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Research Article

First report of *Oswaldofilaria chlamydosauri*, Breinl, 1912 (Nematoda: Onchocercidae) from a new host *Paralaudakia caucasia*, Eichwald, 1831 (Squamata: Agamidae) and its prevalence and intensity in Mashhad, North-eastern Iran

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Abstract

Paralaudakia caucasia, a Caucasian agama, plays an important role as a transport and reservoir host for different kinds of parasites, especially nematodes. We sampled 98 specimens of this lizard between March 2010 to July 2011 at Mashhad, northeastern Iran. Parasites specimens were collected and morphological studies related to their host, the Caucasian agama were carried out. Results showed a first report of nematodes, *Oswaldo filaria chlamydorauri*, with high prevalence of infection (95.3%). The prevalence of infections was 97.6 and 94.1% in male and female hosts, respectively. The highest parasite intensity was in subcutaneous tissue of the pectoral and pelvic girdle. Variations in prevalence and mean intensity of the parasite were influenced by period (month) of sampling ($p<0.05$). The highest prevalence and mean intensity was observed in June.

Keywords: Parasite, Lizard, Prevalence, Mashhad.

Introduction

The Caucasian lizard, *P. caucasia* (Eichwald 1831) belongs to the Family Agamidae and has been reported in Eastern Anatolia, Armenia, Georgia, Azerbaijan, Russia (Daghestan), Northern and Central Iran, South Turkmenistan, Southwestern Tajikistan, North and Northeastern Afghanistan; doubtfully in Western Pakistan; probably in Eastern Iraq and Eastern Iran and Uzbekistan(Sindaco and Jeremčenko 2008). So far, 17 helminths species have been reported in *P. caucasia* including: *Abbreviata uzbekistanica, Foleyella candezei, Parapharyngodon dogieli, P. kasauli, P. szczerbaki,Saurocistis agamae, Thelandro smarkovi, T. ospopovi,Thubunae abaylisi, A. achari*, *Oxyuris cincta, Oochoristicatu berculata, F. candezei, P. dontyche, T. sbaylisi, Skrjabinodon pigmentatus, S. lacertae* (Bogdanov and Markov 1955, Goldberg and Bursey 2003, Rezazadeh *et al*. 2012, Sharpilo 1962, Sharpilo 1976, Sulahian and Schacher 1968, Yildirimhan *et al*. 2006).

Rezazadeh *et al.* (2012) compiled a list of helminths in *P. caucasia* from Northwestern Iran, Ardabil province. They reported that the species harbored three nematode species including *Skrjabinodon pigmentatus, Spauligodon lacertae*, and *T. baylisi*. They showed that Iran is a new locality for all three nematode species. However, Filarioids are a kind of nematodes which is very common in the tissues and tissue spaces of all classes of vertebrates (Anderson 2000, Kamali 2016).

Two families of the parasites are recognized: Filariidae and Onchocercidae. Moreover, the Onchocercidae have evolved blood or skin-inhabiting microfilariae and are transmitted by arthropods. *Oswaldofilaria* is a genus of the Family and includes five species (Kamali 2016) thus: 1) *O. bacillaris* (Molin 1858) is a parasite of the thoracic muscles and lungs of Crocodilians (*Caiman crocodilus, C. sclerops* and *Champsanigra)* . 2) *O. belemensis* (Mullen 2011) is a parasite of the heart, aorta and vena cava of the lizard *Dracaena guyanensis* in South America. 3) *O. petersi* (Mullen 2011) exists in the mesentery, intestinal wall and thigh muscles of *Tupinambisni gropunctatus* (Teiidae). 4) *O. spinosa* (Mullen 2011) is found in the armpit ('aisselle'), aponeuroses and body cavity of the lizard *Mabuyamabouia* (Scincidae). 5) *O. chlamydosauri* (Breinl 1912) inhabits the subcutaneous tissue, body cavity and lungs of *Amphibolurus barbatus, A. muricatus* and *Chlamydosaurus kingi* of Agamidae. The present study reports the finding of *O. chlamydosauri* in a new host, *P. caucasia* . The aim of this study was to survey and identify parasites, prevalence rates and relationship between them and their host.

Material and Methods

In total, 98 specimens of *P. caucasia* (Eichwald, 1831) were collected during field work in 2011-2012 from Mashhad, Northeastern Iran (36°12' N and 59°38' E). Mashhad has a low mean annual precipitation of about 250 mm. This area has an arid climate, with relatively scanty annual precipitation occurring from October to April. The mean temperatures recorded in Mashhad for the periods of March to July 2011 was 23°C. In order to minimize distress to the animals, all specimens were anaesthetized using chloroform and then dissected to search for parasites.

First, internal organs including liver, intestine, caecum, heart, lung and stomach and the general thoracic and abdominal cavities were collected to survey the kinds of parasitic specimens of *O. chlamydosauri* discovered in the subcutaneous tissues of pectoral and pelvic girdles in 98 lizards. Then, the size (Snout-Vent Length, SVL) of these lizards were recorded according to the method of Adeoye and Ogunbanwo (2007); the organs were excised into different Petridishes containing reptilian saline. Collected parasites were recognized, counted and conserved in formal acetic acid (FAA) and pure ethanol. The parasites were placed in lacto phenol for clear microscope observation. All specimens were deposited at Mashhad Branch of the Islamic Azad University's Zoological Laboratory.

Results

Among Onchocercid filarial worms, the subfamily *Oswaldofilariinae* Pereira , 2011 is distinct in that the vulva is situated very far from the head (Anderson 2001). The thirty-two species within *Oswaldofilariinae* represent seven genera; two of which are monotypic (Mullen 2011). All discovered filariae from Crocodylia and Sauria (lizards) belong to this subfamily. *Oswaldofilaria* Travassos, 1933 has a gondwanian-type geographical distribution and is represented by thirteen species (Pereira *et al*. 2010). Members of the *Oswaldofilariinae* are found in lacertilians and crocodilians and distinguished morphologically from other Onchocercids by the posterior position of the vulva, which is generally in the middle or posterior region of the body.

Oswaldofilaria chlamydosauri (Breinl, 1912) (type-host *Chlamydosaurus kingii* Gray, 1825) lives in the subcutaneous tissue. These round worms are whitish in color; males are smaller than females with high sexual dimorphism. Besides, their bodies are fusiform, covered with cuticle from behind the cephalic extremity, up to the anus. The oral opening is not surrounded by lips. Additionally, the caudal alae is narrow or absent; and the body is swollen at the excretory sinus (Fig 1.). According to Pereira et

al. (2010), the female *Oswaldofilaria chlamydosauri* (redescribed by Manzanell 1982) has a long tail but the male possesses a distinctly attenuated tail, a gubernaculum, and the left spicule has a membranous extremity. The buccal cavity is laterally flat and deirids are absent in both sexes (Figs. 1 and 3).

Figure 1. Mushroom-shaped structure at the anterior end of the muscular esophagus of *Oswaldofilaria chlamydosauri*

Prevalence of infection in the 98 host specimens (63 males and 35 females) showed 97.6 and 94.1% in males and females, respectively. The results mentioned the presence of *Oswaldofilaria chlamydosauri* in 95.3% of the lizards. The number of female parasites was one to three specimens in each lizard and the number of male parasites was three to five individuals in each lizard. However, the male lizards were longer than the females (female: 9-13cm and male 13 cm or more). The highest intensity and prevalence of *Oswaldofilaria chlamydosauri* were approximately found in large lizards with body sizes of 12.0-12.9 cm, and 13.0-13.9 cm in females and males, respectively (Fig. 2). The relationship between parasite intensity and the lizard length was positive and significant ($p<0.05$); the number of parasites increased with body size of the lizards. However, there were significant differences in the overall intensity of parasites and the gender of the lizard species ($p<0.05$) (Fig. 4).

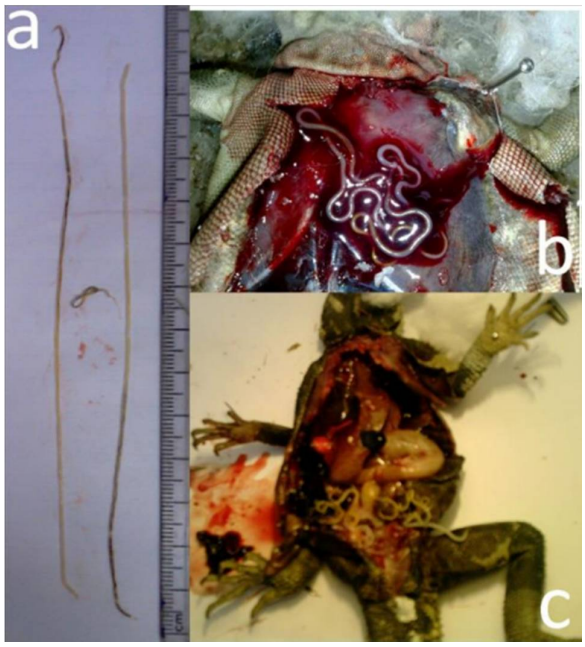

Figure 2. a) size of the parasite b) parasite in the pectoral girdle c) Parasite in the pelvic girdle in *Paralaudakia caucasia*

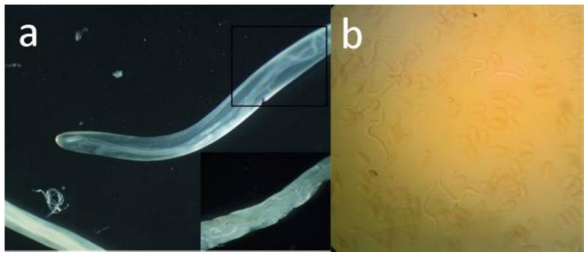

Figure 3. a) Larvae in Female body, b) in peripheral blood of Paralaudakia caucasia

Discussion

In this study, a new record of nematode *Oswaldofilaria chlamydosauri* was found in the subcutaneous tissue of the pectoral and pelvic girdles of the Caucasian agama. At necropsy, 10 - 20 filarioid nematodes (Onchocercidae, Dirofilariinae, *Oswaldofilaria* sp.) were seen in the abdominal cavity under the serous membrane and pleura in ten monitors (Rataj *et*

al. 2011). A high number of eggs of Onchocercidae, Dirofilariinae and *Oswaldofilaria sp*. were found in the abdominal cavity and nodules on pleura, peritonea and lungs of monitors, *Varanus bengalensis* (Kamali 2016, Rataj *et al.* 2011). The neotropical region showed the highest diversity of *Oswaldofilaria* parasite in Sauria (Bursey and Goldberg 2004, Bursey and Goldberg 2005, Pereira *et al*. 2010, Silva and Kohlsdorf 2003, Vicente J.J., Rodrigues *et al*. 1993).

Our finding showed that male lizards had a higher level of infection intensity than the female conspecifics. The difference was statistically significant between intensity of infection and gender. On the contrary, another study by Fadiel *et al.* (2005) showed an insignificant difference between intensity and prevalence in sexes. It seems that male and female lizards were subjected to similar parasitic infection, prevalence and infection intensity (Amo *et al.* 2005). On one hand, males are more at risk of parasitic infection since testosterone probably suppresses the immune system, especially during reproduction (Roberts *et al.* 2004, Uller and Olsson 2003). On the other hand, the development of eggs needs a great amount of energy and metabolites during the pregnancy period in females, which cannot be dissipated to defend against parasites. Therefore, both sexes seem to be weaker in their defense against parasites during their reproductive periods (Amo *et al.* 2005). Our results and other studies showed specialization between species and organs, thus parasites are usually organ-specific. Therefore, the organ in which the parasite resides helps to determine the intensity of infection.

However, our data showed that lizards within the size range of 12 and 13.9 cm have more parasites. The smallest infected lizards were 11 cm long. A significant relationship between size of lizards (SVL) and number of parasites suggest that older lizard hosts have higher intensity of infection. Our findings are completely in congruence with similar studies (Adeoye and Ogunbanwo 2007, Fadiel *et al*. 2005, Ribas *et al.* 1995)which show that the prevalence of infection by helminths was positively correlated with the adult size of the lizard hosts. Accordingly, Ribas et al. (1995) showed that the total mass of nematodes increased significantly with lizard body size. Consequently, significant differences of prevalence of infection in both sexes were observed in the five months of study (March-July). The months of May to June fall within the wet season in Mashhad. However, infection rates could be dependent on the diet of saurian reptiles and habitat exposure to helminths (Goldberg and Bursey 2006, Sanchis *et al.* 2000). On one hand, the parasite, *Oswaldofilaria chlamydosauri,* requires insect vectors. On the other hand, *P. caucasia* is omnivorous, feeding on insects and plants (Rezazadeh *et al.* 2012). Therefore, the prevalence of infection during the wet season and simultaneously, the breeding season of *P. caucasia* explains the high intensity of infection. As we know, insects always depend on water, rainfall and wet season. It seems rainfall in the host's habitat plays an important role in intensity of infection, especially in parasites such as *Oswaldofilaria chlamydosauri* which has insect vectors.

The purpose of the current study was to determine parasites and its prevalence in the Caucasian agama in Northeastern Iran. Our findings interestingly suggest a new nematode and its high prevalence of infection in the Caucasian agama in northeastern Iran. We suggest more sampling for accurate investigation and complementary studies to shed more light on ambiguities of parasitological aspects of the Caucasian agama in Iran.

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