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SHORT COMMUNICATION

Prevalence and intensity of blood apicomplexan infections in reptiles from Romania

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Abstract In order to evaluate prevalence and intensity of apicomplexan hemoparasites in free-ranging reptiles from Romania, blood smears were collected from European pond turtles (*Emys orbicularis*), sand lizards (*Lacerta agilis*), and spur-thighed tortoises (*Testudo graeca*). All three host species were positive for blood parasites, with prevalence of infected individuals between 60.71% and 100% and variable intensity. Similarities and differences with other epidemiological data are discussed.

Introduction

Most of apicomplexan parasites in the blood of poikilothermic vertebrates from Europe have been described over a century ago. In 1850, Chaussat publishes data on parasitic protozoa found in the blood of amphibians. First ever described reptilian hemoapicomplexan in Europe was *Haemogregarina stepanovi* Danilewsky, 1885 in *Emys orbicularis* followed by *Karyolysus lacertae* Danilewsky, 1886 in lacertid lizards and *Hemolivia mauritanica* (Sergent and Sergent 1904) in tortoises of genus *Testudo* (Mihalca 2007). Since their description, these species were recorded all over Europe, and their life-cycle was partly elucidated for some of them. All aforementioned species are heteroxenous parasites and require an invertebrate definitive host to complete the life-cycle, while reptiles are intermediate hosts.

Prevalence and intensity of parasitic infections are considered important parameters in the monitoring of wildlife diseases. Recently, data about prevalence and intensity of helminth infection in reptiles from Romania were published (Mihalca et al. 2007). However, epidemiological data on blood apicomplexan of Romanian reptiles is scarce or old. We aim to describe the prevalence and intensity of infection with blood apicomplexan in three free-ranging reptile species from Romania.

Materials and methods

Between 2002 and 2006, blood smears were collected from three reptile species—European pond turtle (*E. orbicularis*), sand lizard (*Lacerta agilis*), and spur-thighed tortoise (*Testudo graeca*)—and from various geographic locations, according to Table 1. Ventral or dorsal coccygeal veins were used for blood collection in all species, according to the techniques described by Samour et al. (1984). Blood smears were air dried, then fixed in 100% methanol for 1 min, transported to the laboratory in plastic slide boxes, stained using Dia Panoptic (Reagens Kft; Diagon Kft, Hungary) and examined with an Olympus BX41 microscope at ×1,000 oil immersion. Intensity of infection (parasitemia) was estimated for each individual as the percentage of infected red blood cells (RBC) found in an estimated number of 10,000 cells.

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Table 1 Blood samples from examined host species

Population code assigned	Host species	Number of sampled individuals	Year of collection	Location (GPS coordinates)
EO-1	*Emys orbicularis*	8	2002	Drăgăşani (44°39'29"N 24°17'17"E)
EO-2	*Emys orbicularis*	10	2004	Sfântu Gheorghe (44°54'21"N 29°35'51"E)
LA-1	*Lacerta agilis chersonensis*	28	2006	Sfântu Gheorghe (44°54'21"N 29°35'51"E)
TG-1	*Testudo graeca ibera*	45	2006	Greci (45°12'36"N 28°13'65"E)
Total		91		

Results and discussions

Examination of the blood smears revealed the presence of apicomplexan parasites in the RBC of all three examined host species, namely *H. stepanovi* in *E. orbicularis*, *Karyolysus lacerate* in *L. agilis* and *H. mauritanica* in *T. graeca*. Prevalence was found to be maximal for *Haemogregarina* and high for *Hemolivia* and *Karyolysus* (Table 2). Clinical signs were absent in all examined hosts.

Previous reports on epidemiology of *H. stepanovi* infection in *E. orbicularis* in Romania are old (Popovici-Bîznoşanu 1901; Cornelson and Ungureanu 1947) and refer mainly to extinct populations. More recent data (Mihalca et al. 2002, 2004) are focused on infection influence on erythrocytes morphology. Worldwide, most of the epidemiological studies on hemogregarines of turtles reveal high or very high prevalence values. Prevalences of 100% were reported by many authors in various geographical regions: McAllister and King (1980) in *Chrysemys scripta elegans* from USA; Peirce and Adlard (2004) in *Chelodina expansa* from Australia; Siddall and Desser (1992) in *Clemys insculpta* and *Chelydra serpentina* in Canada; Jakes et al. (2001) *Emydura signata* and *Chelodina longicollis* in Australia. Other studies showed also high values for prevalence, even if not maximal (Hahn 1909; Desser and Yekutiel 1986; Siddall and Desser 1992; McAllister et al. 1995; Jakes et al. 2001). Consistency with other studies is also valid for intensity. Most of them report values between 0.05 and 3% infected erythrocytes (McAllister and King 1980; Siddall and Desser 1992; Jakes et al. 2001).

Table 2 Distribution of blood samples in examined host species

Population code assigned	Parasite species	Prevalence	Intensity (mean±SD)
EO-1	*H. stepanovi*	100%	0.08–2.20 (0.75±0.72)
EO-2	*H. stepanovi*	100%	0.20–3.01 (0.87±0.99)
LA-1	*K. lacertae*	60.71%	0.27–4.22 (1.73±1.21)
TG-1	*H. mauritanica*	84.44%	0.04–13.47 (1.60±2.83)

As most of the hemogregarines in turtles are transmitted by leeches, the difference in prevalence and intensity between different host species and populations was hypothesized as being related to leech-feeding behavior. Thus, McAuliffe (1977) suggested that infection rates are lower in turtle species which spend more time basking, while others found no statistical correlations between basking time and prevalence and intensity of infection (Brooks et al. 1990). Another more consistent hypothesis is that prevalence and intensity with blood parasites and leeches is positively correlated with surface of skin available for leeches to feed (Brooks et al. 1990; Siddall and Desser 1992). Nonetheless, leeches are commonly feeding with blood from sinuses of the plastron and carapace bones (Siddall and Gaffney 2004).

Epidemiological data are more heterogenic in the case of blood apicomplexans in free-ranging lizards. No study reports on 100% prevalence, regardless of host or parasite species. In *Lacerta lepida* from Spain, overall prevalence of hemogregarines was 58.9%, with significant statistical differences between adults and juveniles (Amo et al. 2005). Oppliger et al. (1999) found a low prevalence (between 17.8 and 20.0%) of blood parasites in *Gallotia galloti* from Tenerife Island. In France, 30.5% individuals of *Lacerta vivipara* were infected with hemogregarines (Opplinger et al. 1996; Opplinger and Clobert 1997). In *Lacerta monticola* from Spain, prevalence of hemogregarine infection was 77.3%. Intensity ranged between 0 and 2.41% infected erythrocytes (Amo et al. 2004). In all of these studies, no specific or generic identification of blood protozoans is provided, but in some cases, effect on physiology or behavior of host is reported (Opplinger et al. 1996; Opplinger and Clobert 1997; Opplinger et al. 1999). In the population of *L. agilis*, which accounts for this study, host response to parasite infection was not evaluated, but as prevalence and intensity is higher, it might be of interest.

H. mauritanica was first reported in Romania more than 100 years ago (Popovici-Bâznoşanu 1906). This hemoparasite has a similar epidemiological pattern in *T. graeca* and *Testudo marginata* in other countries where the host–parasite associations occur: in Turkey (prevalence 92%, intensity 2.3%), in Greece (prevalence 81%, intensity 1.29%), and in Bulgaria (prevalence 14%, intensity 0.41 (Široký et al. 2005).

This field study expands the knowledge on the distribution and epidemiology of apicomplexan parasites in blood of reptiles.

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