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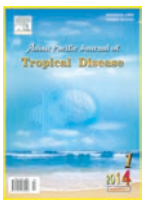

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High helmintic infection of the European grass snake, *Natrix natrix* and the dice snake, *Natrix tessellate* (Serpentes: Colubridae) from Iran

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PEER REVIEW

Peer reviewer

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Comments

In general, this is a good study in which authors try to determine the helminthic parasites of two widely distributed snakes including *N. natrix* and *N. tessellate* in north of Iran. The results are valuable and important. The survey was conducted for the first time on *N. tessellate* in Iran and this is new host and locality records of parasitic helminthes including *T. assula*, *O. europaea* and *R. fuscovenosa* in the country.
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ABSTRACT

Objective: To determine the helminth parasites of *Natrix natrix* Linnaeus, 1758 (*N. natrix*) and *Natrix tessellata* Laurenti, 1768 (*N. tessellate*) in north of Iran.
Methods: Eighteen snakes including nine *N. natrix* and nine *N. tessellata* from Mazandaran Province, north of Iran were collected and examined during March 2011 to October 2011 for helminth parasites. The collected specimens were fixed and preserved in 70% ethanol.
Results: All of the examined snakes (100%) were infected with parasitic helminth. The list of extracted helminths both in *N. natrix* and *N. tessellata* includes one Nematode: *Rhabdias fuscovenosa* (larva), one Digenea: *Telorchis assula* and one Cestoda: *Ophiotaenia europaea*. The infection rate of *Ophiotaenia europaea*, *Telorchis assula* and *Rhabdias fuscovenosa* (larva) from collected snakes were 100%, 83.3% and 61.1%, respectively. Moreover, in the current investigation the morphological characteristics of the collected helminths were described elaborately.
Conclusions: This is the first survey on helminth parasites from *N. tessellata* in Iran and the helminthes are reported for the first time from this host in Iran.

KEYWORDS

Snake, Reptile, Helminth, Parasite, Iran

1. Introduction

The grass snake, European grass snake, *Natrix natrix* Linnaeus, 1758 (Serpentes: Colubridae) (*N. natrix*), sometimes called the ringed snake or water snake occurs from Europe, Northwestern Africa and East to Middle Asia[1]. Grass snake is large reptile, reaching up to 190 cm in total length, though such large specimens are rare. Females are considerably larger than males, typically reaching a size of 90-110 cm when fully grown. Males are approximately 20 cm shorter and significantly smaller in girth. Weight is about 240 g[2,3]. *Natrix tessellate* Laurenti, 1768 (Serpentes: Colubridae) (*N. tessellate*), the dice snake is distributed from Middle and Southern Europe to Western China[4]. Maximum size of this snake is 100-130 cm. *N. natrix* and *N. tessellate* are semi-aquatic snakes

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and observed in most aquatic habitats in Iran. *N. natrix* and *N. tessellate* are the most common snakes in the north of Iran[5,6]. According to our literature review, despite of wide distribution of *N. natrix* and *N. tessellata* in Iran there is not comprehensive and adequate published data about parasitic helminthes of these snakes in Iran. Therefore, the current study was conducted to prepare a list of parasitic helminths of *N. natrix* and *N. tessellata* in Iran.

2. Materials and methods

2.1. Sampling

A total of 18 snakes including nine (3 males, 6 females) *N. natrix* and nine (4 males, 5 females) *N. tessellata* were captured by hand from rural areas of the Mazandaran province, north of Iran, from March 2011 to October 2011. For the purpose of slaughtering an overdose of sodium pentobarbital (Nembutal®) were utilized. To look for endoparasites, dissection was performed from throat to vent meticulously. The body cavity was opened and the digestive tract was removed. After collecting the visible helminthes of each digestive tract (stomach, small intestine and large intestine), the contents were rinsed into sieve Mesh 70 to collect the remaining helminths. In addition, the following organs: esophagus, lungs, liver and urinary bladder separately were opened and examined for helminths at necropsy under a dissecting microscope.

The extracted Digenea and Cestoda helminths were fixed and preserved in 70% ethanol, stained with carminic acid, dehydrated, then cleared and mounted in Canada-balsam. Nematodes were killed in hot saline solution and kept in a solution composed 70% ethanol and 5% glycerin. For examination they were cleared by lactophenol[7]. Identifications of helminths were performed according to available systematic keys[8-10].

2.2. Study area

Mazandaran province (36°33′56″ N, 53°03′32″ E) is located at the northern part of Iran and on the southern coast of the Caspian Sea (Figure 1). It covers an area of 23 842 km^2 and its population is composed by 2 922 432 inhabitants. This province has a particular geographical condition with moderate and subtropical climate with 70%-100% relative humidity, 10-35 °C average temperature and 800-1 200 mm annual rainfall. This province is geographically divided into the coastal plains and the mountainous areas of Alborz Mountains Range. It has diverse ecosystems in many plains, prairies and forests[11].

Figure 1. Map of Iran, Western Asia.
Green shows position of Mazandaran province.

3. Results

Both examined *N. natrix* and *N. tessellata* harbored 1 species of Digenea, Cestoda and Nematoda including *Telorchis assula* (*T. assula*), *Ophiotaenia europaea* (*O. europaea*) and *Rhabdias fuscovenosa* (*R. fuscovenosa*) (larva), respectively. All examined snakes (nine *N. natrix* and nine *N. tessellata*) showed infection by helmintic parasites. The Cestoda, *O. europaea* was observed in all examined snakes. 100% of female snakes were infected by at least two helminth parasites *O. europaea* and *T. assula*. The infection rate of *O. europaea*, *T. assula* and *R. fuscovenosa* (larva) from collected snakes were 100%, 83.3% and 61.1%, respectively. In addition, the mean weight of nine examined *N. natrix* and nine examined *N. tessellate* were recorded 144.4 g and 143.3 g. Table 1 depicts the extracted helminths from examined *N. natrix* and *N. tessellate* according to sex and organ involvement. The morphological characteristics of the extracted helminths were described elaborately below.

3.1. O. europaea

The scolex was observed unarmed encompassing four suckers. Neck was observed short in length, strobile acraspedote; Immature proglottids had 390 μm length and 1 820 μm width; mature proglottids was measured 1626 μm length and 2084 μm width; gravid proglottids 1 780 μm long and 2 161 μm wide; genital pore was distributed irregularly, opening in the middle of the proglottids; vagina anterior or posterior to the cirrus pouch; cirrus pouch was 461 μm length and 191μm width; testicle was observed in two separated parts; the ovary width was 1 512 μm and vitellines

Table 1
The extracted helminths from examined *N. natrix* and *N. tessellate* based on sex and infected organ (*n*=18).

Helminth	Site of infection	*N. natrix* (%) (3 males and 6 females)		*N. tessellate* (%) (4 males and 6 females)		Total (%)
		Male	Female	Male	Female	
T. assula (Trematoda)	Small intestine	1/3 (33.3)	6/6 (100.0)	3/4 (75.0)	5/5 (100.0)	15/18 (83.3)
O. europaea (Cestoda)	Small intestine	3/3 (100.0)	6/6 (100.0)	4/4 (100.0)	5/5 (100.0)	18/18 (100.0)
R. fuscovenosa (Larva) (Nematoda)	Lung	3/3 (100.0)	3/6 (50.0)	1/4 (25.0)	4/5 (80.0)	11/18 (61.1)

scattered as a lateral line. Moreover, numerous lateral branches were seen distinctively in uterus of gravid proglottids (Figure 2).

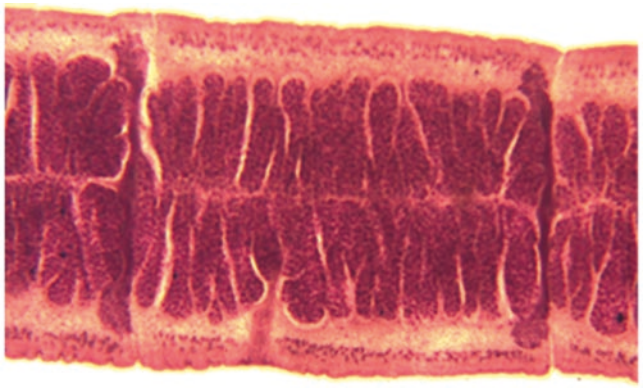

Figure 2. Gravid proglottid of *O. europaea* from *N. tessellate*.

3.2. T. assula

The body was elongated with parallel margins, which appeared to be lingual shape. Prepharynex were observed in all of specimens. Furthermore, oesophagus of *T. assula* appeared moderately long. In addition, caeca seemed slender and gut was looked a little thicker at the end of body. Testes were observed oval, tandem and also contiguous. Concerning cirrus sac, it appeared elongate, large, either straight or sinuous, mainly was appeared in posterior to ventral sucker and contained sacculate seminal vesicle, elongate ejaculatory duct lined with prostatic cells. Cirrus often was seen protruded. Genital pore was seen in median part. Uterus showed descending and ascending symmetrical that firmly packed transverse coils between ovary and anterior testis. In middle of body, vitellarium forms contiguous lateral fields of small follicules (Figure 3).

Figure 3. *T. assula* from *N. tessellate*.

3.3. R. fuscovenosa

Oral opening was seen round and equipped with 6 weakly developed lips which had equal size and shape. Vulva was situated in equatorial position. In esophagus, a posterior bulb was observed. Female parasites were viviparous. Uteri was thin walled and filled with numerous eggs. The shell of egg was thin, smooth, clear and their egg size was 50-70 and 30-45 μm. Male parasites revealed a pair of almost equal spicules. A long spike was observed in the end of tail in both sexes (Figure 4).

Figure 4. Tail of *R. fuscovenosa* (male) from *N. natrix* intestine.

4. Discussion

In the current survey the following helminthes were collected from *N. natrix* and *N. testella*: *O. eropaea* and *T. assula* from small intestine and *R. fuscovenosa* larva from lungs. In this work, *O. europaea* was the most dominant species (100.0%) which is followed by *T. assula* (83.3%) and *R. fuscovenosa* larva (61.1%). According to our review literature, although there is not any report on helminth parasites of *N. testella*, two published studies were carried out on parasitic infection of *N. natrix* in Iran. In one case report described *O. europaea* from one *N. natrix* from north of Iran[5]. And also three helminths including *O. eropaea*, *T. assula* and *R. fuscovenosa* were found from three European grass snakes (*N. natrix*) in Iran[12]. A similar investigation was carried out on 21

N. natrix at 6 locations in neighbor country, Turkey and the following helminthes were reported: *Astiotrema monticelli, T. assula, Encyclometra colubrimurorum, Spirometra erinaceieuropaei* and *O. europaea* from small intestine, *Paralepoderma cloacicola* from large intestine, *R. fuscovenosa*, *Macrodera longicollis* from lungs and *Eustrongylides excisus* (larva) from musculature. The prevalence rate of *O. europaea* from *N. natrix* and *N. testella* were recorded 81% and 85%, respectively that our results are in agreement with the above mentioned study[13]. These results indicate that *O. europaea* is the most prevailing parasite of examined snakes.

Nematodes of the genus *Rhabdias* are considered as a common lung parasites of amphibians and reptiles with over 40 species described throughout the world[14]. *R. fuscovenosa* has been reported frequently from Palearctic and Nearctic snakes[9,14]. Histologic examination of *N. natrix* which was infected with *R. fuscovenosa* revealed vacuolar degeneration of the respiratory epithelium, hemorrhage, faveolae necrosis and obstruction[15]. The infection rate with *R. fuscovenosa* in our specimens was 61.1% which mostly corresponds with Yildrimhan results (43%)[13]. In addition, the Trematoda, *Macrodera longicollis* and the nematodes *Hedruris* sp. and *Oxysomatium brevicaudatum* were extracted from *N. natrix* in Turkey[16,17]. In another survey *Renifer aniarum* (Digenea: Reniferidae) was obtained from *N. natrix* in Calabria, southern Italy[18]. Severe granulomatous lesions in *N. tessellate* was attributed to *Eustrongylides* larvae and we did not observed this larva[19]. Members of the genus *Telorchis* are plagiorchiform intestinal parasites occurring worldwide. They are common inhabitant of North American freshwater turtles and occasionally, snakes and salamanders. *T. assula* were reported from Europe and Turkey[13,20]. From collected samples 83.3% harbored *T. assula* that is noticeably higher compared to Yildirimhan finding 52%.

In this survey we observed the exactly same species in both examined *N. natrix* and *N. tessellate*. A similar diets and food preferences are considered as two causative agents of this helminthes coinfection of these snakes[1]. Based on the current investigation results, all snakes were shown infection by helminth parasites and this infection rate was considerably high in Mazandaran province. Moreover, a significant difference in the infection rate or parasite burden was generally noticed in accordance with the sex of the host. Female specimens harbored at least two helminth parasites *O. europaea* and *T. assula*.

Authors draw a conclusion that this is the first comprehensive study on the helminth parasites of *N. natrix* and *N. tessellate* in order to prepare and complete the list of parasites fauna in Iran, but it represents the first report of *O. europaea*, *T. assula* and *R. fuscovenosa* from *N. tessellate* in Iran. Finally further helminthological investigations are highly suggested to provide enough information about reptiles and amphibians parasites in this area and other regions of country.

Conflict of interest statement

We declare that we have no conflict of interest.

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Comments

Background

Snakes are carnivorous reptiles of the suborder Serpentes. Snakes can carry bacteria, virus and parasites. Some of zoonotic parasitic pathogens cause diseases such as sparganosis, mesocestodiasis and pentastosomiasis. Among snakes *N. natrix* (European grass snake) Linnaeus, 1758 and *N. tessellate* (dice snake) Laurenti, 1768 have a considerable distribution in the world and particularly in Iran. *N. natrix* and *N. tessellate* are the two most common snakes in north of Iran due to having proper geographical condition of this area. Having enough parasitic information about these snakes and their potential risk of zoonotic disease is significant.

Research frontiers

The study was carried out on eighteen snakes encompassing nine European grass snakes and nine dice snakes that were collected from Mazandaran province, North Iran. The samples were dissected and all organs were scrutinized for helminth parasite. The obtained parasites were preserved, cleared, fixed and indentified. In addition, the morphological features of extracted helminthes were described elaborately.

Related reports

The findings of paper is in agreement with Halajian (2013) and Youssefi (2010) investigations on *N. natrix* in Iran. The infection rate of the present work is higher than the study which was carried out by Yildirimhan (2007) in Turkey. But nine helminthes species were recorded from *N. natrix*.

Innovations & breakthroughs

Innovation in the study is that although *N. natrix* and *N. tessellate* have a considerable distribution in Iran there is no information on *N. tessellate* helminthes parasite and there is just limited data on *N. natrix* in Iran. Therefore, authors tried to fill this gap which is so valuable point of view.

Applications

Due to lack of enough information about parasitic burden of *N. natrix* and *N. tessellate* the current study could fill this gap and also clarifies the condition of parasitic diseases in Iran. The work provides fundamental data on these reptiles which are necessary. Furthermore, the paper provides a comprehensive morphological description of parasites which help researchers for better identification.

Peer review

In general, this is a good study in which authors try to determine the helminthic parasites of two widely distributed snakes including *N. natrix* and *N. tessellate* in north of Iran. The results are valuable and important. The survey was conducted for the first time on *N. tessellate* in Iran and this is new host and locality records of parasitic helminthes including *T. assula*, *O. europaea* and *R. fuscovenosa* in the country. Authors gave a comprehensive morphological description of recovered helminthes which taxonomically are valuable.

The findings indicate a high parasitic infection (100%) of examined snakes. And these results are so promising for further investigations for researchers in the future.

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