RESEARCH NOTE

PREVALENCE OF *HEMOLIVIA MAURITANICA* (APICOMPLEXA: ADELEINA: HAEMOGREGARINIDAE) IN NATURAL POPULATIONS OF TORTOISES OF THE GENUS *TESTUDO* IN THE EAST MEDITERRANEAN REGION

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Abstract. *Hemolivia mauritanica* (Sergent et Sergent, 1904) infections were found in 14% (n = 14) of *Testudo graeca* Linnaeus, 1758 tortoises in Bulgaria and in 92% (n = 26) of those in Turkey. *Hemolivia mauritanica*-like gametocytes were found in 81% (n = 47) of wild *Testudo marginata* Schoepff, 1792 tortoises in Greece. Parasitaemia intensity (the percentage of infected red blood cells found in approximately 10^4 cells) was 0.03–22.4% in *T. graeca* and 0.06–12.27% in *T. marginata*. On the other hand, all blood samples from 40 Bulgarian, 38 Greek, and 18 Croatian specimens of *Testudo hermanni* Gmelin, 1789 were negative.

Little is known about the occurrence of *Hemolivia mauritanica* (Sergent et Sergent, 1904) all over the wide range of its type host (Brumpt 1938), despite its century-old description from an Algerian specimen of the spur-thighed tortoise *Testudo graeca* Linnaeus, 1758 (Sergent and Sergent 1904). *Hemolivia mauritanica* is a haemogregarine with an indirect life cycle, involving the tortoise *T. graeca* as the intermediate vertebrate host and the ixodid tick *Hyalomma aegyptium* (Linnaeus, 1758) as the definitive invertebrate host. Merogony and cyst formation take place in the parenchymatous organs of the tortoise. The tick becomes infected when it feeds on a haemogregarine-positive tortoise, ingesting erythrocytes with gametocytes inside. *Hemolivia mauritanica* then reproduces within the gut cells of the tick sexually and asexually to produce infective sporocysts containing sporozoites (Sergent and Sergent 1904, Brumpt 1938, Landau and Paperna 1997, Široký et al. 2004). Ingestion of the infected tick is the only route of transmission of *Hemolivia* spp. to their vertebrate hosts known to date (Smallridge and Bull 1999).

Our field studies were intended to expand the knowledge on the distribution and prevalence of *H. mauritanica* in natural populations of *Testudo* Linnaeus, 1758 tortoises. Three species of the genus were sampled in four different areas of the east Mediterranean region. Two field trips (16–26th June 2001, 30th May–14th June 2004) were carried out to Greek localities in the vicinity of Volos, Platamonas, Sparti and Kardamili, to collect blood samples of marginated tortoises, *Testudo marginata* Schoepff, 1792 (47 samples), and Hermann's tortoises, *Testudo hermanni* Gmelin, 1789 (38 samples). A series of samples from *T. hermanni* (40 samples) and *T. graeca* (14 samples) were obtained in an area around Melnik, and between Melnik and the southern slopes of the Pirin Mountains in southern Bulgaria (16–31st July 2002). Furthermore, 18 samples of *T. hermanni* were obtained during May 2003 at Žuljana, Pelješac Peninsula, Croatia. An additional 26 *T. graeca* imported from Anatolia, Turkey, in September 2003, were examined.

Each sampled tortoise was sexed using the usual morphological criteria for *Testudo* spp. (Fritz 2001). Small specimens without expressed secondary sexual traits were considered as juveniles. Tortoises from Turkey were not sexed. Every specimen was individually marked by temporal painting (retaining for 1–2 months) on its carapax to prevent repeated recording. Blood was collected from the dorsal coccygeal vein. The resulting blood smears were air dried and then fixed in absolute methanol for five minutes. The smears were stained later with Giemsa (diluted 1:10 in buffered water, pH 7) for 20 min and examined under an Olympus CX 40 microscope at 1,000× magnification. The intensity of parasitaemia was estimated for each infected tortoise as the percentage of infected red blood cells found in approximately 10^4 cells. Mean, standard deviation (SD), and range of parasitaemia intensity (calculated in *Hemolivia*-positive specimens), as well as parasite prevalence (the percentage of infected tortoises) were counted for every sampling area, each species and sex separately.

As it is difficult to determine haemogregarine species based solely on the gametocytes in vertebrate erythrocytes (Desser 1993), engorged *H. aegyptium* female ticks were collected from haemogregarine-positive specimens of each tortoise species to confirm conspecificity of isolates with *H. mauritanica*. The ticks were dissected in laboratory by removing the mouthparts with fine ophthalmological scissors and a smear of the gut contents was prepared on a microscope slide by pressing the tick body. This smear was examined unstained for the presence and morphology of *Hemolivia* sp. developmental stages.

We found *H. mauritanica* gametocytes in 14% (n = 14) of Bulgarian specimens of *T. graeca* and 92% (n = 26) of those from Turkey. Moreover, gametocytes, morphologically indistinguishable from those of *H. mauritanica*, were found in 81% (n = 47) of blood smears collected from marginated tortoises *T. marginata* in Greece. On the other hand, 40 examined Bulgarian, 38 Greek and 18 Croatian specimens of *T. hermanni* were negative. Results of these examinations of wild tortoises are given in Table 1.

We found no substantial difference in the prevalence of *H. mauritanica* between males, females, and juveniles of *T. marginata* from Greece (X^2-test, P>0.05). The relatively small Bulgarian sample of *T. graeca* was composed of only six adults. Four of the eight juveniles were hatchlings or yearlings that were all negative.

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Table 1. Occurrence of *Hemolivia mauritanica* in *Testudo* tortoises from the east Mediterranean region.

Host species (Country)	Host category	Number examined	Number infected	Prevalence (%)	Parasitaemia (%): mean; SD; range
T. graeca (Bulgaria)	male	3	0	0	0; – ; –
	female	3	1	33	0.66; – ; –
	juvenile	8	1	13	0.16; – ; –
	total	14	2	14	0.41; 0.354; 0.16–0.66
T. graeca (Turkey)	total	26	24	92	2.3; 5.298; 0.03–22.4
T. marginata (Greece)	male	23	19	83	1.25; 1.867; 0.06–7.93
	female	20	16	80	1.47; 3.166; 0.07–12.27
	juvenile	4	3	75	0.6; 0.18; 0.46–0.8
	total	47	38	81	1.29; 2.411; 0.06–12.27
T. hermanni (Bulgaria, Greece, Croatia)	total	96	0	0	–

The size and overall morphology of intraerythrocytic gametocytes did not differ among the examined *T. marginata* and *T. graeca* tortoises. They were oval or cylindrical, 12.5 (10–14) × 5 (4–7) µm (n = 30). They had a stain-resistant capsule and nucleus located in a polar position. No evident differences were recorded between sporocysts obtained from dissected engorged *H. aegyptium* ticks collected from either *T. graeca* or *T. marginata*. Sporocysts were elongately ellipsoidal, 31.5 (28–35) × 16.4 (12.5–21) µm (n = 30), containing numerous banana-shaped sporozoites.

These data on the morphology of gametocytes from *T. graeca* and *T. marginata* fit well those given for *Hemolivia mauritanica* by Michel (1973). The conspecificity of haemogregarines from both tortoise species with *H. mauritanica* is further supported by the identical morphology of sporocysts and sporozoites from *H. aegyptium* ticks collected from *T. graeca* and *T. marginata*. However, further identification of these haemogregarines, employing molecular genetic methods, and experimental transmission between tortoise-host species, would be helpful to decisively confirm their presumed conspecificity and to describe any possible intraspecific variability. It is notable that recent populations of *T. marginata* and *T. graeca* live parapatrically (Fritz 2001, Hailey and Willemsen 2003), thus their direct contact in the wild is not possible, also making the studied populations of *H. mauritanica* parapatric.

Our field data suggest a high prevalence of *H. mauritanica*, at least in some natural populations of tortoises, upon comparison with data from Smallridge and Bull (2000) on the prevalence of *Hemolivia mariae* Smallridge et Paperna, 1997 in field populations of the Australian sleepy lizard *Tiliqua rugosa* (Gray, 1825). This fact could be explained by the long-term persistence of parasitaemia by *H. mauritanica* in naturally and experimentally infected tortoises (Široký et al. 2004).

On the other hand, we found no haemogregarine-positive specimens of *T. hermanni* (n = 96), in spite of the fact that all examined *T. hermanni* from Bulgaria and Greece (n = 78) originated from populations occurring syntopically with *Hemolivia*-positive populations of *T. graeca* or *T. marginata*, respectively. This finding suggests that *T. hermanni* does not represent a natural host of *H. mauritanica*.

Our results suggest a wide geographic distribution of *H. mauritanica*, described originally from Algeria, within the range of tortoises of the genus *Testudo*, considered for decades to contain five, partially polytypic species (Loveridge and Williams 1957, Fritz and Cheylan 2001). Recently, numerous additional *Testudo* species have been described or revived, resulting in an extreme polytypy of the genus (Perälä 2002). This new result offers —together with the large geographic range and high host specificity of *Hyalomma aegyptium* that lives predominantly on tortoises— a unique model for the complex co-evolution of a host, vector and transmitted pathogen.

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