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A preliminary study of diversity, prevalence and mean intensity of haemoparasites in green bellied lizards (*Darevskia chlorogaster*) from north of Iran

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Abstract

Reptiles are exposed to inter and intracellular apicomplexan blood parasites mainly haemogregarines and haemosporidians, as well as inter cellular kinetoplastida and microfilarians. In the present study diversity, prevalence and intensity of haemoparasites in 50 specimens of Darevskia chlorogaster from north of Iran were investigated. The specimens were captured, blood collected, thin blood smears prepared, stained with Giemsa, and screened with light microscope. According to the morphological characteristics 4 group of apicomplexan blood parasites were detected which belong to haemogregarines and haemosporidians. The prevalence and intensity of apicomplexan blood parasites were consistent with infection of European lacertid lizards. Moreover, we detected one species of trypanosome in red blood cells. To our knowledge this is the first report of trypanosome in Darevskia genus. Molecular methods are needed to identify of apicomplexan parasites as well as trypanosome species due to complexity in life cycle, flexibility in shapes of parasites and few useful characters to differentiate between species.

Keywords: Haemogregarine, Haemosporidia, Lizard, Parasite, Trypanosome.

Introduction

Reptiles globally are exposed to intracellular apicomplexan blood parasites mainly haemogregarines and haemosporidians as well as inter cellular kinetoplastida and microfilariae[1]. Haemogregarines parasites belonging to the suborder Adeleorina are common, widely distributed in reptiles. Haemogregarines are heteroxenous parasites that merogony and the formation of gametocytes occure in vertebrate intermediate host, while gamogony and sporogony within the digestive system of an invertebrate definitive host[2]. Ticks, mites, leeches, and mosquitoes are known to transmit these parasites to vertebrate host. The transmition take place by ingestion of an infected mite or a tick by lizard, or by the biting of an infected arthropod or leech[1]. Lizards from the family Lacertidae especially infected by the genera *Hepatozoon* and *Karyolysus*[3]. Among these parasites *Karyolysus* frequently reported from palearctic lizards[4].

Haemosporidians also are obligatory heteroxenous parasites that have been described from a broad range of vertebrate host (mammals, birds and reptiles) and vectors worldwide[5]. The haemosporidian parasites are transmitted to vertebrates during blood feeding by dipteran vectors. Mosquitoes of the family Culicidae (Genera: *Culex*, *Aedes*, *Anopheles)* are the definitive hosts, in that in this host occurs the sexual part of the parasitic life cycle[5]. *Haemocystidium* and *Plasmodium* are two main and diverse group of haemosporidains that are recognized from lizards[5]. According morphological and morphometric blood features, over than 250 species of these parasites have been described in lizards, while little information is known about their harm to their hosts[6].

The *Trypanosoma* genus from the family Trypanosomatidae and the order of Kinetoplastida have been reported as inter cellular haemoparasites infecting the blood circulatory system of all vertebrates classes[7]. In their life cycle asexual reproduction occure by multiple and binary fission in the gut and salivary glands of the invertebrate host. The potential vector may include haematophagous dipteran or leeches. In addition the direct transmission between different species of *Trypanosoma* have been reported through cannibalism or coprophagy in *T. cruzi* and *T. equiperdum*[8]. Among reptilian, more species of *Trypanosoma* have been reported from lizards than in snakes, chelonians or crocodilians. More species of trypanosomes was found in African and Asian lizards[1]. The shapes of this haemoparasites are influenced by exogenous factor such as temperature, environment pollution, maturity of host and hosts population density[9]. Trypanosomes are known agents of disease in humans, domestic animals and wildlife particularly in the tropics[7].

The prevalence and intensity of blood parasite have been used as a main parameter in considering of wildlife diseases. Because it was known parasitism can be reason of deleterious effect on several aspect of ecology and evolution of host diseases[3]. There are some evidence points that in lizards haemogregarines and haemosporidians can destroy nucleus of erythrocytes and can lead to reduced in hemoglobin and oxygen transportation capacity[10]. Alongside this, some species of haemosporidians can be causing haemolytic anaemia, livestock and malaria disease[6]. Some species of trypanosomes are cause of disease and it was known lizards could be a potential vector of some trypanosomes species such as *T. cruzi*[11]. In recent decade identified vector borne disease can be spread and establishment on endemic populations by climate change, expanding civilization and increasing international trade[12]. Thus our knowledge of the haemoparasites of reptiles is important due to habitat degradation, extinction and decries of population and sensitive to environmental variations[12]. Most records of blood parasites have been reported from erythrocytes of European lizrads. However haemoparasites of reptiles are largely understudied and little is known of the prevalence and intensity of these parasites especially in Iran[13]. In the present study we examined the diversity, prevalence and intensity of haemoparasites in *Darevskia chlorogaster* from north of Iran.

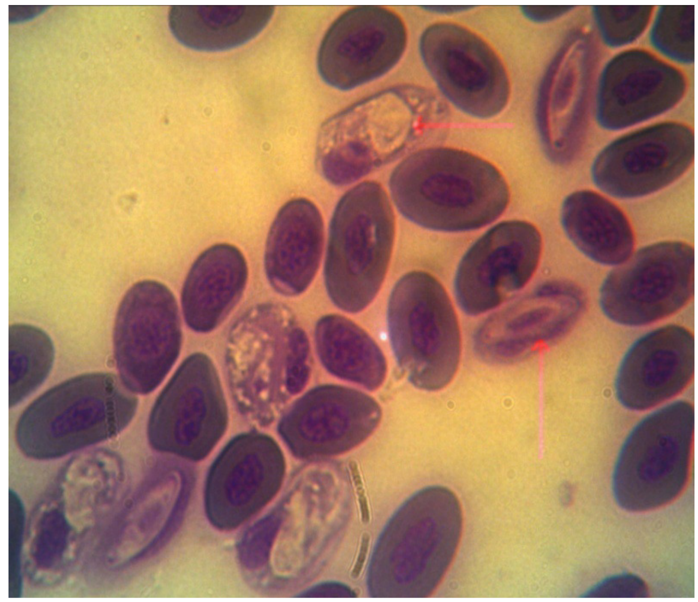
Materials and methods

Fifty samples of *D. chlorogaster* were sampled from Rasht (N 49°33′, E 37°17′) in north of Iran. The lizards were captured by hand and net during April to July 2018. Sex was determined by the presence or absence of hemipenis. No animal was sacrificed specifically for the purposes of this study. Blood was taken from caudal vein and smears were prepared. Smears were fixed in absolute methanol for 3 minutes and stained with Giemsa for 15 minutes. The blood smears were examined with a light microscope, 1,000X magnifcation, for the diagnosis of haemoparasites. Parasites was identified by Telford[1]. The prevalence of haemoparasites was estimated as the percentage of infected lizards. The intensity of haemoparasites was estimated for each infected host as the percentage of infected red blood cells found in approximately 10^4 red blood cells (RBC). All photographs of parasites were taken using TSVIEW software (version 6.2.4.5).

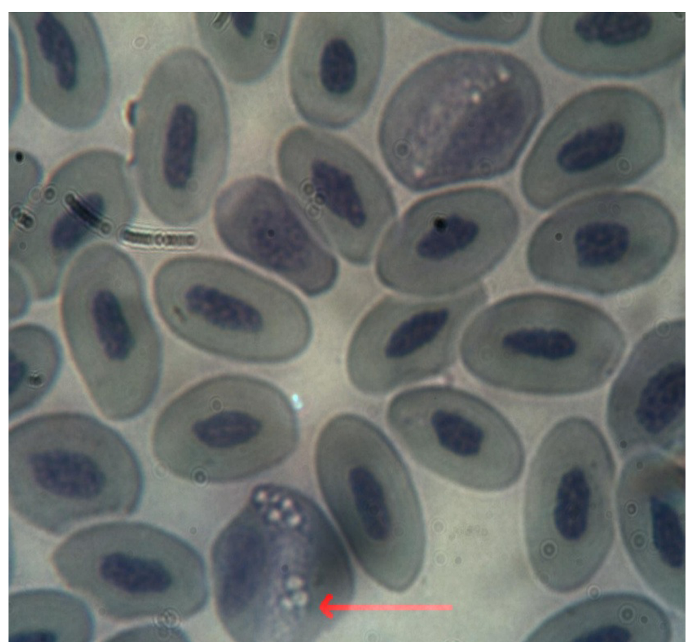
Results and discussion

Examination of the blood samples in *D. chlorogaster* revealed the presence of haemogregarine and haemosporidian parasites along with inter cellular trypanosomes in the blood cells (Figure 1a-d). According with morphological characters of haemoparasites, we identified three types of haemogregarines belong to genera of *Karyolysus* and *Hepatozoon* and one type of haemosporidian belong to *Haemocystidium* or *Plasmodium* along as one type of *Trypanosoma* species (Figure 1 a-d). We did not identify the parasites in exact genus and species level due to the morphological complexity and shape flexibility of haemoparasites in blood cycle.

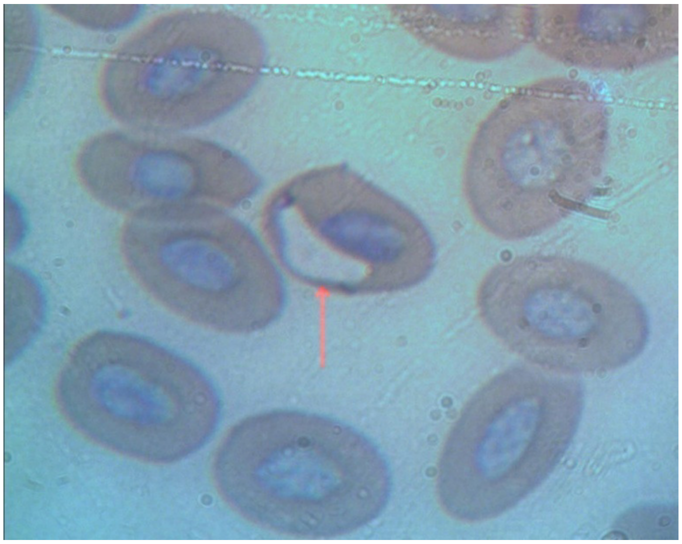
The prevalence of infection of *D. chlorogaster* by haemogregarines was about 8% (4 of 50 individuals) for haemogregarines and five of 50 for haemosporidian (10%). One of the specimens was co-infected by two types of haemogregarines parasites (Figure-1a). The intensity of infection for haemogragarines ranged from one to 10 from 10^4 red blood cells. One of the samples displayed a high level of infection (850 from 10^4 RBC). From five infected lizards by haemosporidian, the intensity was 10 or less infected cells in 10^4 red blood cells in all individuals. Only one out of 50 lizards was infected with *Trypanosoma* sp. The intensity of this parasite was very low.



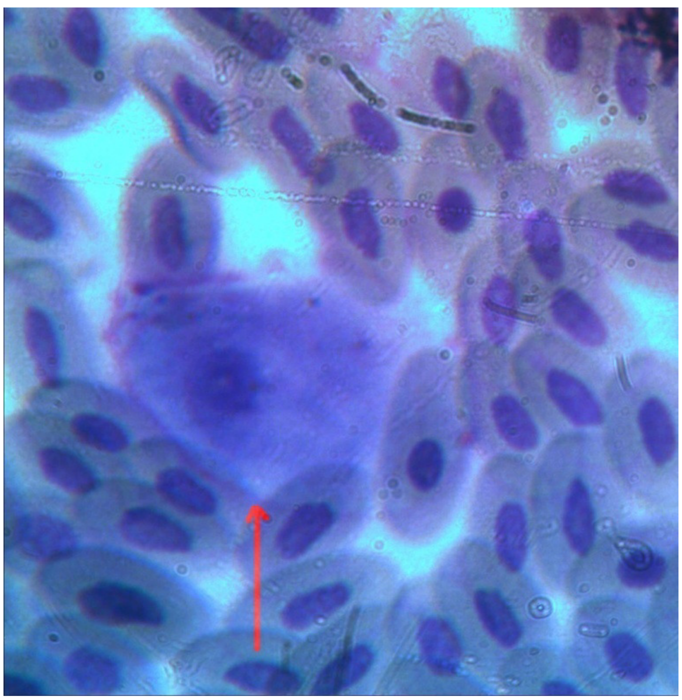
(a) Two types of haemogregarines in blood cells of *D. chlorogaster*



(b) Haemogregarine in blood cells of *D. chlorogaster*



(c) Haemosporidia in blood cells of *D. chlorogaster*



(d) Trypanosome in blood cells of *D. chlorogaster*

Figure-1: Blood parasites in *D. chlorogaster* from north of Iran. a and b) three types of haemogregarines, c) haemosporidia and d) Trypanosome.

Discussion: *D. chlorogaster* is common lizards in north of Iran. It is widespread in border of Caspian coast of northern Iran and Azerbaijan into Hyrcanian forest on the lower slops of the Alborz Mountains and in the Kopet Dagh.[14] This species considered to be Threatened Species in IUCN red list. Nevertheless population of this species is declining in some area due to general habitat destruction[14]. Lacertid species are parasited by haemogregarines commonly members of the genera *Karyolysus* and *Hepatozoon*. Study of three species of genus *lacerta* (*Lacerta agilis*, *Lacerta viridis* and *Lacerta trilineata*) revealed that *Karyolysus* is most prevalence of blood parasites in lacertid lizards of Europe and Asia and nine valid species of parasites have been considered in these lizards[4]. In a study on 1300 individuals of rock lizards of *Darevskia* genus (*Darevskia valentini, D. portschinski, D. raddei, D. nairensis*, *D. armeniaca, D. unisexualis, D. dahli, D.* rostombekowi) from Armenia six morphotypes of haemogregarines parasites were described[16]. Our results indicate that all three haemogregarines species that observed in this study were similar with what has been reported by Harutyunyan[15]. Nevertheless we found one morphotype of haemosporidia different from that parasites which reported by Harutyunyan[15]. Many researcher have been discussed that it is not possible to identify haemogregarine and hamosporidian parasites by morphological characteristics of gametocytes[3,16] because different species may have similar gametocytes[16] as well as mature and immature gametocytes and micro or macrogametocytes may identified as different species[16].

Most of the haemogregarines in lizards are transmitted by mites and ticks. They may acquire these parasites, when they use the same location for basking spots and sharing favorable refuges. However, transmission of *Hepatozoon* has been reported by a wide range of arthropod vectors (mosquitoes to ticks)[1]. Adults harbor probably higher prevalence and intensity of parasites because they have more mobility and more often in contact with parasites[17].

In the present study 18% of lizards were infected by blood parasites. It is very low in comparison with prevalence of 77.3% blood parasites on Iberian rock lizard, *Lacerta monticola* detected by Amo[17]. Moreover, study of blood parasites on European green lizards (*L. viridis*), showed that 96% of individual were infected.[18] In Iberian and Canarian lizards, 74.7% individual of *Podarcis bocagei* and 69.7% of *P. carbonelli* were infected with haemogregarines[3].

In contrast, in a study on *Karyolysus* haemoparasites in different lizards genus of the family Lacertidae and Scincidae (*Darevskia*, *Lacerta*, *Podarcis* and *Mabouia*) in several European country indicated that between 0-64% of individual were infected and two *Karyolysus* species including *K. latus* and *K. lacazei* were identified[4]. They assumed yearly temperature incrising, may be important for development of haemoparasites and altitude may be key factors for the variability of haemoparasites.

In the present study the intensity of haemogregarines were between 0.01-0.1% per infected individual. However we have one sample with high density of haemogregarines with 8.5% intensity in red blood cells. The intensity of haemosporidians were less than 0.01% in infected red blood cells. Overall, intensity was low in the present study, which it is in concordance with the morphological studies on lacertid lizards from the Europe. For example study on intensity of haemogregarines in *Lacerta monticola* were ranged between 0-2.41% infected erythrocytes[17]. Intensity of haemoparasites in sand lizards (*Lacerta agilis*) from Romania recorded between 0.27–4.22 infected erythrocytes[3]. In another microscopic survey of haemoparasites in lizards of the western Mediterranean region indicated that 0.17-6.28% of individuals were infected with haemogregarines[19]. However, intensity of infection between localities was varied. This variety has been shown could be affected with some factors such as feeding habits and seasonality[19], microhabitat characteristics and body condition of lizards[17].

In our study 10% (5 out of 50) of lizards was infected by haemosporidian parasites. The intensity of hamosporidia was less than 0.1 of red blood cells. These results are comparable with the results of Maia [19] for haemosporidian parasites in gecko lizards from Oman in which that study from 80 individual of 17 species, only two species was infected with haemospordia. The prevalence and intensity were 50% and 0.18 respectively for *Hemidactylus luqueorum* (from 2 specimens) and 5% prevalence and 0.34 intensity from *Ptyodactylus hasselquistii* (for 19 specimens). However a few descriptions of haemosporidia in lizards showed that it is not possible to estimat the real diversity of haemosporidian parasites by microscopic methods alone[19].

In the present study we detected *Trypanosoma* sp in one lizard individual. To our knowledge, this is the first record of trypanosome in the genus *Darevskia*. The intensity was very low and only one sample was infected by trypanosomes. Only one morphotype of trypanosomes was observed in this study.

Identification of trypanosomes species is confusing because of morphological polymorphism in this group and inadequite studies on their՚s life cycle. In concequence, many misidentifications were occurred in same parasites and different names were used in same species for different hosts in different geographic localizations. Moreover it has been known that their shape can be influenced by some exogenous factor such as temperature, environment pollution, maturity and host population density[9]. According to Hamilton[11] phylogenetic position of trypanosomes in lizards is varying dependent to genes, taxa and phylogenetic method inferences. Telford[1] registered 48 species of lizard trypanosomes. However due to great variability in form some of described species should be synonymies.

Conclusion

In conclusion, this work represents the first report of diversity, prevalence and intensity of blood parasites in *Darevskia chlorogaster* in Iran. It is a simple fact that we know very little about the distribution of haemoparasites in reptiles host in Middle East especially in Iran. The haemogregarine and haemosporidian parasites as well as trypanosomes are largely understudied due to complexity in life cycle, flexibility in shapes of parasites and few useful characters to differentiate between species. Molecular studies are needed in future works.

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