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Protozoan parasites of four species of wild anurans from a local zoo in Malaysia

Mohammad, K.N.[1,3*], Badrul, M.M.[2], Mohamad, N.[3] and Zainal-Abidin, A.H.[4]
[1]School of Bioscience and Biotechnology, Faculty of Science and Technology, Universiti Kebangsaan Malaysia, 43600 Bangi Selangor, Malaysia
[2]School of Environmental and Natural Resource Sciences, Faculty of Science and Technology, Universiti Kebangsaan Malaysia, 43600 Bangi Selangor, Malaysia
[3]Zoo Veterinary Hospital, Zoo Negara Malaysia, 68000 Ampang Selangor, Malaysia
[4]Faculty of Medicine, Universiti Teknologi Mara (UiTM), 40450 Shah Alam, Selangor, Malaysia.
[*]Corresponding author email: zainalaah@gmail.com
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Abstract. The parasitic protozoan fauna in sixty-six anurans comprising of *Duttaphrynus melanostictus*, *Phrynoidis juxtaspera*, *Hylarana erythraea* and *Polypedates leucomystax* collected from Zoo Negara Malaysia was investigated. The distribution and prevalence rate of parasitic species in the digestive tract and blood were examined. Seven species of intestinal protozoa (*Opalina ranarum*, *Cepedea dimidiata*, *Nycthetorus cordiformis*, *Entamoeba ranarum*, *Iodamoeba butschlii*, *Endamoeba blattae*, and *Tritrichomonas* sp.) and two species of blood protozoa (*Lankesterella* sp. and *Trypanosoma* sp.) were recorded. *Opalina ranarum* was the most common protozoan found in the rectum and intestine (prevalence rate: 34.8%) infecting all host species, with *P. juxtaspera* heavily infected with the parasite, whereas *Tritrichomonas* sp. was the least prevalent intestinal species infecting only *D. melanostictus*. Both *Lankesterella* sp. and *Trypanosoma* sp. were found in the blood of *H. erythraea*.

INTRODUCTION

Parasitic infections have always been a problem in zoos where animals are usually kept in captivity and in close contact with one another. Infections can be disseminated between these captive animals in the enclosure or perhaps between these animals and free-range non-captive wild animals living within the vicinity of the zoo. In addition to birds, anurans (frogs and toads) are known to inhabit zoos most of the time.

Reports on parasitic fauna of anuran species are still lacking in Malaysia especially regarding those living within the vicinity of zoos. There have been some reports on intestinal parasites of various other animals (Lim *et al.*, 2008) and the prevalence of cryptosporidiosis in Zoo Negara Malaysia (Lim *et al.*, 2007), but only Wahab *et al.* (2008) recently described parasitic fauna of two species of wild anuran collected from a riverine area in Penang.

The presence of parasitic infection in non-captive wild animals such as anuran living in close contact with captive animals may pose a great concern since these animals may act as a reservoir host for a parasite which can be spread to other animals or even to human beings. A recent study on two zoos in Italy indicated that zoonotic protozoans and gastrointestinal helminthes were common in zoo animals which may serve as potential reservoir and transmit these parasites to humans (Fagiolini *et al.*, 2010). Study on zoonotic infections in Auckland Zoo also found that zoo animals had infections with potential zoonotic agents such as

Giardia lamblia, *Salmonella* spp., *Compylobacter* spp *and Toxoplasma gondii* (Forsyth *et al.*, 2012).

In our study, protozoan parasitic fauna in four species of wild anurans collected from within the vicinity of Zoo Negara Malaysia were examined and identified as to indicate its potential role as possible zoonotic agents in the zoo.

MATERIALS AND METHODS

A total of 66 anurans comprising of aquatic, terrestrial and arboreal species were randomly collected within the vicinity of Zoo Negara in Hulu Kelang Selangor, Malaysia in May, June and July 2008 and November and December 2009. The anurans were caught by hand at night. Most of the specimens were obtained near water sources such as the artificial pond, river, and lake around the zoo. Different anuran species were kept in different plastic sampling bags in order to avoid death among individuals due to the toxic secretions produced by some anurans as a self-defence mechanism. The species of the anurans was identified during sampling according to the descriptions provided by Inger & Stuebing (2005).

In the laboratory, the anurans were anesthetized and dissected within 24 hours. The heart was punctured with a needle and fresh blood was used to prepare thick and thin blood smears on glass slides. The smears were air-dried, fixed with methyl alcohol and stained with Giemsa stain.

The alimentary tract was separated into its three anatomical parts, i.e. stomach, intestine and rectum. A small amount of the contents from each part was examined using direct smear with normal saline and iodine. The remaining contents were scraped into 3-ml Bijou bottles containing Polyvinyl Alcohol (PVA) as preservative. These fixed contents were stained with permanent Trichrome stain. The specimens were then observed under light microscope at x4, x10, x40 and x100 magnifications for parasite identification using guidelines by Kudo (1971).

RESULTS

The individual anurans were identified and categorised into four species of which nine (13.6%) were *Phrynoidis juxtaspera*, 13 (19.7%) *Duttaphrynus melanostictus*, 15 (22.7%) *Hylarana erythraea* and 29 (43.9%) *Polypedates leucomystax*. *Hylarana erythraea* is an aquatic species, *P. juxtasper* and *D. melanostictus* both are terrestrial while *P. leucomystax* is arboreal. There were nine species of parasitic protozoa identified of which seven were collected from the intestine and the rectum and two from the blood. There were no protozoan parasites found in the stomach. Of all four species of anurans, no one species was infected by all nine species of parasites, although *D. melanostictus* and *H. erythraea* harboured six species of parasites each (Table 1).

Phrynoidis juxtaspera was infected with five different species of protozoa confined to the rectum, i.e. *Opalina ranarum*, *Cepedea dimidiata*, *Entamoeba ranarum*, *Iodamoeba butschlii* and *Endamoeba blattae* (Tables 1 and 2), with *O. ranarum* being the most prevalent (55.6%). *Duttaphrynus melanostictus* was infected with six species of protozoa: four infecting the rectum (*E. ranarum*, *I. butschlii*, *E. blattae* and *Tritrichomonas* sp.) and two (*O. ranarum* and *Nycthetorus cordiformis*) infecting the intestine. Two species of protozoa (*O. ranarum* and *N. cordiformis*) were isolated from the intestine of *H. erythraea* and two species (*E. ranarum* and *E. blattae*) from the rectum. Two species of blood protozoa (*Lankesterella* sp. and *Trypanosoma* sp.) were also recorded from this host. The prevalence of *N. cordiformis* (66.7%) was the highest in *H. erythraea*. The tree frog, *P. leucomystax* was infected with only three species of intestinal protozoa (*O. ranarum*, *C. dimidiata and N. cordiformis*) and one species in the rectum (*E. ranarum*).

The results also indicated some variation occurred in the parasitic location of the species inhabiting the digestive tract viz. five species (*O. ranarum*, *C. dimidiata*, *E. ranarum*, *E. butschlii* and *E. blattae*) were

Table 1. Protozoan parasites, hosts, infection site and prevalence rate in four wild anuran species from Zoo Negara Malaysia

Protozoan Parasites	Hosts	Infection sites	Number infected hosts / total	Prevalence (%)
Opalina ranarum	*Phrynoidis juxtaspera*	Rectum	5/9	55.6
	Duttaphyrynus melanostictus	Intestine	4/13	30.8
	Hylarana erythraea		5/15	33.3
	Polypedates leucomystax		9/29	31.0
Cepedea dimidiata	*P. juxtaspea*	Rectum	1/9	11.1
	D. melanostictus	Intestine	0/13	0
	H. erythraea		0/15	0
	Polypedates leucomystax		10/29	34.5
Nycthetorus cordiformis	*P. juxtaspera*	Rectum	0/9	0
	D. melanostictus	Intestine	1/13	7.7
	H. erythraea		10/15	66.7
	Polypedates leucomystax		3/29	10.3
Entamoeba ranarum	*P. juxtaspera*	Rectum	1/9	11.1
	D. melanostictus		1/13	7.7
	H. erythraea		1/15	6.7
	Polypedates leucomystax		1/29	3.5
Iodamoeba butschlii	*P. juxtaspera*	Rectum	1/9	11.1
	D. melanostictus		2/13	15.4
	H. erythraea		0/15	0
	Polypedates leucomystax		0/29	0
Endamoeba blattae	*P. juxtaspera*	Rectum	1/9	11.1
	D. melanostictus		1/13	7.7
	H. erythraea		1/15	6.7
	Polypedates leucomystax		0/29	0
Lankesterella sp.	*P. juxtaspera*	Blood	0/9	0
	D. melanostictus		0/13	0
	H. erythraea		1/15	6.7
	Polypedates leucomystax		0/29	0
Trypanosoma sp.	*P. juxtaspera*	Blood	0/9	0
	D. melanostictus		0/13	0
	H. erythraea		1/15	6.7
	Polypedates leucomystax		0/29	0
Tritrichomonas sp.	*P. juxtaspera*	Rectum	0/9	0
	D. melanostictus		2/13	15.4
	H. erythraea		0/15	0
	Polypedates leucomystax		0/29	0

recovered from the rectum of one or more host species, one species (*N. cordiformis*) recovered only from the intestine of one host species and the other two species (*O. ranarum* and *C. dimidiata*) were recovered from both the intestine and rectum.

The ciliated protozoan *O. ranarum* was widespread in all species of anurans in this

Table 2. Overall prevalence rate of protozoan parasites in four wild anuran species from Zoo Negara Malaysia

Protozoan Parasites	Number of infected hosts / total	Prevalance (%)
Opalina ranarum	23/66	34.8
Cepedea dimidiata	11/66	16.7
Nycthetorus cordiformis	14/66	22.2
Entamoeba ranarum	4/66	6.1
Iodamoeba butschlii	3/66	4.6
Endamoeba blattae	3/66	4.6
Lankesterella sp.	1(66)	1.5
Trypanosoma sp.	1(66)	1.5
Tritrichomonas sp.	2(66)	3.0

study (prevalence 34.8% in Tables 1 and 2). This protozoan was multi-nucleated with variable shapes and sizes (592.50 ± 23.71 x 57.50 ± 48.66 µm). The other species *C. dimidiata* had a higher prevalence rate (34.5%) in *P. leucomystax* than in *P. juxtaspera* (11.1%) and had an elongated body. *Nycthetorus cordiformis* was relatively large ciliated protozoan had a distinct kidney-shaped body.

Among the three amoebas detected, *E. ranarum* was the smallest in size (8 to 15 µm in diameter). Its nucleus was spherical, the chromatin peripheral and the karyosome central. For *I. butschlii*, the nucleus had large karyosome and large and bright vacuole but peripheral chromatin was absent. In *E. blattae*, the nuclear membrane and peripheral chromatin were visible with a large, central chromatin dot. There were granules around the peripheral chromatin. Its cytoplasm was highly vacuolated or reticulated.

Tritrichomonas sp. found only *in D. melanostictus* (prevalence 15.4%), was characterised by the presence of three flagella at the anterior end. The prevalence of both of the blood protozoa in *H. erythraea* was low (6.7%). The extracellular trypanosome had a wavy undulating membrane producing a leafy body shape with a short free flagellum at the pointed and narrow end. The banana-shaped merozoite of intracellular *Lankesterella* sp. was found in the cytoplasm of the erythrocytes.

DISCUSSION

Opalina ranarum has been found in *Rana ridibunda*, *Rana temporaria* and *B. viridis* in Bulgaria (Tomova & Golemansky, 2001) and also in *Rana ridibunda* in Turkey (Senler & Yildiz, 2000). A local study found that this species was present in large numbers in *Rana limnocharis* and *D. melanostictus* (Wahab *et al.*, 2008). In the study the parasite was found in all the four anuran species and was the most common parasite.

Nyctotherus cordiformis mostly occurred in *H. erythraea* and not found in *P. juxtaspera* but co-existed with *O. ranarum* as also noted earlier by Jim'enez *et al.* (2001) in *Rana perezi* in Spain. Both of these ciliated protozoa are common protozoa inhabiting the digestive tracts of frogs and toads (Wahab *et al.*, 2008) and are believed to have a commensalism relationship with their hosts, causing no ill effects even though they may be present in high numbers (Poynton & Whitaker, 2001). As an opalinid protozoa, *C. dimidiata* may also play the same role as that of the above two species.

The presence of *Tritrichomonas* sp. may indicate that a possible transmission takes place between surrounding animals and the anurans because a related species *Tritrichomonas foetus* in particular is known to cause reproductive disease in cattle (Felleisen, 1999) and is common in cats (Tolbert & Gookin, 2009)).

It is interesting to note the presence of three species of amoebas in this study. *Entamoeba ranarum* was found in all four species of the anurans. This species was believed to cause amoebiasis in marine toads (*Bufo marinum*) and in frogs (Richter *et al.*, 2008). *Iodamoeba butschlii* is a non-pathogenic amoeba that usually occurs in humans, other primates and pigs (Roberts & Janovy, 2010). Likewise, the presence of *E. blattae* was reported in cockroach colon (Kudo, 1971). To our knowledge, there are no record of the presence of these two species in anurans. It is possible that the anurans may acquire the parasites through their feeding activities. This observation may implicate a possible transmission of these parasites

between other animals and humans with the anurans in Zoo Negara.

Observations of the protozoa found in the blood or rectum as noted in this study were no less important. *Lankesterella* sp. is known to be disseminated by a leech *(Placobdella marginata)* to anurans through blood sucking activities (Kudo, 1971). *Limnonectes kuhlii* and *Limnonectes blythii* have been recorded in the blood of other anurans from Thailand (Chutmongkonkul *et al*., 2006). Most *Trypanosoma* species found in anurans were non-pathogenic (Smyth & Smyth, 1980) in contrast with *Trypanosoma* species infecting humans. According to Kudo (1971), *T. rotorium*, which infects tadpoles and various other frog species, is also spread by the leech which acts as an intermediate host. We believe that leeches may also transmit both *Lankesterella* sp. and *Trypanosoma* sp. in *H. erythraea*. As an aquatic species, *H. erythraea* spends nearly all its time in bodies of water providing a much greater chance of being infected by leeches. This may explain the only infection of blood protozoa in frogs that inhabit water. It is not known whether infection of the other host species, i.e. *P. leucomystax* takes place through other intermediate hosts such as insects.

The protozoan parasites found in this study offer little evidence of strict host specificity in the anurans examined. Of the nine species of protozoa found, only three were restricted to a single host. The blood protozoa, *Lankesterella* sp. and *Trypanosoma* sp. were only found in *H. erythraea*, while *Tritrichomonas* sp. was only confined to *D. melanostictus*. On the other hand, multiple infections of protozoan parasites commonly occurred in the anurans examined (Table 1). Two species of protozoa were noted to be the parasites of different anuran hosts. *Duttaphyrynus melanostictus* and *P. leucomystax* were new hosts for *I. butschlii* while *P. juxtaspera*, *D. melanostictus* and *H. erythraea* were also new hosts for *E. blattae*. Further studies are required to confirm this.

In conclusion, nine species of protozoa were described in this study of which seven species were isolated from the intestinal tract and two species from the blood of the anurans. *Opalina ranarum* was the most prevalent (34.8%) protozoan species in the four species of anurans. The least input that this observation may offer is that some protozoan parasites are spreading between anurans which they may have acquired them from other surrounding animals or humans. Continuous monitoring of the presence of these parasites, in particular *Tritrichomonas* and *I. butshlii*, may be useful in an effort to prevent and control future possible outbreak of parasitic infections at the zoo.

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