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ORIGINAL PAPER

Blood parasites in two co-existing species of lizards (*Zootoca vivipara* and *Lacerta agilis*)

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Abstract We investigated the occurrence of blood parasites of two lizard species: the common or viviparous lizard (*Zootoca vivipara*) and the sand lizard (*Lacerta agilis*) in western Poland. Selected traits of lizard body morphology were studied with respect to the presence and intensity of haematozoan infection in blood samples collected from 218 adult lizards; 88 of the common lizard and 130 of the sand lizard. Haemogregarinid blood parasites were found to be the common parasite of both lizard species in studied locality with prevalence 39.8 (95% CL, 29.5–50.8) for *Z. vivipara* and 22.3 (95% CL, 15.5–30.4) for *L. agilis*. Incidence of parasitemia did not differ between sexes and was not correlated with morphological traits or presence of ectoparasites—*Ixodes ricinus* ticks. However, a significant difference between the two species of lizards was a greater frequency of haemogregarinid parasitism in *Z. vivipara*.

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Introduction

Parasites play an important role in the evolution of host life-history traits because they often impose important selective pressures on them. Parasites remove resources from their hosts that could otherwise be used for host growth, maintenance, or reproduction (Price 1980). The prevalence and the intensity of infection provide basic information about status and the possible impact of parasites in the lizard population in natural conditions. Parasite interactions influence numerous aspects of the ecology of their hosts (Smallridge and Bull 2000). Haemogregarinid blood parasites (Apicomplexa, Adeleorina) are the most common parasites of reptiles with worldwide distribution. This group comprises the families Hepatozoonidae, Karyolysidae, and Haemogregarinidae. Species of *Haemogregarina* parasitize turtles and possibly other reptiles in contact with leeches which transmit these haemoparasites (Telford 2008). *Hepatozoon* species are transmitted by various haematophagous arthropods such as mosquitos, mites, and ticks. *Karyolysus* species are transmitted by mites. The precondition for the successful transmission is ingestion of the vector invertebrate host (Telford 2008). The pathogenicity of haemogregarines for their hosts is poorly known. Low levels of parasitism do not appear to affect health or blood chemistry of the host organism. Blood protozoa may affect reptiles by reducing their ability to transport oxygen (Caudell et al. 2002). However, parasites will inevitably compete for energy and nutrients with the host, which consequently must resolve

trade-offs between the amount of energy invested in the reproductive effort and any immunological battle against parasites (see Møller 1997). Adaptive immune responses against parasites are costly, manifested by, e.g., reduced body condition and a compromised defense against secondary infections by haemoprotid parasites (Olsson et al. 2005). Co-occurrence of different species of parasites in the same host can alter the life-history traits in ways different from effects of a single species.

Host organisms adaptively respond to a parasitism by altering particular life-history traits (Minchella 1985). Reports have documented that haemogregarinid parasites influenced several life-history traits, such as body mass condition (Amo et al. 2004), age-dependant host mortality (Sorci 1996), colour, composition of femoral secretions (Martin et al. 2008), and reduction of tail regeneration after autotomy (Oppliger and Clobert 1997).

In the current study, we investigated simultaneously the relationship among morphology and prevalence and intensity of blood parasite infection in two co-existing lizard species.

Materials and methods

Study species

The sand lizard (*Lacerta agilis*) is a short-legged, rather robust, small- to medium-sized lizard (up to 90 mm snout to vent length (SVL)) from the family Lacertidae. The sand lizard is ground-dwelling and a strongly diurnal species with one of the widest distribution ranges of all reptiles (Bischoff 1984). Sand lizards are largely insectivorous, actively chasing and consuming a range of spiders and insects (Corbett and Tamarind 1979).

The common lizard (*Zootoca vivipara* formerly *Lacerta vivipara*) is a small lacertid (adult SVL 50–70 mm) with allopatric oviparous (egg-laying) and viviparous (live-bearing) populations. It inhabits fragmented habitats such as peat bogs and heath lands. Common lizards are widely distributed throughout Europe and Asia, and their distribution overlaps the polar circle. *Z. vivipara* has the most extensive range of all lacertids, significantly larger than *L. agilis*. They actively forage on invertebrates, especially on insects.

Study area

The 70-km^2 study area was located in southern Wielkopolska near the town Odolanów (51° 34′ N, 17° 40′ E). The mean density, established by 84 transect lines in 200-m lengths randomly covered the study area, was 0.85±1.35/200 m of transect line for the common lizard and 0.31±0.71/200 m for the sand lizard (Ekner et al. 2008). For more details about the study area, see Antczak et al. (2004). Lizards were captured in April of three consecutive years (2006–2008).

Blood collection and parasite detection

Lizards were captured using landing fishnets, by hand or by noosing, where a loop made from fishing nylon was attached to the end of a wooden stick and dangled in front of a lizard, who would be captured upon walking through the loop. Animals were sexed, aged (adult, sub-adult, juvenile), and examined for the presence of ectoparasites, including ticks *Ixodes ricinus*. All ticks were counted on the particular part of the body where they were found, removed manually with forceps, and stored in 70% ethanol for further identification.

Blood samples from each adult lizard specimen were obtained by ventral puncture of the caudal vein with disposable sterile syringes. Blood smears were made and air-dried immediately in the field. Slides were stored and transported to the laboratory in plastic slide boxes. Slides were stained with Giemsa's solution (Sigma) for 30 min and examined with a light microscope at ×200 magnification. Approximately 50 microscopic fields on each smear were examined for the presence of blood parasites. When no parasites were detected by this method, the smear was considered negative. Intensity of infection (parasitemia) was estimated for each individual as the percentage of infected red blood cells found in an estimated number of 10,000 cells at ×1,000 magnification with oil immersion. Parasite scores were blind in the sense that the observer (BH) was given the ring number and no other information about the lizards from which samples were taken.

Measurement of morphometric traits

For each individual, we measured SVL with an accuracy of 0.5 mm, with digital callipers and body mass (± 0.1 g) with a digital scale. After measurements, the lizards were released in the same location they were captured. Collar scales, used for a different examination (Majláthová et al. 2008), were taken from each lizard. This avoided the possibility that the same lizards were considered twice in the analyses.

Statistical analyses

To improve sample size and show a more general pattern, data from three breeding seasons were pooled before analysis. Statistics were performed using SPSS for Windows (http://www.spss.com/), and tests are two-tailed. Confidential

limits (95% CL) were for binary, absence–presence; data were calculated in Excel Macro (http://office.microsoft.com/pl-pl/excel). Data are presented mainly as means SD; however, abundant presence of haemogregarinid blood parasites allowed a rough quantification from microscope counts, with very few lizards falling into the last highest counting categories (Fig. 1). Hence, as in many other studies (see discussion in Votýpka et al. 2003; Olsson et al. 2005), we decided to divide the data into only two groups: infected and non-infected individuals. Because not all data were available for every captured individual, sample size differs slightly between analyses.

Results

Ectoparasites

Two species of ectoparasitic arthropods were found on both lizard species. Developmental stages of *I. ricinus* ticks as well as *Ophionyssus saurarum* mites were found.

Blood parasite prevalence

In the blood smears of 218 examined lizards, the haemogregarinid blood parasites were found in 138 individuals (63.3%, CL=56.5–69.7; Fig. 2). The level of parasite prevalence differs significantly between the two studied species (logistic regression, Wald=27.049, B±SE=1.57±0.30, $P<0.0001$) but not significantly between sexes in both species—compare 95% confidential limits in Table 1.

The mean number of haemogregarinid blood parasite count per 10,000 erythrocytes was 73.8±110.4 for *Z. vivipara* (kurtosis=2.85) and 85.7±115.2 for *L. agilis* (kurtosis=15.79).

Haemogregarinid infection vs. morphometric traits and number of ticks

No significant relationships between incidence of blood parasites and morphometric traits were found in both studied species and sex categories (Table 2).

Moreover, no significant relationship between the *I. ricinus* tick load on lizard and blood parasite occurrence was found in either lizard species (Table 2). Additional logistic regression analysis between the presence of blood parasites and number of ticks (log-transformed+1 before analysis) gave insignificant results as well ($P=0.08$ in sand lizard, and $P=0.67$ in common lizard).

Discussion

In our study, blood from two co-existing lizard species, the viviparous lizard (*Z. vivipara*) and the sand lizard (*L.*

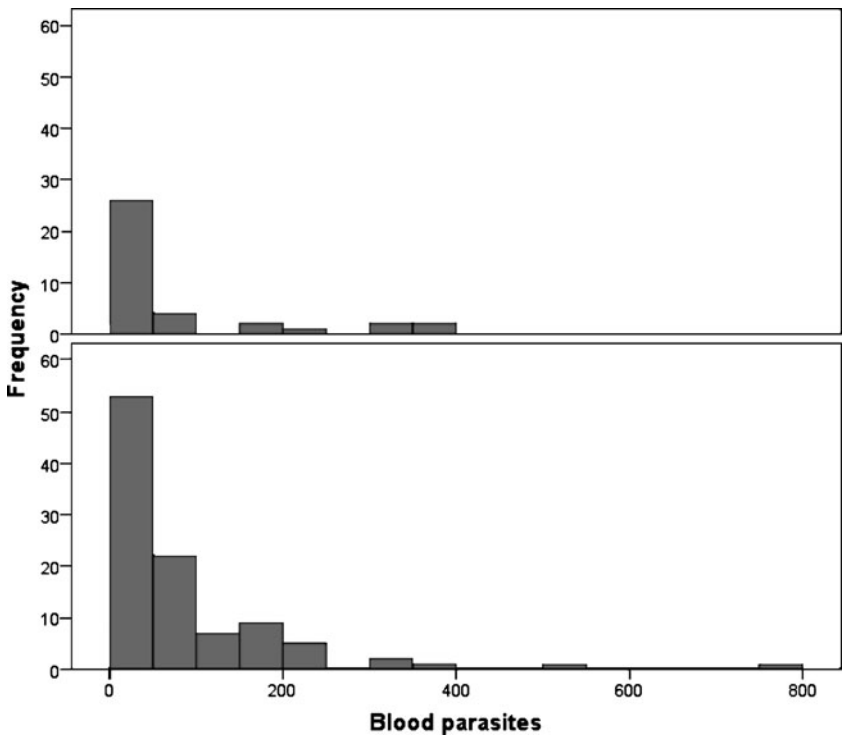

Fig. 1 Distribution of number of blood parasites in two lizard species. *Upper panel—Z. vivipara*; *bottom panel—L. agilis*

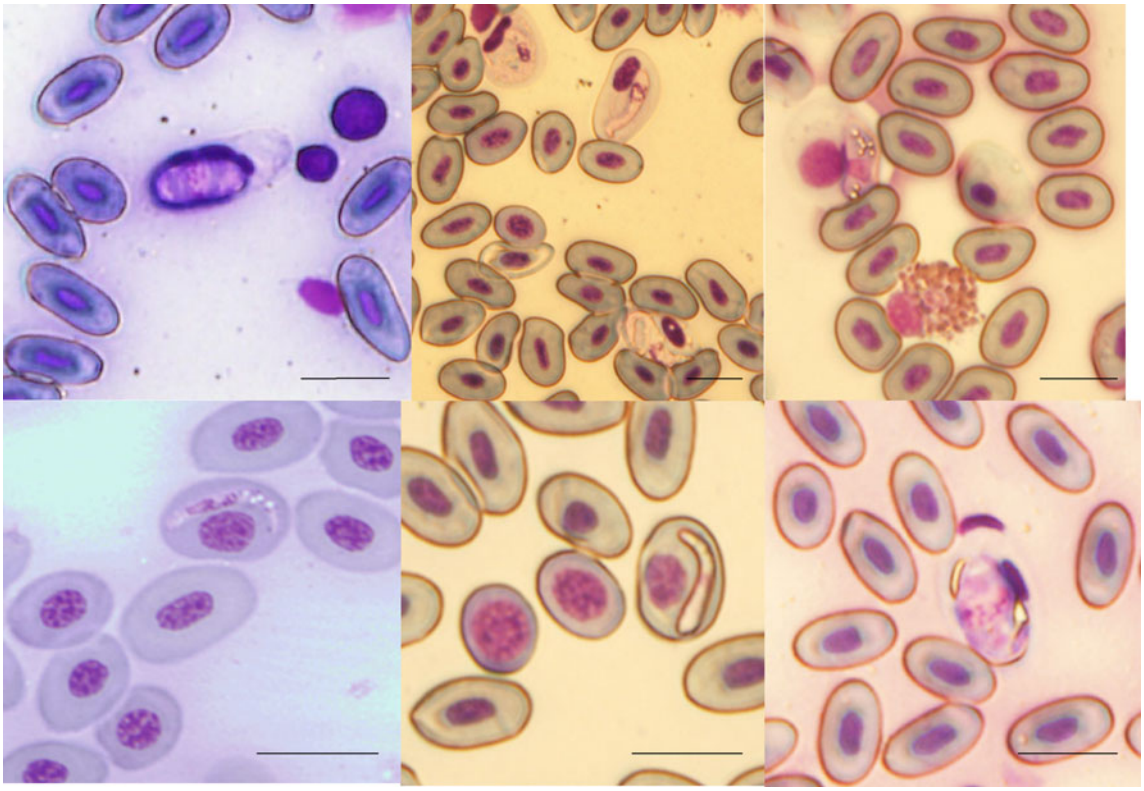

Fig. 2 Various forms of haemogregarinid blood parasites in erythrocytes of sand lizard (*L. agilis*) in Western Poland. Scale bar is indicating 10 μm length

agilis) in Poland was sampled. Observed parasites were termed as haemogregarinids because it is difficult to determine the parasites to species based only on the morphology of gametocytes in erythrocytes. The precise identification is possible only with combination of description of sporogonic stages (Olsen 1974). *O. saurarum* mites, found on lizards in studied locality, are known as vectors for *Karyolysus* sp. (Reichenow 1913). Based on the observed morphology of blood parasites, together with the finding of the *O. saurarum* mites, we assume that at least two different species, probably from the genus *Karyolysus*, were present in erythrocytes of lizards. Differences in prevalence of infection between the two species were found with 39.8% of *Z. vivipara* parasitized compared to 22.3% of *L. agilis*. The prevalence of blood parasites in comparison to other studies carried out all over the world was low. Amo et al. (2004) found almost 80% of *Lacerta monticola* in Spain infected with haemogregarines. The prevalence of blood parasite infections is often relatively stable for long periods (Bennett and Cameron 1974; Eisen 2000; Schall et al. 2000; Smallridge and Bull 2000) and independent of seasonal climatic changes in temperature (Schall 1986). In our studied lizard population, prevalence of

Table 1 Prevalence of blood parasites in two studied lizard species

Lizard species	Females	Males	Total
Z. vivipara			
+	15	20	35
−	31	22	53
% (+)	32.6 (19.5–48.0)	47.6 (32.0–63.6)	39.8 (29.5–50.8)
L. agilis			
+	11	18	29
−	41	60	101
% (+)	21.2 (11.1–34.7)	23.1 (14.3–34.0)	22.3 (15.5–30.4)

"+" denotes parasite presence, "–" denotes parasite absence, and "% (+)" denotes prevalence (with 95% CL) of a given parasite

Table 2 Mean (± SD) morphometric traits of lizards in relation to presence of blood parasites

	Uninfected	Infected	*t* and *P* values
Z. vivipara females			
Body length (mm)	54.8±6.2 (30)	55.0±6.3 (15)	$t=-0.118$; $P=0.91$
Body mass (g)	3.76±0.86 (28)	3.95±1.12 (14)	$t=-0.608$; $P=0.55$
No. of ticks	5.5±5.2 (31)	5.4±6.4 (14)	$t=0.070$; $P=0.94$
Z. vivipara males			
Body length (mm)	48.5±4.8 (20)	51.0±3.4 (22)	$t=-1.951$; $P=0.06$
Body mass (g)	3.04±0.61 (17)	3.61±2.03 (21)	$t=-1.108$; $P=0.27$
No. of ticks	8.3±8.5 (20)	7.2±6.7 (18)	$t=0.432$; $P=0.67$
Lacerta agilis females			
Body length (mm)	76.3±7.0 (11)	75.2±6.1 (41)	$t=0.522$; $P=0.60$
Body mass (g)	11.87±2.87 (11)	11.15±2.76 (38)	$t=0.760$; $P=0.45$
No. of ticks	6.1±5.6 (11)	3.7±5.5 (34)	$t=1.263$; $P=0.21$
L. agilis males			
Body length (mm)	70.2±7.4 (16)	67.3±6.3 (59)	$t=1.581$; $P=0.12$
Body mass (g)	10.29±2.82 (15)	9.28±2.27 (58)	$t=1.465$; $P=0.15$
No. of ticks	8.1±8.3 (15)	7.6±12.6 (46)	$t=0.125$; $P=0.90$

Sample size in parenthesis

t Test value of *t* test

haemogregarinid blood parasites did not change significantly over the period of our study.

Factors that can influence parasite abundance include host sex and age (Schall et al. 2000; Smallridge and Bull 2000), host reproductive effort (Norris et al. 1994; Nordling et al. 1998; Veiga et al. 1998), host condition and physiology (Salvador et al. 1997; Appleby et al. 1999; Dowell 2001), vector biology (Sol et al. 2000; Readon and Norbury 2004), and host density (Arneberg et al. 1998). Infection of *Z. vivipara* was found to be significantly higher than *L. agilis*. Differences between species may be either due to differences in life history, behaviour, immune system, changes in diet, or simply an increased chance of exposure to vector invertebrates in different habitats over time (Eisen and Wright 2001). For example, it was shown previously that high stress conditions such as predation pressure increased the load of haemogregarines in *Z. vivipara* (Oppliger et al. 1998; Martin and Lopez 1999). Our study was conducted in the farmland habitats in Western Poland where *Z. vivipara* is under high predation pressure by great grey shrike (*Lanius excubitor*) here (pers. observation).

Previous studies have demonstrated that the prevalence of blood parasites in lizards is frequently independent of environmental seasonal conditions (Schall 1986; Schall and Marghoob 1995). Geographical patterns in parasite infection prevalence may be more dependent on the specific host–parasite relationship. Parasite prevalence showed no geographical pattern in Australia relative to the parasite load which was higher in lizard populations in the tropics (Salkeld et al. 2008). Our study area was characterised by intensively farmed land that may influence blood parasite prevalence with anthropogenic factors. Insecticides may reduce vector abundance, and habitat fragmentation may impact parasite transmission dynamics (Allan et al. 2003).

Both genders were similarly susceptible to the infection, the prevalence was only slightly higher in males. Several authors similarly reported no intersexual differences in parasite prevalence (Smallridge and Bull 2000; Amo et al. 2005). On the other hand, significant differences were demonstrated between lizard gender, being higher in males which can be explained by the level of immunosuppressive testosterone in males (Belliure et al. 2004). Previous studies have demonstrated that an increase in host reproductive effort decreases parasite defense and, thus, increases parasite load (Gustafsson et al. 1994; Oppliger and Clobert 1997; Oppliger et al. 1998; Fargallo and Merino 2004).

Lack of correlation among blood parasites, tick load, and morphological traits was observed. Resistance to ectoparasites was found to be costly, as manifested by a reduced body condition and a compromised defence against secondary infection by blood parasites (Olsson et al. 2005). It was shown previously that in the population of the Swedish sand lizard, two distinct genotypes with respect to the major histocompatibility complex determined by restriction fragment length polymorphism differed in capacity to combat ticks. Males with genotype "O" were more resistant to ectoparasites but suffered from the higher haemoprotid parasite load (Olsson et al. 2005). However, we found no relationship between blood parasite prevalence and ectoparasite load or with body size. Thus far, *I. ricinus* was not proved to be the vector of any haemoparasites of lizards. Disproportion in results of different studies dealing with the relation of body size and blood parasite load exist.

Among the lizards, *Tiliqua rugosa*, there was a decline in the prevalence of *Haemolivia mariae* infection with increasing lizard size (Smallridge and Bull 2000), while the prevalence of infection was positively correlated with the adult size in *Lacerta lepida* (Amo et al. 2005).

In conclusion, haemogregarinid blood parasites are common parasites of the sand lizard (*L. agilis*) and common lizard (*Z. vivipara*) in habitats in Western Poland. Intensive farming and the high predation pressure in the studied localities may worsen impact of parasitism on the fitness of these protected animals.

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