**Endoparasites (Helminths) of Toad (Anurans) in the Dir Lower, Khyber Pakhtunkhwa, Pakistan**

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**Abstract**

Twenty-seven specimens of *Bufo stomaticus,* (Anura: Bufonidae) were collected in the Dir Lower, Khyber Pakhtunkhwa province of Pakistan in the period from April to August 2016, and examined for helminths (endoparasites) parasites. Twelve (44.44%) specimens were infected by two types of helminths parasites. Total of forty-one helminths parasites were collected, among them six were identified Cestode and the remaining thirty-five were nematodes. The dominant parasites were nematodes (*Cosmocerca sp.* with 56.09% followed by *Cosmocercoides sp.* with 29.26%). But the Cestodes were found with single specie (*Nematotania dispar* with 14.63%). Female toads showed higher parasite diversity than male toads.

**Key words**: Helminths parasites, *B. stomaticus*, Biometry, Toad, Dir Lower and Pakistan

**Introduction**

The amphibians are unique group of animals provided an evolutionary evidence for the link between aquatic and terrestrial mode of life (Vitt and Caldwell, 2014). According to the international union for conservation of nature and natural resources (IUCN) in July 2012, about 41% of amphibians’ species are at risk of extinction (Ceballos et al., 2017). Amphibian conservation is ignored and little work has been conducted on the local threat faced to amphibians in Pakistan (Crump & Fenolio, 2015). The estimated species of amphibians are 7481 worldwide. About 787 species of amphibians endangered (Vaughan & Harris, 2014), while about 1,900 species are identified to be threatened (Morrison, 2012). Due to arid condition, Pakistan is less favourable for survival of amphibian fauna but the only Order Anura is found here (Sarwar et al., 2016). However, there are 25 species of anura reported still from Pakistan. Among them, 9 species are endemic (Portik & Papenfuss, 2015).

Helminths infect and produce diseases occasionally include Cestode and acanthocephalans. Cestodes are not commonly isolated from amphibian species, but these may produce gastrointestinal lesion, gastrointestinal obstruction and may cause death in case of heavy infection, (Wright, 2006). Acanthocephalan or thorny headed worms produce lesion in juveniles and adults’ intestine of amphibians (Densmore & Green, 2007). The Helminths are important parasites of free ranging amphibians (Johnson & McKenzie, 2009; Bursey et al., 2001) with an ecological role in the regulation of amphibian population. Most of the studies analysing community structure of helminth parasitizing amphibians were conducted in temperate regions e.g. (Akani et al., 2011; Comas & Ribas, 2015), whereas studies on trophical helminths and their amphibian hosts are common in the international literature and mainly confined to the Central and South American study systems e.g. (Johnson & McKenzie, 2009; Johnson et al., 2004).

Many types of Helminths parasite infect amphibians (Koprivnikar, 2012). Trematods are may be widely recognized parasite of amphibians. Nematodes are common helminths parasite which infect amphibians (Dorny, 2009). Anura amphibians have a rich parasitic fauna due to their typical association with both aquatic and terrestrial habitats and more susceptibility to microbial and parasitic infections. The nematodes parasites of amphibians have usually a direct life cycle without an intermediate host (O’Rourke et al., 2015; Blaustein, A et al. 2012). Few reports are available on the occurrence of Trematods, Nematodes, Cestodes and acanthocephalan parasites from frogs and toads from Pakistan (Imkongwapang et al., 2014; Goldberg et al., 2009; Rizvi & Bhutia, 2014).

Due to the availability of water bodies and rice fields there are two anurans (*E. cyanophlyctis* and *Bufo stomaticus)* species found in the Dir Lower. So it was felt necessary to undertake research work, to recognize the prevalence and diversity of helminth parasites and infection in toads in the study area, to develop literature for the conservation of this ignorant group of vertebrates.

**Materials and Methods**

The present study was carried out at the Dir Lower, Khyber Pakhtunkhwa, Pakistan. The coldest months are December, January and February. During this period temperature falls below the freezing point (11.22co and -2.39co-8co). The rainfall is received throughout the year but mostly rainfall occurs in the month of July, August and December to March. During winter season the average rainfall is more than of summer. The maximum rainfall is recorded in the March is 242.22co mm. Humidity is quite throughout the year, (Khan, 2013).

 The toads were hand-captured from seven selected localities in the Dir Lower i.e. Munda, Kambat, Mayar, Maskeni, Samarbagh, Timargara and Chakdara, from April to August 2016. The specimens were deposited in boxes and then transported to the laboratory of Parasitology, Department of Zoology, University of Malakand, where they were euthanatized with help of Chloroform (CHcl3) solution. In biometry the body was measured with the help of graduated scale to the nearest 0.01mm and bodies were weighted with the help of a digital balance to the nearest 0.1g in the laboratory. Each specimen was dissected and the sexes were determined.

Subsequently, the digestive tract (Oesophagus, stomach, small and large intestines and cloaca) was examined for helminth parasites. All helminths were isolated with the help of forceps and fine hair brush and wash with normal saline (0.9% Sodium chloride solution) in room temperature and then preserved in 70% GL ethyl alcohol. Later on, the parasites were then classified and studied under the electric compound microscope. The collected parasitic data was interpreted and analysed with the help of computer package MS office.

**Results**

A total 27 collected *Bufo stomaticus*, 14 (51.85%) were recorded male, while 13 (48.14%) were female. The average body weight was 37.46g of female toad was higher than of male was 34.26g. The body length was also measured where average body length was 53mm and 56mm for male and female toad respectively. The average mean length of fore limbs of male was 24mm and female was 28mm. On the other hand, mean average length of hind limb of male was 45mm and 48mm of female toad. From these measurements, it was concluded that the length of limbs was longer in females than that of male toads.

A total of 27 collected and examined toads, 12(44.44%) were infected by helminths parasites in larval or adults form. Among the infected specimens 6 (42.85%) out of 14 were male and 6(46.15%) out of 13 were female (Fig. 1). The above figure shows that the prevalence of infection was higher in female toads as compare to male sex because the percentage prevalence of female toads’ infection was 46.15% and male was 42.85%. In the total infected toads, two were infected with both Nematodes and Cestodes while the remaining were infected were infected with Nematodes parasite only in adult or in larval stage.

During the experimental work total 41 of helminths parasites were collected from the digestive tract of toads. Out of these, 6 were identified Cestode and the remaining 35 were identified Nematodes. All identified Cestodes were single species *Nematotaenia dispar* while the nematodes belong to two different genera of *Cosmocerca* and *Cosmocercoides*. We found total of 41 parasites in which the prevalence of *Cosmocerca sp.* (56.09%) followed by *Cosmocercoides sp.* (29.26%) and *Nematotaenia sp.* (14.63%) as shown in the Table 1.

**Table 1:** Prevalence and percentage of each Helminth parasite and their site of infection.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **S/No** | **Type of parasite** | **No. of parasites** | **Total No. of toad** | **No of infected toad** | **Prevalence** | **Site of infection** |
| 1 | *Cosmocerca* | 23 | 27 | 6 | 56.09% | Large intestine |
| 2 | *Cosmocercoides* | 12 | 27 | 4 | 29.26% | Large intestine |
| 3 | *Nematotania dispar* | 6 | 27 | 2 | 14.63% | Small intestine |
| Total | 3 Genera | 41 | 27 | 12 | 100% | 2 organs |

Discussion

Our study reflects that*Bufo stomaticus* feed on snails and insects like ants, beetles, cockroaches, wasps, spiders, butterflies, flies, mosquitoes and cockroaches similar with the observations of Boulenger (1897) and Noble (1918).

The different varieties of prey consumed suggests that the species feed on wide varieties of invertebrates’ species and each toad’s diet depended on the size of toad and locality. The availability of food was different for each individual toad. Beetles were constituting an important part (39.41%) of its diet. In other species such as *Mantella*, the proportion of mites and ants are much higher, ranging from 14% to 74% (Clark et al., 2005: Arenas Chaves, 2010) and they were considered as microphagous.

**Figure 1: Shows the sex wise prevalence of infection in *Bufo stomaticus* toad.**

The presence of some stone pieces and pebbles along with plants leaves has been reported from the stomach contents of some anuran species (Sabagh, & Carvalho, 2008; Goldberg et al., 2008; Düşen & Öz, 2006), and its ingestion has been considered to be accidentally ingested when they feed and swallows the prey among the dead leaves or in sand (e.g. Kühn et al., 2015; Parker et al., 2005), which seems same to be in case of*Bufo stomaticus*.

Our results indicate that *Bufo stomaticus* mostly feed on arthropods’ insects and crustaceans. Among these insect of frog diet have great economic importance like mosquitoes and flies. The present study on the feeding habit of *Bufo stomaticus* revealed that toads were not belong to the beneficial group. Thus, this species of anuran may be considered as a useful amphibian for the control of pests and other insects and therefore plays a very important role in the economy of nature. Therefore, the removal of large numbers of toads from their environment will upset the existing balance of nature as well as of cultivated crops areas. Now a day it is globally accepted that biological control is the only safe method which does not produce any single harmful reactions and effects with contras to other methods.

*Nematotaenia dispar* was also been reported from the *Rhinella icterica* toad intestine by (McAllister et al., 2013) as well as by Mohammad and Ahmad (2010). In addition, it also has been reported that there is greater incidence of infection of anurans with nematodes than that of Cestodes infections (Mohammad, 2010). It has been also reported from North Central Texas that nematodes are dominant in infection as compare to Cestode in Spotted Chorus frog, *Pseudacris clarkii* frog (Vhora 2012). *Nematotaenia dispar* was found to the most dominant species of Cestodes.

*Nematotaenia dispar* covers broad geographical regions throughout the countries of North Africa, the Middle East, Europe, India and South East Asia (Mohammad et al., 2010). Host records to date imply that *N. dispar* is primarily a parasite of Bufonidae (KARAKAŞ 2015) and it is also recorded from the other anuran families (Ranidae and Hylidae), and from caudated amphibians as well. *Lanfrediella spp* of nematode has been recorded from the toad in Shendi Sudan (Sulieman Y et al, 2015) and *Baerietta jaegerskioelii* Cestode has been reported from African common toad. *N. dispar* Cestode was isolated and recorded from some Iraqi Amphibians species i.e. *Rana ridibund, Bufo viridis and Hyla Arborea* species (Iyaji et al. 2015).

**Conclusion**

The present study showed that *Bufo stomaticus,* in the region of the Dir Lower, Khyber Pakhtunkhwa province of Pakistan show a high rate of endoparasites (helminth) infection. Helminths that parasitized were nematodes of the genera *cosmocercoides*, *Cosmocerca* and one species of Cestode, *Nematotania dispar*. Average body length of female *B. stomaticus* toad was higher as compared to male toad. In case of limbs length, the length of hind limbs was higher in female toads. The results emphasise the significance of further studies in the Dir Lower to get well understanding of the helminth parasites with ecological relations of their hosts.

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**Declaration of competing of interest**

The authors declares no conflict of interest.

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