the genus *Karyolysus* (Coccidia, Adeleina) in Scandinavian lizards: description and lifecycle. Norw J Zool 23: 277–295

Thrall PH, Biere A, Uyenoyama MK (1995) Frequency-dependent disease transmission and the dynamics of the *Silene-Ustilago* host-pathogen system. Am Nat 145: 43–62

Zung JL, Lewengrub S, Rudzinska MA, Spielman A, Telford SR, Piesman J (1989) Fine structural evidence for the penetration of the Lyme disease spirochete *Borrelia burgdorferi* though the gut and salivary tissues of *Ixodes dammini*. Can J Zool 67: 1737–1748