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Case Report

Blood parasites in European legless lizards (*Pseudopus apodus*) from north of Iran

Hossein Javanbakht *, Hamidhossein Khezri

Department of Biology, Faculty of Science, University of Guilan, Rasht, Iran

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ABSTRACT

Parasites are a very diverse group of organisms that play a vital role in ecosystems. The *Apicomplexa* phylum is a large group of obligate intracellular single-celled parasites found in invertebrates and vertebrates. They can infect a wide range of animals from fish to mammals. The present study investigated the *Apicomplexan* blood parasite in legless lizards, *Pseudopus apodus*, from the north of Iran. Blood smears of caudal venous blood from 14 legless lizards were collected, prepared by fixation with methanol, and stained with Giemsa for 15 minutes. Morphological examination of the prepared smears by microscope confirmed the presence of species of *hemogregarine* blood parasites belonging to *Hepatozoon ophisauri*. This parasite is commonly placed in the concave part of the red blood cell in gametocyte form. Hemoparasites infected 14.2% of lizards. The mean intensity of hemoparasites was 0.084% in each infected individual. In this study, the infection and intensity of hemogregarine parasites were reported from the legless lizard. The intensity of blood parasites compared with Palearctic lacertid lizards was very low. A molecular study with more specimens alongside vectors is needed to identify all *hemogregarine* parasites in these lizards.

انگل های خونی در مارمولک بی دست و پا (*Pseudopus apodus*) از شمال ایران

حسین جوان بخت *، حمیدحسین خضری

گروه زیست شناسی، دانشکده علوم ، دانشگاه گیلان، رشت، ایران

چکیده

انگل ها گروههای متنوعی از ارگانیسم ها هستند که نقش مهمی در اکوسیستم ها بازی می کنند. شاخه *آپی کمپلکسا* گروهی بزرگ از انگل های تک سلولی اجباری داخل سلولی هستند که در مهره داران و بی مهره گان یافت می شوند. آنها می توانند طیف وسیعی از جانوران از ماهیان تا پستانداران را آلوده کنند. در این مطالعه انگل های خونی *آپی کمپلکسا* در مارمولک بدون دست و پای ***Pseudopus apodus*** , در شمال ایران مورد بررسی قرار گرفت. اسمیرهای خونی از سیاهرگ دمی ۱۴ مارمولک بی دست و پا جمع آوری شد. به مدت ۱۵ دقیقه جهت رنگ آمیزی در گیمسا قرار گرفت. بررسی مورفولوژیکی اسمیرهای تهیه شده بوسیله میکروسکوب، حضور انگل های خونی *هموگری گارینی* متعلق به گونه ***Hepatozoon ophisauri*** را مورد تایید قرار داد. این انگل ها عموما در گوشه کاو رو به داخل گلبول قرمز بصورت گامتوسیت حضور داشتند. انگل های خونی ۱۴/۲ درصد از مارمولک ها را آلوده کرده بودند. میانگین شدت انگلی ۰/۰۸۴ درصد در هر فرد آلوده بود. در این مطالعه آلودگی و شدت انگل های خونی هموگریگارینی از مارمولک های بدون دست و پا گزارش شد. شدت انگل های خونی در مقایسه با مارمولک های پالئارکتیک بسیار پایین بود. داده های مولکولی با نمونه های بیشتر در کنار ناقل ها برای شناسایی همه انگل های هموگری گارینی در این مارمولک ها الزامی است.

واژه های کلیدی: *هموگری گارین*، ***Hepatozoon, Karyolysus***، خزنده، انگل خونی

* Corresponding author: h.javanbakht@guilan.ac.ir

INTRODUCTION

Reptiles globally are exposed to several intercellular and intracellular apicomplexan blood parasites in their terrestrial habitats. Species of *Hepatozoon* and *Karyolysus* are frequently reported from reptiles, which serve as intermediate hosts, and a variety of hematophagous vectors, including mosquitoes, ticks, mites, and flies, serve as definitive hosts of the parasites [1]. *Hepatozoon* and *Karyolysus* species are he*mogregarines*, an informal grouping of three families of the order *Adeleorina* that infect the red blood cells of their vertebrate hosts. They share many morphological and life cycle traits. *Hemogregarines* are heteroxenous parasites, and presently three groups of these families *are* described, namely the *Hemogregarinidae Léger*, 1911, *Hepatozoidae Wenyon*, 1926, and *Karyolysidae Wenyon*, 1926 [2]. Six genera of blood parasites within these families are differentiated on the sporogonic development in their invertebrate hosts [2, 3]. In spite of numerous records and the wide distribution of blood parasites of reptiles in the world, there is not enough information about blood parasites of reptiles in Iran. While numerous data on the biology, zoogeography, and evolution of reptiles have been published in recent years, there are few data on blood parasites in reptiles [4, 5]. Thus, our objective was to carry out a preliminary study of the blood parasites of the European legless lizard, *Pseudopus apodus*, in the north of Iran. *P. apodus* inhabits open country, such as short grassland or sparsely wooded hills. The distribution area of *P. apodus* includes the Balkan, Crimean peninsulas, and Ciscaucasia region in Europe, and Asia Minor, and the Middle East [6]. In Iran, *P. apodus is* reported from all borders of the northern country, including Damghan, Salehabad of Torbat-e-Jam, Khorasan Razavi province, North Khorasan, Semnan, Golestan, Mazandaran, Gilan, Ardabil, West Azarbaijan, East Azarbaijan, Lorestan, and Tehran Provinces [7].

CASE PRESENTATION

Fourteen samples of *P. apodus* were examined from around Rasht [N 49°34′, E 37°21′] in the north of Iran. The lizards were found in grassland and were captured by hand and net from April to September 2021. No animal was sacrificed specifically for the purposes of this study. Blood was taken from the caudal vein, and smears were prepared. Smears *were* dried in the air and were fixed in absolute methanol for 2 minutes and stained with Giemsa for 15 minutes. The blood smears were examined with a light microscope, 1000X magnification, for the diagnosis of hemoparasites. Parasites were identified by Telford [2]. The infection of hemoparasites was estimated as the percentage of infected lizards [4]. The intensity of hemoparasites was estimated for each infected host as the percentage of infected red blood cells found in approximately 104 red blood cells (RBC). All photographs of parasites were taken using TSVIEW software (version 6.2.4.5). Examination of the blood smears in *P. apodus* revealed the presence of species of hemogregarine parasites in the blood cells. These parasites are commonly placed in the concave part of the red blood cell nucleus in gametocyte form. According to the morphological characteristics of hemoparasites, we identified them *as belonging* to the species of *Hepatozoon ophisauri* (Figure 1a-d). The measures were: size of gamonts: 15.34±0.65, 4.21±0.53 (14.35–16.25× 3.20-4.82 µm), LW 45.92–78.32 µm, L/W 4.48–3.37 (n=10). Notable effects on the host cell nucleus and an observable heavily vacuolated area on two sides of parasites were observed (Figure 1 b-c). The cytoplasm of parasites was stained whitish-blue, and no visible nucleus was observed. The

infection of lizards was estimated at 14.2% (2 of 14 individuals). The intensity of haemoparasites was 0.084%.

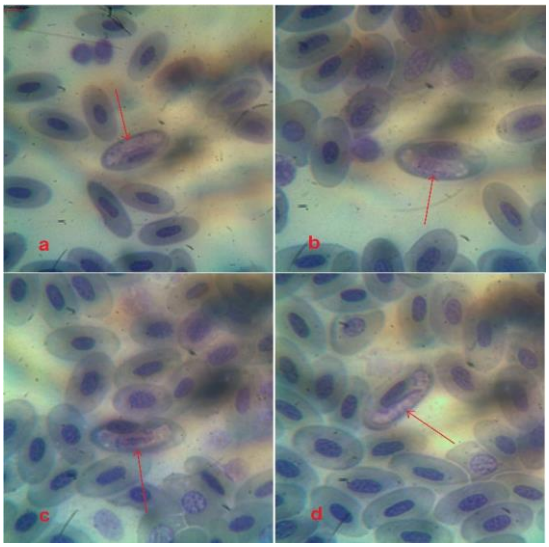

Figure 1: *Hepatozoon ophisauri* in red blood cells (arrow) of *Pseudopus apodus*

DISCUSSION

Using an integrative approach, including morphological characters, geographical features, and host specificity, we reported *Hepatozoon ophisauri* from common legless lizards in northern Iran. Identifying *hemogregarine* parasites by an integrative approach has been advised by many authors [8, 10] because it has been shown that some species of haemogregarines may have similar gametocytes in their different life cycles [11]. On the other hand, mature and immature gametocytes and micro or macro gametocytes may be misidentified as different species [9]. Thus, other traits, such as the life cycle in different hosts, the developmental stage in vertebrates and invertebrates, and the biogeography and ecology of the vertebrate hosts, are necessary to identify these blood parasites. However, it has been shown that European legless lizards are the only host of *Hepatozoon ophisauri* [12]. In recent decades, biological species differentiation and phylogenetic studies have been broadly used to better identify haemogregarine species [13].

Mites and ticks are considered the vectors of most *hemogregarine* parasites in lizards. They transmit the parasites by sharing favorable places such as basking spots or refuges. However, a wide range of arthropod vectors (from mosquitoes to ticks) have been reported to transmit *Hepatozoon* in lizards [2]. These arthropods can transfer the parasites both by biting and even by being used as food by the host. A study on P. apodus in Turkey revealed a range of arthropods belonging to the orders *Coleoptera*, *Orthoptera*, *Hemiptera*, *Gastropoda*, and *Isopoda* (5.6%) are the most common prey groups in these lizards [14]. However, no mites or ticks have been found in the food habitat of *P. apodus*. It is possible that ticks or mites are occasionally digested [2]. According to Telford [2], in experimental environments, *Palearctic Karyolysus* species completed their life cycles in Ophionyssus mites. Different experimental infections have proved that infection of the lizard can only take place by ingestion of nymphs or mite parasite generations [15]. It seems transmission of a

blood parasite by the bite of an infected tick is much more efficient than ingestion of mite nymphs. In support of this theory, ticks stuck to lizards are much more abundant in nature than molesting mite nymphs [16]. In the present study, we reported the percentage of infection and intensity of *hemogregarine* parasites in P. apodus from Iran. Hemoparasites infected 14.2% of lizards. To the author's knowledge, there are not many studies about the prevalence and intensity of haemoparasites in anguid lizards. Only two studies were done by Aliyev et al [17] and Zechmeisterova et al. [12], who reported P. apodus as infected by *Hemogregarina ophisauri* using a limited number of samples. However, a study of haemoparasites in Darveskia cholorogaster in the north of Iran showed 18% of lizards were infected by haemoparasites [18]. It was quite low in comparison with the prevalence of 77.3% blood parasites on the Iberian rock lizard, *Lacerta monticola*, and 96% infection on European green lizards (*L. viridis*) [19, 20]. Previous reports on the epidemiology of *hemogregarine* parasites in three species of snakes in the north of Iran revealed the same prevalence values. A prevalence of 16.66% in *Natrix natrix* and *N. tessellata* was reported in the same geographical regions [5]. Nevertheless, a prevalence of 66.66% was recorded in the *Aesculapian snake* (*Zamenis longissimus*) [5]. These differences can be a result of differences in the mobility of species that lead to an increase in exposure to parasites of infected conspecifics [21, 22]. In the present study, the intensity of hemoparasites was 0.084% of red blood cells. Noghnchi and Javanbakht [18] showed the intensity of *hemogregarines* was 0.01-0.1% per infected individual in D. cholorogaster. Nevertheless, the intensity of *hemogregarine* parasites in *Lacerta monticola* ranged from zero to 2.41% infected erythrocytes [19]. According to Hassl [23], microscopy estimation of *hemogregarine* parasites may be an underestimation of the real diversity of this group of parasites. A study on lacertid lizards from middle Europe, including *Lacerta agilis*, *L. viridis*, *Podarcis muralis*, *P. tauricus*, *P. siculus*, and *Zootoca vivipara* revealed all four *hemogregarine* species were members of the genus *Karyolysus* [16]. Also, only ticks but no mite nymphs were found in the parasite lizards. Therefore, it is possible to hypothesize that more than 90% of infected lizards with *Karyolysus* were found to be transferred with ticks [23]. In a similar study by Haklova-Kocikova et al. [24], *K. latus* was detected as the only *hemogregarine* species in five species of lizards (*L. agilis*, *L. viridis*, *P. muralis*, *Z. vivipara*, and *L. trilineata ssp.*) from six European countries. However, in contrast to previous studies, *Ophionyssus* mites were found as vectors of *Karyolysus* parasites to these lizards. Their results confirmed mites and ticks as hosts of *Karyolysus* and *Hepatozoon* respectively. They introduced temperature and altitude as two key factors for the variable occurrence of blood parasites. Also, a study on the life cycle between *Karyolysus*, *Hepatozoon*, and *Hemolivia* showed transovarial transmission occurs in *Karyolysus*, while this type of transmission was not described in *Hepatozoon* or *Hemolivia*. However, in the present study, we could not find any mites and ticks on the surface of *P. apodus*. In conclusion, this study revealed the morphological data as well as the percentage of infection and intensity of *Hepatozoon* parasites infecting *P. apodus* from Iran. The simple fact is that we know very little about the blood parasites in reptiles of Iran. Molecular studies and DNA sequencing of mitochondrial genes, comprising more specimens and studying vectors, are recommended to investigate hemoparasite species infecting this lizard in subsequent studies.

ETHICS

Approved.

CONFLICT OF INTEREST

None.

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