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Original article

Endo-helminth parasites of frog (Rana cyanophlyctis) from the Gurez Valley of Jammu & Kashmir, India

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ABSTRACT

Abstract: The present study carried out from May 2013 to May 2015 on helminth parasites of Rana cyanophlyctis of Gurez Valley. Out of 135 specimens of frog examined, 89 specimens were infected by one type of helminth parasite. Only three species of helminth parasites i.e. one cestode species and two nematode species were observed. Out of 135 specimens examined, 59 of Rana cyanophlyctis were infected with cestode Nematotaenia kashmirensis, 77 with nematode Cosmocerca kashmirensis and 86 with Rhabdias bufonis. Thus the prevalence of nematodes was much higher than cestodes; however the overall prevalence of helminth infection was 65.92%. The prevalence of 70.17% and 47.36% (in summer), 63.63% and 45.45% (in autumn), and 52.94% and 35.29% (in spring) of nematodes and cestodes respectively. In conclusion the amphibians showed a high prevalence of GIH infection. This can be due to the fact that amphibians are confined to small restricted areas nearer to the human habitations. Thus anthropogenic activities which facilitate the environmental pollution can be a major cause of high level of infection in these frogs of the Gurez valley.

Introduction

Amphibians are ecologically and economically important group of animals. Among the amphibians, anurans have been exploited for food and as medicine. They play a significant role in controlling harmful insects and pests that damage crops (Ray, 1999). Amphibians are one of the most fascinating groups of hosts for parasitologists to study. Aho (1990) showed that amphibians represent excellent system for the study of host parasite relationships, because they occupy a wide variety of habitats, exhibit different life cycle patterns with diverse reproductive strategies and hold different positions within ecosystem food webs. Wild amphibian populations are declining worldwide (Whiles et al., 2006; Wake, 2007) and factors such as global change and pollution may magnify the effect of pathogens and disease in this animal class (Harvell et al., 2002; Taylor et al., 2005; Paull and Johnson, 2011; Macnab and Barber, 2012; Tinsley et al., 2011). For example, higher water temperatures lead to a rise in the incidence of parasitic diseases due to increased pathogen development transmission and host susceptibility (Karvonen et al., 2010). Parasites can have a significant influence on the population dynamics of the host species (Longshaw et al., 2010) and understanding how host-parasite interactions will change in relation to host traits is one of the cornerstones of parasitology (McAlpine, 1997; Dobson, 2009; Lafferty, 2009). The relationship between prevalence and factors such as age or sex is not clear. In some studies an age-related increase in prevalence in amphibians has been reported (McAlpine, 1997; Campiao et al., 2009). Nevertheless, some studies describe a decrease in prevalence (Ibrahim, 2008; Raffel et al., 2009; Tinsley et al., 2012), while others report no age-related patterns (Garvin et al., 2003; Hasselquist et al., 2007). Age-related changes in prevalence may be due to several causes. An increase in prevalence with age may be caused by prolonged exposure to parasite accumulation (Sanchis et al., 2000), while a decrease in prevalence with age may be linked to differential survival rates between individuals with and without parasites (parasitized individuals may be more likely to die and so over time there will be more non-infected individuals). A decrease in prevalence with age may also be influenced by changes in the immune response capacity of infected individuals. Levels of disease risk in hosts of different ages will vary due to differences in their susceptibility to infection and in their age-acquired immunity (Raffel et al., 2009; Tinsley et al., 2012). Nonetheless, prevalence may also decrease with time post-exposure due to parasite mortality, especially in the case of parasites that do not reproduce within a host (Telfer et al., 2008; Holland, 2009).

Thus this little confined valley being geographically isolated and with less anthropogenic load has a great potential for helminth parasites and as such is a great concern for researchers and helminthologist. There is a possibility in some cases to check and control the incidence of parasitism as the prevalence and intensity are very low.

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The present study has been worked out keeping in view the following objectives:

To carry out a general survey of helminth parasites, with regard to their species diversity and developed a database of the region. To make a detailed study on the distribution of Helminths (Cestodes and Nematodes) with reference to season, gender, and age of the host animals. To study the morphological and taxonomical status of the observed gastrointestinal helminth parasitic fauna of the said region. To devise recommendations and various prophylactic measures for control of parasitic infestation in this hidden paradise on earth.

MATERIAL AND METHODS

Study area and sampling

Gurez is a valley located in the high Himalayas on banks of river Kishenganga, about 86 km from Bandipore and 123 km from Srinagar in northern Jammu and Kashmir, India. In northeast of Srinagar, the main valley of Gurez extends between (340 30′ to 340 41′ N latitudes) and (740 37′ to E 740 46′E longitudes) at an average altitude of about 2370 m.a.s.l (about 8,000 feet). It is surrounded on its north by Ladakh, by Bandipora on the south, by Ganderbal on its southeast and on the west by Kupwara with its peripheries touching Line of Control (LoC) that divides the states of India and Pakistan. The valley is nestled among high towering peaks and lofty and glaciated snow capped mountains which are not just an unvarying landmass but show great differences in elevation, aspect, rock type, ruggedness and glacial work which coalesce to make contrasting land surfaces. Gurez is divided into three regions; Baghtor to Sharda Peeth, Baghtor to Abdullae Tulail, Chorwan to Burzil pass.

Frogs were collected from the study sites with the help of locals or personally by insect nets and were brought alive in medium sized containers containing water to the temporary laboratory maintained at Dawar - the capital of Gurez and later were subjected to chloroform treatment to effect anaesthesia or death. On an average 40-45 specimens were collected and dissected on seasonal basis except winter-the hibernation period. Immediately after killing, the frogs were visually examined for any ectoparasite and then a thorough examination of the whole body for helminth infestation was done with the help of hand lens or dissecting microscope etc.

For the collection of endoparasites, the frogs were dissected mid-ventrally and before removing the internal organs, the body cavity was thoroughly examined for parasites. Various organs like liver, bladder, heart, etc were removed and kept in separate petridishes containing normal saline (0.65% NaCl). The alimentary canal being the obvious part of entry for many kinds of parasitic infestations was removed completely and split open longitudinally and placed in petridishes containing saline water to which a few crystals of menthol were added so that the parasites which were firmly anchored to the lumen of the intestine were easily detached without causing any damage or distortion to the body of the parasite. Other organs were examined in the same manner by dissection and teasing with the help of brushes and needles. However, maximum numbers of parasites were collected from the alimentary canal of fish. Parasites collected from each fish were counted separately and regular record of the collection was maintained.

Photography & Photomicrography

The Photographs were taken with the help of Sony Digital SLR Camera Model Number (DSLR – A200). Photomicrography was conducted with DP – 12 Digital Camera attached to Olympus Research Microscope in the department of Zoology.

Statistical analysis

The mean prevalence of each parasite and its percentage was calculated by using appropriate formula (Steel et al., 1980) and data were analyzed by analysis of variance (ANOVA). The whole data was fed into a Microsoft Excel 2010. A computer program (SPSS 11.5 for windows) and Primer software was used for data analysis. Student's t-test was used for the analytic assessment. The prevalence was calculated by dividing the number of animals harboring a given parasite by the total number of animals examined for a particular parameter. Percentage (%) to measure prevalence was also studied. The differences were considered to be significant when the p-value obtained was less than 0.05.

RESULTS

During the present study on helminth parasite infection in the common frog Rana cynophylctus of Gurez, only cestode and nematode parasites were recovered. The recovered cestode was Nematotaenia kashmirensis and nematodes were Rhabdias bufonis and Cosmocerca kashmirensis. The epidemiology of gastrointestinal helminth parasites is governed by host- parasite relationship and reaction with environmental conditions. The epidemiology of GIT parasites of frog in Gurez has been studied taking into consideration the overall prevalence (overall, seasonal, age-wise, gender-wise) and the associated risk factors with GIT parasites.

Overall Prevalence

Out of 135 specimens of frog (Rana cyanophlyctis) examined during the present sudy, 89 specimens (65.92%) were infected atleast by one type of helminth parasite. Only three species of helminth parasites i.e. one cestode spp. and two nematode spp. were observed. Out of 135 specimens examined, 59 (43.70%) of Rana cyanophylctis were infected with cestode Nematotaenia kashmirensis, 77 (57.03%) with nematode Cosmocerca kashmirensis and 86 (63.70%) with Rhabdias bufonis. Thus the prevalence of nematodes was much higher than cestodes; however the overall prevalence of helminth infection was 65.92% (89 out of 135) (Tables 1 & 2).

In 89 (65.92%) specimens of Rana cyanophylctis infected with Helminth parasites, a mean intensity & relative abundance of 11.57 & 7.62 respectively was observed. However the mean intensity and relative abundance of Rhabdias bufonis, Cosmocerca kashmirensis & Nematotaenia kashmirensis was 4.60, 2.93; 3.85, 2.2 & 5.71, 2.49 respectively (Tables 3 & 4)).

During the present study, no trematode parasite was observed in Rana cyanophlyctis from the study area which might be attributed to the fact that there are very less stagnant water bodies which act as reservoirs of infective stage trematode larvae and secondly the frogs remain for short time in the cold running water streams, nallas which are prevalent in the area. Further, higher infection was reported in areas with high anthropogenic load than in areas with less anthropogenic load. It may be of the reason that there are less stagnant water reserves/bodies, ponds in the vicinity of human habitations which act as breeding pools for frogs, as well as the reservoirs of helminth infective stages. In general the area, however isolated geographically from rest of the world, showed a high prevalence of helminth infection wherein the nematodes being more common than cestodes. This can be due to the fact that cestode Nematotaenia has a long life cycle and requires numerous intermediate hosts which are not readily available here in this particular area. The present study clearly indicates that Gurez being an isolated region is not in any way devoid of helminth populations, but has a good potential for helminth parasite proliferation.

The present study reveals that Rhabdias bufonis was the most common nematode parasite in the lungs of the host frog (Rana cyanophlyctis) in Gurez with a prevalence of 63.70%. The is in agreement with that of Ragoo and Maharaj, (2003) who observed a very high prevalence of Rhabdias fuelleborni in Bufo marinus (62.70%); Kloss (1971) reported a prevalence of 63%; Linzy et al (1998) reported prevalence of 67% for species collected from Bermuda. Gendron et al (2003) while studying the infection dynamics of lung worm Rhabdias spp. in adult leopard frogs (Rana pipiens) exposed to agricultural pesticides reported that high concentrations of pesticides leads to high establishment of adult worms in frogs of 21 days post-infection.

Single and Multiple species infection

Single and multiple species infection indicate that single species infection was higher than the corresponding multiple species infection, however a low prevalence of 5.10% was observed when all the three observed parasites were reported from the same host. These observations indicate that the parasites do enjoy their micro habitats more freely when singly than in multiple species **combination (Table 5).**

Season-wise prevalence

It was observed that the highest prevalence of infection was during summer followed by autumn and spring. However no amphibian host was studied during winter, because of hibernation and also because of disconnectivity of the study area from rest of the world. The prevalence of 70.17% and 47.36% (in summer), 63.63% and 45.45% (in autumn), and 52.94% and 35.29% (in spring) of nematodes and cestodes respectively was observed during the present study. It is pertinent to mention here that breeding season starts from late May to ending June which is a bit later than the adjacent valley habitats because of the temperature variations (Table 6).

Age-wise prevalence

The host animals were grouped into 3 categories viz, Below 6 months, 6 months to 2 years, and above two years. These three groups were examined for helminth infections. Out of 51 specimens belonging to age group below 6 months, only 18 (35.29%) were infected with cestodes, and 27 (52.94%) were infected with nematodes. Among nematodes, Cosmcerca spp. infection (52.94%) was found higher than Rhabdias spp. infection (50.98%). In the second age group i.e. 6 months to 2 years, out of 46 specimens examined 23 (50.00%) were having cestode infection, while 33 (71.73%) were infected with nematodes. However, Rhabdias infection was higher i.e. (71.73%) than Cosmocerca (58.69%). In all the 38 specimens of above 2 years age group, only 18 (47.36%) were infected with cestode (Nematotaenia spp.), whereas 27 (71.05%) were infected with nematodes. Among nematodes, Rhabdias infection was higher (71.05%) than Cosmocerca infection (60.52%). Thus a slight trend of increase in Nematode infection with increase in age and vice versa for cestodes was observed during the present study (Table 7).

Gender-wise prevalence

Out of the total 135 frogs examined, 74 were males and 61 were females. It was observed that prevalence of infection was slightly lower in males i.e. 43.24% (cestode) and 63.51% (nematode) than in females i.e. 44.26% (cestode) and 68.85% (nematode) which indicates that male and female frogs were almost equally prone to helminth infections. The observed prevalence of infection does not differ significantly in male and female hosts and as such of no significant value (Table 8).

DISCUSSION

While working on helminth parasitism in Amphibian host (Rana cyanophlyctis) in Gurez valley during the present study, about 66% (65.92%) hosts were found to be infected with atleast one or the other helminth parasite. The recovered parasites were only Nematodes & Cestodes. The observed parasites were Nematotaenia kashmirensis among cestodes, and Rhabdias bufonis and Cosmocerca kashmirensis from Nematodes. These findings are in accordance with those of Vashetko & Siddokov (1999) who reported Nematotaenia dispar in the small intestines of toads irrespective of the type of habitat, and Fotedar (1966) who reported new species of Nematotaenia kashmirensis in Bufo viridis. Studies conducted in many other parts of the world showed similar parasites infecting different amphibian species. Baker (1978) reported population changes in Rhabdias ranae in Rana sylvatica. Nematotaenia dispar was found to be the most dominant species of helminths parasitizing different amphibian hosts (Al-Sorkhy and Amr, 2003). High prevalence of Nematotaenia dispar and Rhabdias bufonis was reported by Mashaii (2005). Yildirimhan (1999) reported Nematotaenia dispar, Rhabdias bufonis and Cosmocerca commutate in Bufo viridis. Goater and Ward (1992) reported presence of Rhabdias in almost all areas of the world where the host species (frogs and toads etc) are present. Vashetko and Siddikov (1999) while studying distribution of helminthes in toads revealed a high prevalence (70.22%) of Rhabdias, besides cosmocerca spp. as well. This is in close conformity with the present study where the authors have found high prevalence of Rhabdias bufonis followed by Cosmocerca kashmirensis and Nematotaenia kashmirensis.

63 out of 135 examined host frogs were having mixed infection, i.e. having more than one type of helminth species. 19 out of 135 frogs were infected with cestodes only, whereas 38 out of 135 were infected with nematodes. This could be because of the fact that cestodes do have a complex type of life cycle and do require a number of intermediate hosts for completion of their life cycle.

Seasonal prevalence and distribution of helminth parasites showed that the infection rate was higher during summer followed by autumn and then spring. This is in agreement with the findings of Baker (1978); Barton (1998). The winters being harsh here and the frogs undergo hibernation and do not come out so the incidence of infection can not be assessed. Moreover there is no possibility of getting any host specimen due to snow everywhere. The spring starts a bit late than the adjacent Kashmir valley because of varied temperature, as such show low infection during this season. It can however be supported by the findings of Comas and Ribs (2013) which reveal that the low prevalence of helminth parasites in Pyrenean brook newt (Calotriton asper) was due to long hibernation period, the species, the lotic habitats and its reophilous lifestyle.

With regard to the age of the host it was observed that there was a marked increase in helminth infection with increase in age of the host. However, age resistance shown against Nematotaenia kashmirensis and Rhabdias bufonis was well marked. The young frogs showed slightly low prevalence of helminth infection for which the probable reason could be that the newly metamorphosed young frogs have not been so exposed to the infective helminth stages as compared to their respective elder ones. As these are now in terrestrial habitats and suddenly show a high helminth infection while these have crossed their first 5-6 months of their young age. Further the cestodes do have many life cycle stages and probably fail to get the specific intermediate hosts at proper time which may result in their failure to reach to the final definite host i.e., frogs. Thus exposure time to different intermediate hosts and increase in immunity with advancement of age of the host could be the probable reasons for such an increase in infection in these hosts and slight decrease in the advanced age respectively. Bursey and Dewolf (1998) studied the helminth fauna of the frogs, Rana catesbeiana, Rana clamitans and Rana palustris, from Coshocton country, Ohio and observed that the metamorphosing frog's contained no infections. Comas et al. (2014) in Italian edible frog Pelophylax kl. hispanicus found that prevalence and mean species richness increased with age.

The present study also reveals that males and females do not show any significant difference in the prevalence of infection which is in accordance with the studies of Ragoo and Omah-Maharaj (2003) who also reported similar rates of infection in both the sexes. Comas et al. (2014) in Italian edible frog Pelophylax kl. hispanicus observed that prevalence of helminth infection was positively correlated with snout-vent length and weight, but did not differ with body condition or sex.

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