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SHORT COMMUNICATION

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Prevalence and intensity of blood and intestinal parasites in a field population of a Mediterranean lizard, *Lacerta lepida*

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Abstract We describe the blood and intestinal parasites in the Ocellated lizard, *Lacerta lepida*, examining the factors that determine the prevalence and intensity of infection of haemogregarines, and the prevalence of coccidia and nematodes. In relation to haemogregarines, no juveniles were detected as being infected, whereas 71.7 % of adults were infected. The prevalence of infection was positively related to the size of the adults. There were no differences between seasons or sexes in the prevalence or intensity of infection in adults. There were no significant differences in the prevalence of infection by nematodes between ages or sexes, nor in relation to the size of adult lizards, but adult lizards excreting coccidian oocysts tend to be smaller. During the mating period, reproductive activities lead to a decrease in the body condition. However, neither the intensity of haemogregarine's infection nor the prevalence of intestinal parasites was related to the lizards' body condition.

Introduction

The study of the relations between parasites and their host populations is essential for understanding their role in host's population dynamics and ecology. Although the effect of virulent parasites species on humans and domestic animals is relatively well understood, the effect of less virulent parasites causing subtle but chronic diseases in natural populations, especially in reptiles is however little known. Parasites compete with the host for resources; it has been evidenced that parasites affect host population growth and regulation (Holmes 1995; Krebs 1995; Hudson et al. 1998), spatial distribution (Price 1980; Van Riper et al. 1986), individual reproductive success (Schall 1996; Pacejka et al. 1998), and sexual selection (Hamilton and Zuk 1982; Møller et al. 1999). Prevalence and intensity of a parasitic infection provides a first approach to understand the parasite impact on a natural population (Smallridge and Bull 2000).

Lizards are one of the more prominent groups of animals in Mediterranean climates. The Ocellated lizard (*Lacerta lepida*) is the largest European lizard distributed in the Mediterranean habitats of the Iberian Peninsula, southern France, and northern Italy (Pérez-Mellado 1998), from sea level to high mountains, reaching the highest densities in Mediterranean forests and shrublands (Castilla and Bauwens 1992). Although this especies is strictly protected, its populations are decreasing mainly due to habitat destruction (Pérez-Mellado 1998). Therefore, it is especially important for conservation the knowledge of factors, such as parasites, that can be affecting the maintenance of populations. Anecdotal records have indicated that Ocellated lizards are infected by blood and intestinal parasites (Roca et al. 1986; Pérez-Mellado 1998), but neither the prevalence of a population nor the intensity of infection of individuals have been examined.

We aim to describe the prevalence and intensity of infection of haemo- and endoparasites in a montane population of Ocellated lizards. We also aimed to

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analyse which factors related to the reproductive period or the lizard's characteristics (i.e. age, sex, size, body condition) determined the parasite status of lizards (infected vs. uninfected) and the intensity of infection.

Materials and methods

We performed the study in an extensive montane grassland in the region of Campo Azálvaro (40°40′N, 4°20′W; 1,300 m. a.s.l.; Ávila and Segovia provinces, central Spain, see Fargallo et al. 2001). In this area, *L. lepida* lizards mate in May and produce a single clutch in June (Pérez-Mellado 1998).

Lizards were collected with Sherman traps in two trapping seasons among the mating season, in spring (June) and in summer (July–August). Traps were operated for three consecutive days. Each trapping session consisted of ten trapping plots, each consisting of ten Sherman traps following a line, with traps spaced at 15 m. Traps were baited with a mixture of tuna, flour and oil and with some small pieces of apple, and were set under the cover of herbs to provide camouflage and thermal insulation. Traps were checked twice daily at 14:00 h and 19:00 h. and closed daily at the last revision to avoid the capture of small mammals at night.

Each captured lizard ($n=56$) was individually marked by toe clipping, sexed, weighed, and measured for its snout-vent length (SVL). Lizards were classified as non-matured juveniles (SVL1998). A smear was made on a microscope slide from blood taken from the postorbital sinus by using one 9 μl heparinised hematocrit tube. Lizards were released at the point of capture. Blood smears were air-dried and fixed in the laboratory with absolute ethanol for 10 min and then stained in Giemsa diluted 1:9 with phosphate buffer (pH 7.2) for 40 min before their examination for parasites. On mounted slides, half a smear, chosen at random, was scanned entirely at 200× along the longitudinal of slide, looking for extraerythrocytic protozoa (Merino and Potti 1995). Number of intraerythrocytic parasites was estimated at 400× by counting the number of parasites per 2,000 erythrocytes. The only haemoparasites found were haemogregarines. Because haemogregarina genus can only be characterized by oocyst stages within the invertebrate host (Levine 1982; Barnard and Upton 1994), we could only identify these parasites as haemogregarines.

We also take samples of faeces from 25 lizards captured in summer, to determine the prevalence and intensity infection of intestinal parasites in the lizard population. We analysed faecal samples in order to quantify the excretion of parasite propagules by the classical technique of flotation and counting in MacMaster chamber. Propagules were identified following Melhorn et al. (1992). We found coccidia oocysts and two types of nematode eggs (families Oxyuridae and Ascarididae).

To compare the prevalence of different parasites between lizard ages, we used chi-square tests. We calculated logistic regression by using Generalized Linear Models (GLZM; logit link function, binomial distribution) in STATISTICA Software (StatSoft Inc. 1984–2001, Tulsa, OK, USA) to assess what variables better explain the variation in the infection (presence vs. absence) by haemogregarines, coccidia or nematodes. As potential explanatory variables, we used sex, season (factors) and body size (continuous). Differences in the intensity of haemogregarines infection among adult lizards, in relation to their sex, size and season were analysed by using General Linear Models (GLM), including all the interactions between these three variables.

To examine the variation of body condition of adult lizards in relation to parasites, we used General Linear Models (GLM) in which body mass was included as response variable; sex, season and intensity of infection by haemogregarines as factors; and body size (SVL) as a covariate. We also analysed variation of body condition of adult lizards in relation to the presence or absence of intestinal parasites (coccidia and nematodes) by using GLM, with body mass as the response variable, sex and parasite status as factors and body size as a covariate.

Lizards can autotomize the tail. This could affect the body condition. Therefore, we initially compared the body mass of adult lizards with an analysis of covariance, using SVL as a covariate and taking into account the tail condition (original complete, complete but regenerated, incomplete and in process of regeneration) as a categorical independent factor. There was a significant positive correlation between body mass and SVL

Table 1 Parasitic status (presence vs. absence) and intensity of infection by blood (Haemogregarines) and intestinal (Coccidia and Nematodes) parasites in the Ocellated lizard, *Lacerta lepida* in relation to age (juveniles vs. adults) and sex of adult lizards, and to the season (spring vs. summer) for blood parasites

	Infected vs. non infected	Infection intensity (mean ± SE)
Haemogregarines		
Juveniles	0/10	0
Adults	33/13	12.4 ± 3.3
Adult males	15/10	12.5 ± 5.2
Adult females	18/3	12.3 ± 3.7
Adults in spring	14/4	9.5 ± 3.4
Adults in summer	19/9	14.3 ± 4.9
Males in spring	7/3	6.7 ± 3.2
Males in summer	8/7	16.4 ± 8.4
Females in spring	7/1	13.0 ± 6.5
Females in summer	11/2	11.9 ± 4.7
Coccidia		
Juveniles	2/4	54.1 ± 34.6
Adults	5/14	49.6 ± 26.9
Adult males	3/8	52.3 ± 35.2
Adult females	2/6	45.5 ± 44.5
Nematodes		
Juveniles	2/4	667.8 ± 666.4
Adults	9/10	1504.4 ± 1204.2
Adult males	6/5	2541.8 ± 2061.5
Adult females	3/5	78.0 ± 76.0

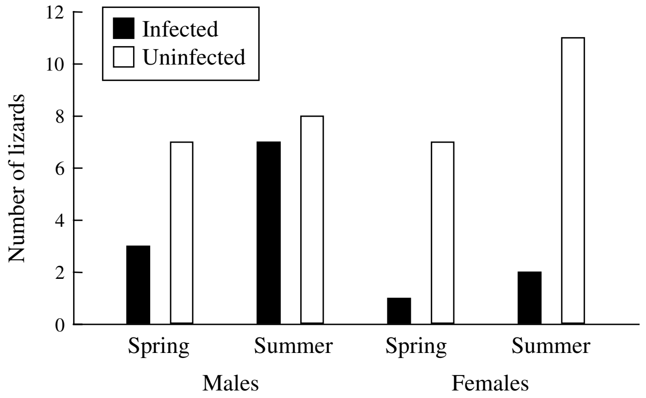

Fig. 1 Number of male and female adult *Lacerta lepida* lizards infected (*black bars*) or uninfected (*open bars*) with Haemogregarines during the mating season (spring) or after the reproductive season (summer)

(GLM, $r = 0.89$, $F_{[2,43]} = 99.71$, $P < 0.0001$), but there were no significant differences in relation to the tail condition ($F_{[2,43]} = 0.52$, $P = 0.60$). Thus, we did not include the tail condition in subsequent analyses.

Results

Overall population data

Thirty-three of 56 lizards (58.9%) were found infected by haemogregarines (protozoa) (see Table 1). In relation to intestinal parasites, we found coccidia (protozoa) and oxyurides (nematoda). The prevalence of coccidia was 28% ($n = 25$) and the prevalence of oxyurides was 40% ($n = 25$) (Table 1). Only one lizard was found infected with Ascarididae (nematoda) eggs.

Parasite infection

There were significant differences in the prevalence of haemogregarines between adults and juveniles ($\chi^2 = 17.47$, $df = 1$, $P < 0.0001$; Table 1). Thus, only adult lizards were detected as being infected by haemogregarines.

The prevalence of haemogregarines did not differ significantly between sexes (GLZM, Wald's $\chi^2 = 0.44$, $df = 1$, $P = 0.51$) (Fig. 1), or seasons (Wald's $\chi^2 = 0.28$, $df = 1$, $P = 0.60$) (Fig. 1). Infected lizards were significantly larger than uninfected ones (Wald's $\chi^2 = 4.23$, $df = 1$, $P = 0.04$). The interactions between sex and season, and between sex and body size were not statistically significant (both $P > 0.3$).

The intensity of infection by haemogregarines in adult lizards did not differ significantly between sexes (GLM, $F_{[1,41]} = 1.27$, $P = 0.27$), or seasons ($F_{[1,41]} = 0.001$, $P = 0.97$), and was not correlated with body size ($F_{[1,41]} = 0.001$, $P = 0.97$). The interaction between season and sex was not significant ($F_{[1,41]} = 0.08$, $P = 0.78$; Table 1).

In the case of coccidia, there was no significant difference in the prevalence of oocyst excretion of juveniles and adults ($\chi^2 = 0.11$, $df = 1$, $P = 0.74$; Table 1). There were not significant differences in the prevalence of coccidia between adult males and females (GLZM, Wald's $\chi^2 = 0.33$, $df = 1$, $P = 0.57$). The prevalence was correlated to the body size of adult lizards (Wald's $\chi^2 = 3.82$, $df = 1$, $P = 0.05$). Infected lizards tended to be smaller.

There were no significant differences in the prevalence of nematodes between adults and juveniles ($\chi^2 = 0.36$, $df = 1$, $P = 0.55$; Table 1), nor between sexes within adults (GLZM, Wald's $\chi^2 = 0.04$, $df = 1$, $P = 0.84$), and was not correlated with body size (Wald's $\chi^2 = 0.42$, $df = 1$, $P = 0.51$).

Body condition

Males showed a higher body condition than females ($F_{[1,42]} = 17.16$, $P = 0.0002$), and all lizards had a significantly lower body condition in summer ($F_{[1,42]} = 7.53$, $P = 0.009$), but the intensity of infection by haemogregarines was not significantly related to body condition ($F_{[1,37]} = 0.00$, $P > 0.99$). Similarly, the presence of coccidia or nematodes was not significantly related to the body condition of lizards (GLM, $F_{[1,16]} = 1.12$, $P = 0.72$; $F_{[1,16]} = 0.74$, $P = 0.40$, respectively).

Discussion

No juvenile lizards were observed infected by haemogregarines. A similar result was found in the skink *Tiquila rugosa* (Smallridge and Bull 2000) and in the alpine lacertid lizard, *Lacerta monticola* (Amo et al. 2004). In addition, the prevalence of infection was positively correlated with the adult size. Since lizard size increases with age, these two results point out to a positive association between haemogregarine prevalence and age. Lizards may acquire mites or ticks (haemogregarine vectors, Paperna et al. 2002) when they share favourable places such as basking spots or refuges, in which mites had detached from a previous occupant. Thus, adults are expected to be more infected because they occupy more frequently these places and interact more often with other adults. On the contrary, juveniles are often relied to suboptimal areas by dominant older male lizards. Therefore, they may be less exposed to vectors, which can explain the different prevalence we found. There were no seasonal differences in the prevalence or intensity of infection in adults. This suggests that infections occur early in the breeding season, when lizards are more active. This consistency, in prevalence and intensity of infection may reflect the stability of the parasite–host interactions in this population. These results are similar to those found in the skink *T. rugosa* (Smallridge and Bull 2000).

An interesting result is that males and females seem to be similarly susceptible to parasite's infection, as the prevalence and intensity of infection were similar in both sexes. Recent studies in lizards (Salvador et al. 1996; Olsson et al. 2000; Klukowski and Nelson 2001; Uller and Olsson 2003) and other organisms (see Zuk 1996) have found that males are more susceptible to parasite's infection probably due to the immune suppressive effects of testosterone at least during the reproductive period (Roberts et al. 2004). Early in the mating period, males maintained high levels of this hormone (Tokarz et al. 1998), thanks to which they are more aggressive, thus, more able to obtain and maintain a territory. Our findings agree with a previous study with the lizard *Psammodromus algirus*, in which there were no differences in the haemogregarine load between males with a testosterone implant and control males (Veiga et al. 1998). In pregnant females, the development of eggs requires a great amount of energy and metabolites, which could not be allocated to defence against parasites. Therefore, both sexes seem to invest more in reproduction than in defence against parasites.

In relation to nematodes, we could not reach the especie level when identifying oocysts in faeces, however, two species of oxyurid have been cited parasitising *Lacerta lepida* in the Iberian peninsula: *Parapharyngodon bulbosus* (Linstow 1899) Teixeira de Freitas 1957; and *Spauligodon extenuatus* (Rudolphi 1819) Skrjabin, Schihobalova et Lagodovskaya, 1960 (Cordero del Campillo et al. 1994). Conversely, no ascarid species has been cited, although an Heterakidae (whose eggs are sligth similar to those of ascarids) has been reported in a Lacertidae species in Madeira Island (Portugal) (Sanchez-Gumiel et al. 1991). There are no previous reports of oocyst faecal excretion in lizards in the Iberian peninsula.

Helminth acquisition appears to be related with the diet of saurian reptiles (Sanchis et al. 2000), for which it would be expected, as our results support, no differences between sexes and age classes. Therefore, even though juveniles feed upon a restricted number of prey types, which were small in size, and adults consume a large number of prey taxa (Castilla et al. 1991), adults and juveniles may have similar diets and food habitat that make them both similarly vulnerable to helminth's infection. On contrast, even though there were no differences in the prevalence of coccidia oocyst excretion between adults and juveniles, adult infected lizards tend to be smaller. Thus, it seems that smaller lizards are more vulnerable to coccide's infection, or that larger lizards have infection intensities under undetectable levels. An alternative hypothesis might be that, since the parasited lizards might allocate resources to fight against infection, they suffered a decrease in their growth rate, and therefore, they showed smaller body sizes. However, we cannot obtain reliable conclusions due to our limited sample and the low level of significance.

The body condition of lizards decreased over the activity season. During the mating period, reproductive activities lead to a decrease in the body condition. However, the intensity of infection by haemogregarines did not affect the body condition of lizards. Therefore, these reproductive costs seem to be more determining to body condition than costs associated to parasite load.

Haemogregarines destroy erythrocytes, which should result in depressed haematocrit levels (Wintrobe 1991; O'Dwyer et al. 2004). Thus, parasited lizards may have reduced haemoglobin concentrations, and reduced capacity for oxygen transportation (Salvador et al. 1996; Oppliger et al. 1996; Veiga et al. 1998). The reduced ability to transport oxygen to the muscle tissue may affect different aspects of lizard's physiology and behaviour with important fitness consequences, such as foraging efficiency or sprint speed (Caudell et al. 2002), which should also affect body condition (Wozniak et al. 1996). However, lizards could compensate this for the loss of their oxygen transport ability by increasing the production of erythrocytes, as it has been observed in American kestrels (*Falco sparverius*) infected with blood parasites (Dawson and Bortolotti 1997). Therefore, additional research should be conducted to examine the physiological mechanisms implicated on the compensation of the deleterious effects of haemogregarine infection. In general, parasites that have evolved with a species will not be overly detrimental to that species (Caudell et al. 2002). Thus, most of these parasites do not produce clinical disease unless the host is highly infected or stressed (Oppliger et al. 1996, 1998; Campbell 1996; Lane and Mader 1996). It has been shown that stress can often magnify the deleterious effects of normally benign parasites (Caudell et al. 2002). For example, the blood parasite, *Plasmodium mexicanum*, contributed to mortality of *Sceloporus occidentalis* lizards under laboratory, and probably stressful conditions, whereas there was no evidence of mortality induction under natural conditions (Eisen 2001).

The lack of effect of infection by haemogregarines and intestinal parasites on body condition suggests the stability of the parasite–host interactions in this lizard population. However, this equilibrium in host–parasite interaction may be broken if any additional factor influence the lizard's condition. For example, an increase in predation pressure, with the subsequent increase in the use of refuges or loss of time to forage has physiological costs, such as a decrease in body mass condition (Martín and López 1999; Perez-Tris et al. 2004). This could imply that lizards with decreased body condition may not be able to allocate resources to parasite defence. For example, *Lacerta vivipara* lizards submitted to high stress conditions due to a low habitat quality have higher levels of haemogregarines than non-stressed individuals (Oppliger et al. 1998). Therefore, if the stability of host–parasite interactions is modified by external factors, such as habitat disturbance or predation pressure, probably the deleterious effects of parasitism may be more evident. This could have important implications related to conservation of lizards.

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