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Original Article

Infection of Afghan Pika (*Ochotona rufescens*) with Oxyurid Worms in Northeastern Iran

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Introduction: The Afghan pika (*Ochotona rufescens*) is a small mammal in the family Ochotonidae. This small mammal occurs in Afghanistan, Iran, Pakistan, and Turkmenistan. Here, we investigated the helminth infections among the Afghan pikas from northeastern Iran. **Methods:** Fourteen Afghan pikas were entrapped from Bojnord, Mashhad, Birjand, and Tandoureh in the northeast of Iran during 2013-2015. The animals were euthanized, dissected, and their gastrointestinal tracts were removed, which were then cut longitudinally, and inspected under a stereomicroscope for the presence of helminths. Also, using the formalin-ether concentration technique, the intestinal contents of the animals were examined microscopically for helminths eggs. **Results:** Out of 14 pikas examined, 11 (78%) harbored at least one helminth species and mixed infections with two helminths were observed in 3 (21%) animals. Four oxyurid species including *Cephaluris chabaudi*, *Cephaluris akhtar*, *Fastigiuris prudhoei*, and *Dermatoxy schneider* were identified. Also, the Oxyurid eggs were recovered from the intestinal contents of four pikas. No significant difference ($P>0.05$) was observed in infection rate between male and female animals and between the areas. **Conclusion:** Oxyurid worms were the only prevalent infection among the Afghan pikas in northeastern Iran. The absence of other helminthic infections in pikas of the study area might be attributed to various factors like the lack of hosts species, high-elevation, and adequate moisture which facilitate the life cycle of many helminths species. *J Med Microbiol Infec Dis, 2018, 6 (1): 31-34.*

Keywords: Afghan pika, Oxyurid worms, Iran.

INTRODUCTION

The Afghan pika (*Ochotona rufescens*) is a small mammal belonging to the family Ochotonidae. The members of this family have 26 teeth, and along with rabbits and hares from the family Leporidae is categorized in the order Lagomorpha. The members of this order can be separated from the rodents by a second set of incisors called peg teeth [1]. The Afghan pikas occur in mountainous regions of Afghanistan, Pakistan, Iran, and southwestern Turkmenistan. In Iran, they inhabit mountainous areas with the cold and semiarid weather [2]. Some parts of Iran such as Semnan, Tandoureh in Khorasan and different parts of Southern Khorasan are the primary habitats of the Afghan pikas. These animals, unlike the rodents, are strictly herbivores and are considered as pests of crops and compete with livestock for available vegetation [3]. In experimental infections, Afghan pikas were found susceptible to larvae of nematodes like *Haemonchus contortus*, *Ostertagia circumcincta*, *Trichostrongylus axei*, *Trichostrongylus colobriformis* [4]. Previously, in Iran, Afghan pika exhibited infection with some oxyurid worms such as *Fastigiuris prudhoei*, and *Cephaluris chabaudi* [5]. Also, these animals are host to fleas, Ixodid ticks, and red mites [5]. In plateau grasslands, the Afghan pikas are found in company with ruminants and livestock that may lead to transmission of the helminthic parasite to humans and livestock [3]. This study aimed to identify the parasitic helminths among Afghan pikas from Bojnord, Mashhad, Birjand, and Tandoureh in the northeast of Iran.

MATERIAL AND METHODS

Study area. We collected the pikas from four regions in northeastern Iran including Bojnord in North Khorasan Province, Mashhad, and Tandoureh in Razavi Khorasan Province, and Birjand in South Khorasan Province during 2013-2015 (Figure 1).

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North Khorasan is a mountainous region with the cold temperate weather, while Razavi Khorasan and south Khorasan are semi-desert regions with mild weather. The average annual rainfall in North Khorasan Province is 262 mm, in Razavi Khorasan Province 203 mm, and in South Khorasan Province 113 mm.

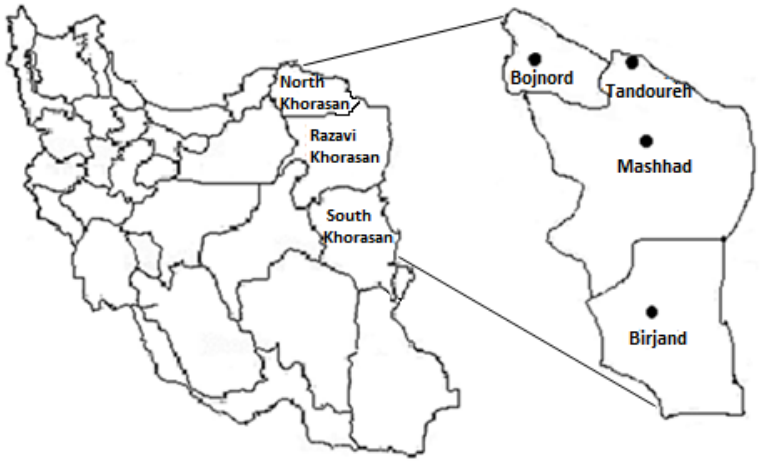

Fig. 1. Map of Iran, the provinces in northeastern Iran from which the Afghan pikas were collected

Collection of animals. The animals were collected by postgraduate students of Zoology Department of Mashhad University aiming to study the biodiversity and genetics of the rodents and lagomorphs. The animals' gender was determined, and the identification of the species was performed based on the morphological features [5]. The pikas were dissected, their gastrointestinal tracts were removed, preserved in a 10% formalin solution, and sent to the Laboratory of Helminthology at Tehran University of Medical Sciences.

Identification of the worms. Each digestive tract was cut longitudinally, the contents were transferred into a Petri dish containing normal saline and examined for the presence of helminths under a stereomicroscope with the magnification 40X [6]. The recovered helminths were cleared with lactophenol as described elsewhere [6]. The transparent helminths were drawn using a microscope equipped with a camera Lucida and identified based on valid morphological keys [7].

Formalin-ether concentration technique. About one ml of intestinal contents were mixed with 10 ml of 10% formalin solution. The suspension was strained through a small funnel containing three folds of gauze, and the effluent was collected in a centrifuge tube. Three ml of ether was added to it, shaken vigorously, and centrifuged at 1500 rpm for 5 min. The supernatant was removed, two drops of the sediment were transferred on a slide, covered with a coverslip, and examined microscopically for helminths egg with the magnification 10X [8].

Statistical analysis. The data were analyzed with STATA software, Version 11.0 (Stata Corp., College Station. Tx, USA). The rate of helminth infections in relation to the gender and the area was analyzed using Fisher's exact test at the 5% levels of significance.

RESULTS

Animals. Overall 14 Afghan pikas (6 male and 8 female) were collected from the study area, 5 from Birjand, 4 from Bojnord, 3 from Tandoureh, and 2 from Mashhad.

Helminth infections. All the 14 examined pikas were found infected with at least one oxyurid worm. Four species of oxyurid worms including *C. chabaudi*, *Cephaluris akhtar*, *F. prudhoei*, and *D. schneider* were identified (Fig. 2). Out of 14 pikas examined, 11 (78%) had an infection with only one helminth species, while 3 (21%) showed mixed infections with two helminths, one (7%) harboring *C. chabaudi* and *F. prudhoei*, and 2 (14%) *C. chabaudi* and *D. Schneider*. No significant difference was observed in the infection rate between male and female and areas ($P > 0.05$) (Table 1).

Table 1. The rate of single and mixed helminth infections according to the gender and area in Afghan pikas collected from northeastern Iran (n= 14)

Helminth species	No. of infected pikas (%)							
	Gender			Study area				
	Male n= 6	Female n= 8	*P* value	Birjand n= 5	Bojnord n= 4	Mashhad n= 2	Tandoureh n= 3	*P* value
Cephaluris chabaudi	2	3	> 0.9	3	2	0	0	0.376
Cephaluris akhtar	2	4	> 0.627	2	0	2	2	0.118
Cephaluris chabaudi* and *Fastigiuris prudhoei	1	0	0.429	0	1	0	0	0.643
Cephaluris chabaudi* and *Dermatoxy schneider	1	1	> 0.9	0	1	0	1	0.615

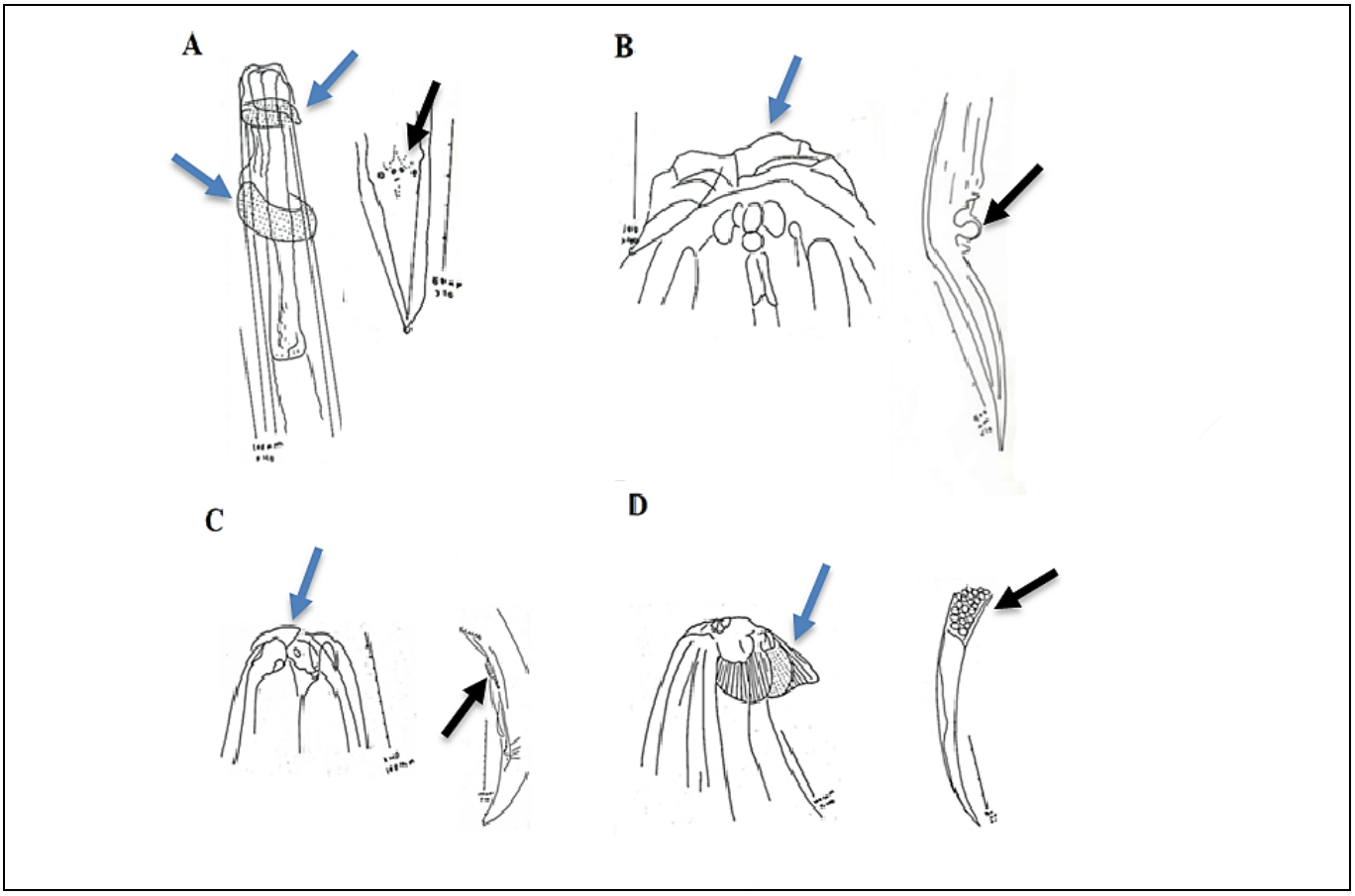

Fig. 2. The helminths recovered from the Afghan pikas in northeastern Iran. (A) *Fastigiuris prudhoei*, alae (blue arrows) and papillae (black arrow); (B) *Cephaluris akhtar*, lip (blue arrow) and papillae (black arrow); (C) *Dermatoxy Schneider*, alae (blue arrow) and sucker (black arrow); (D) *Cephaluris chabaudi*, alae (blue arrow) and eggs (black arrow).

Formalin-ether concentration technique. Oxyurid eggs were detected in gastrointestinal tracts contents of four pikas. One pika showed the adult worms of *C. chabaudi* and *D. Schneider*, two *C. chabaudi*, and one *C. akhtar*.

DISCUSSION

The present study shows the helminthic infections among the Afghan pikas in northeastern Iran. Our results showed that all the 14 examined Afghan pikas were infected with at least one species of oxyurid worms. Four helminth species including *C. chabaudi*, *C. akhtar*, *F. prudhoei*, and *D. Schneider* were found in the studied animals. These helminths have not been reported as zoonotic helminths yet. The oxyurid worms have a direct life cycle [9, 10], and are the most common helminths in lagomorphs. The high prevalence rate of these worms is probably linked to coprophagia, a specific biological trait in this animal that may facilitate the self-infection [11]. Moreover, formalin-ether examination revealed the presence of oxyurid eggs in four pikas, which shows the infection cannot be reliably diagnosed merely by this method as female worms lay eggs on the skin around the anus, and eggs are rarely passed in the feces [12]. Previously, the helminth *C. chabaudi* was reported from Afgan pikas in Iran [13]. Also, *F. prudhoei* helminth has been identified in this animal from various areas of Iran including south of Shahmirzad in the south of Alborz mountain, south of Bojnord in North Khorasan province, and south of Tehran [14]. These helminths are similar to the species we identified in our study. Some helminth species found in the present study were previously reported from other countries, *D. Schneider* from Ochotonidae and Leporidae in North America [15], and *C. akhtar* from Ochotonidae in Canada, Kazakhstan, and Japan [7]. In Qinghai Province of southeastern China, four nematode genera including *Oesophagostomum* sp., *Haemonchus* sp., *Trichuris* sp., and *Chbertiinae* sp., two species of nematodes including *Cephaluris coloradensis*, and *Eugenuris schumakowiescsi*, three genera of adult cestodes including *Schizorchis* sp., *Ochotona* sp., and *Hymenolepis nana* were detected from Plateau pikas. None of the helminths mentioned above was found in the present study, which could be attributed to the distribution of host species, the limited number of examined pikas, geographical and climatic conditions [3]. Previous studies showed that difference in prevalence of helminths in various areas is related to environmental conditions [16]. For example, swampy regions may provide a suitable environment for development and survival of nematodes larvae [3]. Contamination of watering and feeding areas may predispose mammals, fox, wolf to infection by nematodes

such as *Cephaluris* sp. [3]. The Afghan pikas were found in grasslands in company with ruminants. In this study, the helminths found in the gastrointestinal tracts of ruminants did not occur in the Afghan pikas. The absence of some helminth species in Afghan pikas might be due to the annual administration of helminthicide to ruminants [3]. The results of this study indicated that oxyurid worms were the only common helminthic infection among Afghan pikas in northeastern Iran. The absence of other helminthic infections in pikas of the study area might be attributed to various factors like the lack of host species, high-elevation, and adequate moisture which facilitate the life cycle of many helminths species.

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest associated with this manuscript.

REFERENCES

1. Chapman JA, Flux JE. Introduction to the Lagomorpha. Lagomorph Biology: Evolution, Ecology and Conservation. Springer. 2008; 1-9.

2. Sahneha SK, Nourib Z, Shabanic AA, Ahmadid M, Dargahie MD. Bioclimatic niche model to predict Afghan Pika (*Ochotona rufescens*) distribution range in Iran. Biological Forum. An International Journal. 2014; 96 (2): 96-102.

3. Wang C, He H, Li M, Lei F, Root JJ, Wu Y, Qin J. Parasite species associated with wild plateau pika (Ochotona curzoniae) in southeastern Qinghai Province, China. J Wildl Dis. 2009; 45 (2): 288-94.

4. Okamoto M, Kamiya M, Oku Y, Ohbayashi M, Matsuzaki T. Susceptibility of laboratory-reared Afghan pika, *Ochotona rufescens* rufescens (Lagomorpha: Ochotonidae), to gastrointestinal nematodes of ruminants. Nihon juigaku zasshi. 1988; 50 (4): 913-7.

5. Smith AT, Johnston CH, Alves PC, Hackländer K. Lagomorphs: Pikas, Rabbits, and Hares of the World: Johns Hopkins University Press; 2017; 77-8.

6. Rafique A, Rana S, Khan H, Sohail A. Prevalence of some helminths in rodents captured from different city structures including poultry farms and human population of Faisalabad, Pakistan. Pak Vet J. 2009; 29 (3): 141-4.

7. HOBBS RP. A revision of the genus *Cephaluris Akhtar*, 1947 (Nematoda: Oxyuridae) with redescriptions of the North American species. Proc Helminthol Soc Wash. 1976; 43 (2): 151-60.

8. Amin HA, Ali SA. Evaluation of different techniques of stool examination for intestinal parasitic infections in Sulaimani City-Iraq. Int J Curr Microbiol Appl Sci. 2015; 4 (5): 991-6.

9. Najafi F, Rezaie S, Kia E, Mobedi I, Mahmodi M, Salimi M, Hasanpour H, Makki M, Mowlavi G. Intestinal Helminths in Laboratory Mice and Rats in Four Research Centers, Tehran, Iran. J Med Microbiol Infect Dis. 2014; 2 (4): 130-2.

10. Najafi F, Naddaf SR, Rezaie S, Kia EB, Mowlavi G. In Vitro Ovicidal Activity of Nematophagous Fungus Paecilomyces lilacinus on the Eggs of Parasitic Helminths. J Med Microbiol Infect Dis. 2016; 4 (3): 52-6.

11. Hugot J-P, Gardner SL, Borba V, Araujo P, Leles D, Da-Rosa ÁAS, Dutra J, Ferreira LF, Araújo A. Discovery of a 240 million year old nematode parasite egg in a cynodont coprolite sheds light on the early origin of pinworms in vertebrates. Parasit Vectors. 2014; 7 (1): 486.

12. Roberts LS, Janovy J, Schmidt GD. Foundations of parasitology. 6 th ed. McGraw-Hill; 1996; 130-1.

13. Inglis WG. Some oxyurid parasites (Nematoda) from *Ochotona rufescens* vizier (Mammalia: Lagomorpha) in Iran. Bulletin de la Societe Zoologique de France. 1959; 84 (213): 178-87.

14. Sadighian A, Ghadirian E, Sadjadpour E. Two new species of nematodes of lagomorphs and rodents from Iran. J Helmint. 1974; 48 (4): 241-5.

15. Hoberg EP, Pilitt PA, Galbreath K. Why museums matter: a tale of pinworms (Oxyuroidea: Heteroxynematidae) among pikas (Ochotona princeps and O. collaris) in the American West. J Parasitol. 2009; 95 (2): 490-501.

16. Jacobson HA, Kirkpatrick RL, McGinnes BS. Disease and physiologic characteristics of two cottontail populations in Virginia. Wildl Monog. 1978; (60): 3-53.