Int.J.Curr.Microbiol.App.Sci (2014) 3(9) 933-939

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 3 Number 9 (2014) pp. 933-939**
http://www.ijcmas.com

Original Research Article

Endoparasitic Helminths of various Species of Frogs in Penang island, Peninsular Malaysia

Wahab A Rahman* and ZatilShakinah

School of Food Science and Technology Universiti Terengganu Malaysia
Kuala Terengganu, Malaysia
**Corresponding author*

Keywords

Frogs, Penang Island, Peninsular Malaysia, helminthic parasites

A B S T R A C T

From this study a total of 300 frogs comprising of 14 species were collectedfrom eight study sites in Penang Island, Peninsular Malaysia: Duttaphrynus melanostictus, Phrynoidisaspera, Hylaranaerythraea, H. labialis, H. nigrovittata, Polypedates leucomystax, Fejervary acancrivora, F.limnocharis, Limnonectesblythii, L. paramacrodon, L. ibanorum, L. ingeri, Microhylabutleri, and Kaloulapulchra. Eleven (11) species of helminthes were recovered from the frogs: Heterakissp., H. vesicularis, Trichostrongylussp., Pharyngodonspp., Ascarisspp., Oswaldocruzia spp., Rhabdias spp.,Glypthelminsstaffordi, Diplodiscussacculosus, Manodistomum spp., and Macracanthorynchus spp.

Introduction

Taxonomists have described more than 5,000 species of frogs (Duellman and Trueb, 1994). Disease has been a factor in the decline of amphibian populations worldwide, although other factors including habitat loss and fragmentation, chemical pollution, climate changes, introduction of exotic species, increased ultraviolet radiation, and natural pollution have also been responsible for the decline (Hayes *et al*., 2010).Malaysia is a country with an equatorial climate with high annual humidity ranging from 60% to 90% and rainfalls of 2000 to 3000 mm, resulting with a rich diverse biodiversity of wildlife (Department of Meteorology Malaysia, 2011). Malaysia harbors about 165 species from six families of anurans of which more than 150 species are found in Borneo (Inger and Stuebing, 2005), 107 species in Peninsular Malaysia, including 26 species in Penang Island (Ibrahim *et al*., 2008).

As reported by Duellman and Trueb (1986), there are two main threats to amphibians resulting from habitat destruction and environmental pollution. In Malaysia rapid developments for the last fifteen years have led to changes of breeding habitats for survival of amphibians (Ibrahim *et al* 1997).Negative outcomes from forest logging, draining swamps, covering streams of land developments, damming of rivers and draining of irrigation, introduction of weeds and livestock are the main causes that witnessed the inevitable decrease of

amphibianhabitats such as swamps, natural waterways, wetlands, forests, rural areas andagricultural lands; secondly isparasitic infection, which has been a concernfor the causal decline in frog populations in many parts of the world (Taylor*et al*., 2007).

In Malaysia there had been some reports of helminthic parasites recovered from frogs. Some nematodes had been described from the intestines of frogs (Fusco and Palmieri 1979; Moravec*at al*., 2007; Rahman*et al*., 2008). Other investigators had described several species of trematodes (Yuen, 1961; Rahman*et al* 2008) and one species of cestode (Ibrahim *et al*., 1997), also from the intestine of several species of frogs. However, most of these studies were conducted elsewhere in the country, andcurrent knowledge on the helminthic parasites of frogs from the Penang Island, Peninsular Malaysia is lacking.

Materials and Methods

(i) Study Sites

Penang Island, Peninsular Malaysia is located near the northwestern coast of Peninsular Malaysia (5° 24.0' N, 100° 14.0' E). Samplings were carried out during May 2010 to May 2011 from eight (8) areas, randomly selected from various parts of the island.

(ii) Sample Collections

Collection of frogs was carried out at night between 2000h -2300husing torch lights and head lamps. Besides using butterfly nets, frogs were also caught by hand, especially for those frogs found inside rocks cervices. Captured frogs were kept alive in aquarium boxes and transported back to the laboratory.

In the laboratory, the frogs were placed in the sink with running tap water to avoid dehydration of the frog's skin. Species of frogs were identified according to the descriptions of Inger and Stuebing (2005).

Subsequently, frogs were killed by using low doses of chloroform. Frogs were then weighed using a top pan digital electronic balance (SHIMADZU Brand Japan). Frogs were then measured for their snout-vent (cm) and total lengths (cm) by using a ruler scale.

Parasites

Each frog was examined for external abnormalities before the animal was euthanized. The stomach, intestines, rectum, heart and liver were recovered and examined for endoparasites. Blood samples were drawn from the heart.

The frogs as shown in were laid on the dissecting tray with belly facing upwards and their legs were pinned with pins. The first incision was done through the top layer of their skin by using a blade scalpel without damaging the organ. The incision was made vertically down the belly.

Next, the incision was done laterally across the hind legs and across the throat as well. Then the two lateral incisions were connected together with a vertical incision down the belly.

The flaps were opened and pinned them down. Subsequently, the same incision was done on the last layer of frogs' skin by twisting the scissor to avoid cutting organ under the chest bones. The flaps were opened and all fats were removed from the organs.

Results and Discussion

Species composition of frogs

Fourteen species of frogs from the five families were identified :*Duttaphrynus melanostictus* and *Phrynoidisaspera* (Family Bufonidae); *Fejervarya cancrivora, F. limnocharis, Limnonectesblythii, L. paramacrodon, L. ibanorum*and *L. ingeri* (Family Digroglossidae); *Microhylabutleri* and *Kaloulapulchra* (FamilyMicrohylidae)*; Hylaranaerythraea, H. labialis*and *H.Nigrovittata* (FamilyRanidae); and *Polypedates leucomystax* (Family Rhacophoridae) (Table 1).

Duttaphrynus melanostictus from Bufonidae family seemed to be the mostdominant frog species in Penang Island, followed by *H. erythraea*from the Ranidae family. The least common species of frog was *L. ibanorum* from the Dicrossidae family.

Composition of helminthes

A total of 8058 individual helminths were collected comprising of
43.7% nematodes, 36.8% trematodes and 19.5% acanthocephalans (Table
2). The most common nematode species found were *Heterakis*spp.,
*Heterakisvesicularis, Pharyngodon*spp., *Trichostrongylus*spp., *Ascaris*spp.,
*Oswaldocruzia*spp. and *Rhabdias*spp. The three species of trematodes were *Glypthelmins steffordi, Diplodiscus sacculousus* and *Manodistomum*spp. One species of acanthocephalan was collected and identified as *Macracanthorynchus* spp.
The frog species *Duttaphrynus melanostictus* seemed to be infected by almost alls pecies of parasites except for the trematode *Manodistomum*spp. Unlike other parasite species, four in particular are confined to three frog host species: thenematodes *Trichostrongylus*spp *and Rhabdias* spp. and the trematodes *Diplodiscus sacculousus* and *Manodistomum* spp. The acanthocephalan, *Macracanthorynchus* spp. seemed to be able to infect numerous species of frog hosts.

The mean significance between frogs and parasites

One-way ANOVA analysis and LSDtest on significant mean differences between the groups of helminthes parasites andfrogs for *H. vesicularis*,showed there was high mean significance ($p<0.05$) in*D. melanostictus*(63.14 ±24.67), *P. aspera*(48.57 ±20.20) and*H. erythraea*(44.00 ±17.06) compared to the other frog species: *P. leucomystax,F. cancrivora, F. limnocharis, M. butleri, L. blythii, L.ibanorum, H. labialis, L.paramacrodon, L. ingeri, K. pulchra* and *H.nigrovittata*. For *Heterakis* spp., there was high significance ($p<0.05$) in *P. aspera*(33.14 ±27.03) and *H. erythraea*(33.43 ± 13.31). *Ascaris*spp. was the least number of parasites found in frogs. *P. aspera*(29.71 ± 8.59) had a high significance at level ($p<0.05$).
*Macracanthorynchus*spp. in the frog*D. melanostictus*(120.42 ±
93.51) had a high mean significance (0.05) followed by *Phrynoidisaspera* (34.14 ± 18.51). Conversely, *D.sacculousus* numbers was also found to be low in frogs. But it has a high significance ($p<0.05$) in *D. melanostictus*(16.42 ± 5.93). *Glypthelmins staffordi* had high significance($p<0.05$) in *D.melanostictus*(259.85 ± 213.66), followed by *H. erythraea*(78.43 ± 42.14).

There were 300 frogs from 14 species captured in eight localities in Penang
Island, Peninsular Malaysia. Different species of frogs inhabit different habitat areas. Therefore, the distribution of frogs found in every sampling site was different.

Table.1 Number of frogs caught in eight study sites in Penang Island, Peninsular Malaysia

Frog species	**Total numberof frogs caught**
Family Bufonidae	
Duttaphrynusmelanostictus	90(27.0)*
Phrynoidisaspera	47 (14.1)
Family Ranidae	
Hylaranaerythraea	71(21.3)
Hylaranaraniceps	20 (6.0)
Hylarananigrovittata	2 (0.6)
Family Rhacophoridae	
Polypedatesleucomystax	16 (4.8)
Family Dicroglossidae	
Fejervaryacancrivora	10 (3.0)
Fejervaryalimnocharis	25 (7.5)
Limnonectesblythii	25 (7.5)
Limnonectesparamacrodon	3 (0.9)
Limnonectesibanorum	1 (0.3)
Limnonectesingeri	4 (1.2)
Family Microhylidae	
Microhylabutleri	4 (1.2)
Kaloulapulchra	5 (1.5)
Total	333 (100)

*Figure in bracket denotes percentage.

Table.2 Composition of helminthesrecovered from the respective frog hosts

Species of helminths	Total no. of helminthes (%)	Host species
Nematode		
Heterakis spp.	762 (9.4)	a,b,c,d,e,f, g,i,j,l,n*
Heterakisvesicularis	1345 (16.7)	a,b,c,d,e,f,g, h,i,j,k,l,m,n
*Pharyngodon*spp.	1021 (12.7)	a,b,c,d,f,g, h,j,k,l,m,n
*Trichostrongylus*spp.	77 (1.0)	a,c,f
*Ascaris*spp.	272 (3.3)	a,b,c,d,f,h,i
*Oswaldocruzia*spp.	31 (0.4)	a,b,d,f,h,j
*Rhabdias*spp.	13 (0.2)	a,b,n
Percentage	43.7 %	
Trematode		
Diplodiscussacculousus	144 (1.8)	a,c,j
Glypthelminsstaffordi	2601 (32.3)	a,b,c,d,f,j,k,l,m
*Manodistomum*spp.	214 (2.7)	e,f,j
Percentage	36.8 %	
Acanthocephalan		
*Macracanthorynchus*spp.	1578 (19.5)	a,b,c,d,e,f,j,k,m
Percentage	9.5 %	
Total number of helminthes	8058 (100%)	

*Note: Denotes the respective frog species
a: *Duttaphrynusmelanostictus*; b:*Phrynoidisaspera*; c: *Hylaranaerythraea;* d:*Polypedatesleucomystax*; e*:Fejervaryacancrivora*; f: *F. limnocharis*; g: *Microhylabutleri*;h:*Limnonectesblythii*; i: *L. ibanorum*; j: *H. labialis*; k: *L. paramacrodon*; l: *Lingeri; m: Kaloulapulchra, n: H.nigrovittata ingeri*;m:*Kaloulapulchra*; n: *H. nigrovittata*.

*Oswaldocruzia*spp. has not been previously recorded to occur in frog in Malaysia. A previous study discovered *Oxysomatium* spp. and *Rhabdias* spp. from *D. melanostictus* and *F.limnocharis* (Rahman*et al*., 2008); *Batrachonema synaptospicula* and *Paracosmocera* spp had also been described in*Ranamacrodon* spp. (currently*L. paramacrodon*) (Yuen, 1961; Fusco and Palmieri,1979) and *Paracapillariamalayensis* spp. from *D.melanostictus* (Moravec*et al*.,2007).

In a previous study, frog trematodes had been recovered from *Haematoloechu ssingaporensis* from the lungs of *Ranacancrivora* (currently *F.cancrivora*), *D. sacculosus*from the rectums of *R.erythraea* (currently*H. erythraea*) and *G. staffordi*from the intestines of *R. cancrivora, R. erythraea*and *R. macrodon.*

There was only one species of acanthocephalan, ie. *Macracanthorynchu s*spp. that was isolated. This species was abundantly found in the intestines of various species offrogs. Most of the parasiteswere at the larvae stage. A previous study stated that thisspecies was found in pigs and thus the medium of infection mode is unclear. Themost common adult form in amphibians is*Acanthocephalusranae*(DuellmanandTru eb, 1986) as well as *A.bufonis*(Goldberg *et al*., 2007). However, so far, not much has been reportedon *Macracanthorynchus*spp. and knowledge of this species is still lacking.

There was no cestode recorded from this study although the study by Ibrahim *et al*., (1997), reported a species of cestode, *Nematotaenia*spp. in *F. cancrivora*and *F. limnocharis* frogs in northern Peninsular Malaysia.

*Phrynoidisaspera*and *H.erythraea*were mostly exposed to water and the chance to get infected by helminthes is highercomparing to other species. As reported by Brooks *et al*. (2006), parasites require water for transmission when the frogs feed on aquatic intermediate hosts or when the parasite is swimming from one host to another host. Besides that, type of habitat also contributes to the parasite infection. A previous study by Kusrini*et al*. (2003) reported that the higher prevalence in nematode infection is mostly in terrestrial frogs compared to dwelling frogs. Since *D. melanostictus*is a terrestrial frog, thus explaining that *D.melanostictus* was infected with *H. vesicularis*.

References

Brooks, D. R., Leon-Regagnon, V., Mclennan, D. A., Zelmer, D. (2006). Ecological fitting as adeterminant of the community structure of Platyhelminthes parasitesof anurans.*Ecology*, 87: 76-85.

Duellman, W. E. and Trueb, L. (1986).*Biology of Amphibians*. United States. McGraw-Hill Publication. 670pp.

Fusco, A. C. and Palmieri, J. R. (1979). The nematode fauna of *Ranamacrodon* Dumeril and Bibron with supplementary data on *Batrachonema synaptospicula*Yuen, 1965(Nematoda: Amidostomatidae).

*Proc. Helminthological. Soc. Wash*ington,46: 289-292.

Goldberg, S. R., Bursey, C. R. and Kraus, F. (2007). First report of gastrointestinal Helminthes from Wokan Cannibal Frog, *Lechriodusmelanopyga* (Amphibia: Limnodynastidae),from Papua New Guinea. *Pacific Science*, 61: 429-432.

Ibrahim, J., Shahrul, A. M. S., Norhayati, A., Chan, K. O. and Muin, M. A. (2008). *The Common Amphibians and Reptiles of Penang Island, Peninsular Malaysia.* State Forestry Department of Penang.116pp.

Ibrahim, J., Samsudin, R. and Akil. M. M. (1997). Parasite fauna of *Ranacancrivora* Gravenhorst and *R. limnocharis* Boie from District IV of the Muda Agricultural Development Authority (MADA) Scheme, Kedah. *Proceedings of UniversitiSains Malaysia,* 119-29 pp.

Inger, R. F. and Stuebing, R. B. (2005).*A Field Guide to The Frogs of Borneo. Borneo, Sarawak.* Natural History Publication.209pp.

Kusrini, M. D., Suzanna, E. and Satrija, F. (2003).Endoparasites of two species of edible frogs, *Limnonectesmacrodon*, Boie and *Fejervaryacancrivora*, Gravenhorst, from Bogor, Indonesia.*Prosiding Seminar Hasil Penelitian Departemen Konservasi Sumberdaya Hutan*, 53-64.

Moravec, F., Modry, D. and Jirku, M. (2007). A new species of (Nematode:Capillariidae) from the intestine of the toad *Duttaphrynus melanostictus*(Anura) fromthe Malayan Peninsula.*Journal of Parasitology*, 93: 907-909.

Tyler, M. J., Wassersug, R. and Smith, B. (2007). How frogs and human interact: Influences beyond habitat destruction, epidemics and global warming.*AppliedHerpetology*, 4: 1-18.

Yuen, P. H. (1961). Three trematodes from Malayan amphibians including two new species.*The Journal of Parasitology*, 48: 532-535.