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Trypanosome parasites in four species of ranid frogs from Peninsular Malaysia

Akira Miyata[1] and H. S. Yong[2]
[1]Department of Biology, Medical University of Oita, Hazama, Oita 879-56, Japan
[2]Department of Zoology, University of Malaya, 59100 Kuala Lumpur, Malaysia

ABSTRACT Four species of ranid frogs - *Rana cancrivora*, *Rana erythraea*, *Rana hosei* and *Rana limnocharis* - from several localities in Peninsular Malaysia were examined for trypanosome infection. *Rana hosei* is a forest frog, while *R. cancrivora*, *R. erythraea* and *R. limnocharis* are commensal species. All of them were infected with a variety of trypanosomes. The prevalence and type of infection (with single or mixed trypanosome-type) differed from locality to locality. Parasites of *Trypanosoma chattoni* group and *Trypanosoma rotatorium* group were present in all the four species of frogs. The next common group of parasites belonged to *Trypanosoma bocagei*-type which were found in *R. erythraea*, *R. limnocharis* and *R. hosei*. Trypanosomes of *Trypanosoma loricatum*-type were found in *R. erythraea* and *R. hosei*, *Trypanosoma tsukamotoi*-type in *R. erythraea* and *R. limnocharis*, *Trypanosoma hosei* in *R. hosei* and *Trypanosoma mega*-type in *R. limnocharis*. Of these parasites, *T. raksasa* the *T. loricatum*-type found in *R. erythraea*, is perhaps the largest trypanosome of anuran origin in the world. *Trypanosoma hosei* is unique, being very similar morphologically to the trypanosomes known from freshwater fishes.

ABSTRAK Empat spesies katak Ranidae, iaitu *Rana cancrivora*, *Rana erythraea*, *Rana hosei* dan *Rana limnocharis*, dari beberapa kawasan di Semenanjung Malaysia telah dikaji untuk jangkitan tripanosom. *Rana hosei* ialah katak hutan, manakala *R. cancrivora*, *R. erythraea* dan *R. limnocharis* ialah spesies komensal. Kesemua katak ini dijankiti oleh beberapa jenis tripanosom. Kelaziman dan jenis jangkitan (dengan jenis tripanosom tunggal atau bercampur) berbeza dari kawasan ke kawasan. Parasit-parasit daripada kumpulan *Trypanosoma chattoni* dan kumpulan *Trypanosoma rotatorium* wujud dalam keempat-empat spesies katak. Kumpulan parasit dengan kelaziman yang berikut tergolong kepada jenis *Trypanosoma bocagei* yang terdapat dalam *R. erythraea*, *R. hosei* dan *R. limnocharis*. Tripanosom-tripanosom daripada jenis *Trypanosoma loricatum* terdapat dalam *R. erythraea* dan *R. hosei*, jenis *Trypanosoma tsukamotoi* dalam *R. erythraea* dan *R. limnocharis*, *Trypanosoma hosei* dalam *R. hosei* dan jenis *Trypanosoma mega* dalam *R. limnocharis*. Di antara parasit-parasit ini *T. raksasa*, iaitu jenis *T. loricatum* yang terdapat dalam *R. erythraea*, adalah mungkin tripanosom berasal Anura yang paling besar di dunia. *Trypanosoma hosei* adalah unik kerana ia aldalah sangat serupa secara morfologi kepada tripanosom-tripanosom yang terdapat dalam ikan air tawar.

INTRODUCTION

The trypanosomes are members of the blood-inhabiting protozoa with flagella. They occur in humans, mammals, birds, reptiles, amphibians and fish. Some of them cause diseases in humans and livestock, and are therefore of public health and economic importance. These disease-causing trypanosomes are pathogenic and are frequently fatal. The trypanosomes that cause diseases in humans live also in other wild mammals, which serve as natural reservoirs for human infection.

Although human trypanosomiasis has been reported in Peninsular Malaysia, the taxonomic status of the trypanosomes was not clear. The few cases are believed to be secondary infection from a rodent trypanosome, *Trypanosoma lewisi*, which is transmitted by fleas [1]. This rodent trypanosome is however, like most animal trypanosomes, nonpathogenic.

In view of potential public health and/or economic importance, we initiated a study to determine the parasite fauna found in various vertebrates, particularly the commensal animals, such as lizards, frogs and toads, and rodents. It was hoped that the information gathered could enlighten us on the possibility of these parasites being transmitted to humans. One of our main focus was on the trypanosome fauna as there have been no detailed studies in this country.

The present report deals with trypanosome parasites found in four species of ranid frogs, viz. *Rana cancrivora* Gravenhorst, *Rana erythraea* (Schlege), *Rana hosei* Boulenger, and *Rana limnocharis* Boie.

MATERIALS AND METHODS

Frogs were collected from several locaities in Peninsular Malaysia (Table 1). Thin smears were prepared from cardiac blood, fixed in absolute methanol, and stained with 3% Giemsa for one hour. The smears were examined at x200 for the detection of trypanosomes. The parasites were photographed at x500 under oil immerson for measurement and identification.

RESULTS

All the four species of frogs studied were infected with trypanosomes. The prevalence and type of infection (whether infected with a single trypanosome-type or with two or more types in the same frog) are summarized in Table 1. Both the prevalence and type of

infection differed from locality to locality. In *R. cancrivora* and *R. limnocharis*, the frogs from Sekinchan, Selangor Darul Ehsan had the lowest infection rate.

Parasites of *Trypanosoma chattoni* group and *Trypanosoma rotatorium*-type were present in all the four species of frogs (Table 2). The next common group of parasites belonged to trypanosomes of *Trypanosoma bocagei*-type which were found in *R. erythraea, R. hosei* and *R. limnocharis*. Likewise, *Trypanosoma loricatum*-type was found in *R. erythraea* and *R. hosei*. *Trypanosoma raksasa* found in *R. erythraea* belonged to *T. loricatum*-type. In addition, *T. tsukamotoi*-type was found in *R. erythraea* and *R. limnocharis*, *Trypanosoma hosei* in *R. hosei* and *Trypanosoma mega*-type in *R. limnocharis* (Table 2).

Table 1. Prevalence and type of trypanosome infection (with single or mixed trypanosome-types) in four species of ranid frogs collected from various lacalities in Peninsular Malaysia.

Frog	Locality	Total No.	No. +ve	Type of infection Single	Mixed
R. cancrivora	Desa Pahlawan	3	2	2	0
	Ampang	3	3	3	0
	Sekinchan	47	4	3	1
R. erythraea	Desa Pahlawan	36	17	14	3
	Univ. Malaya	27	21	6	15
	Taman Templer	46	39	6	33
	Gombak	6	4	1	3
	Penang	21	12	9	3
R. hosei	Ampang	8	7	6	1
	Gombak	22	22	6	16
	Ulu Langat	4	3	0	3
	Kota Tinggi	13	12	3	9
	Cameron	19	10	6	4
	Bukit Rengit	47	35	28	7
R. limnocharis	Desa Pahlawan	2	1	1	0
	Taman Templer	10	7	3	4
	Sekinchan	11	0	0	0
	Batang Berjuntai	3	1	0	1
	Tanjung Karang	20	2	1	1

Table 2. Trypanosomes detected in four species of ranid frogs from Peninsular Malaysia.

Frog	Trypanosome
R. cancrivora	*chattoni*-type, *rotatorium*-type
R. erythraea	*chattoni*-type, *rotatorium*-type, *bocagei*-type, *tsukamotoi*-type, *raksasa* (*loricatum*-type)
R. hosei	*chattoni*-type, *rotatorium*-type, *loricatum*-type, *bocagei*-type, *hosei*
R. limnocharis	*chattoni*-type, *rotatorium*-type, *bocagei*-type, *tsukamotoi*-type, *mega*-type

DISCUSSION

About 60 species of anuran trypanosomes have been described from various parts of the world [2]. Prior to our studies, there was only a short report which dealt with anuran trypanosomes in Malaysia [3]. The trypanosomes were, however, not identified to the species or species groups.

In the present report, the trypanosomes in four species of ranid frogs from Peninsular Malaysia were identified to at least species groups. The exact number of species involved, however, remain to be ascertained.

Trypanosoma chattoni Mathis and Leger is a round trypanosome with a short free flagellum and without an undulating membrane. The round nucleus is situated near the central part of the body, and the kinetoplast is on the nucleus. Trypanosomes belonging to *T. chattoni*-type are cosmopolitan and are very common in frogs and toads.

Trypanosomes belonging to *T. rotatorium*-type are very common in Asia, Europe, Africa and South America. The distinctive feature of this group is the presence of a fusiform nucleus running parallel to the axis of the oval or elliptical body. Undulating membrane is conspicuous, with 3-6 folds. Free flagellum is present but sometimes unstained.

Trypanosoma loricatum (Mayer) is a cosmopolitan species, except North America and Australia. It is a monomorphic trypanosome with elliptical or oval body and costate surface. The kinetoplast is situated very near the round nucleus. Both ends of the body are rounded and the undulating membrane has many folds. *Trypanosoma raksasa* belongs to this *T. loricatum*-type.

Trypanosoma bocagei-type is distinguished by the kinetoplast being situated near the nucleus. In *Trypanosoma tsukamotoi*-type, the kinetoplast is situated close to the posterior edge of the nucleus. And in *Trypanosoma mega*-type, the nucleus is elliptical or lens-shaped.

Of the trypanosomes found in the ranid frogs, two species, viz. *Trypanosoma raksasa* Miyata and Yong and *Trypanosoma hosei* Miyata and Yong, are noteworthy. *Trypanosoma raksasa*, detected in the blood of the frog *R. erythraea* is apparently the largest species of anuran origin known in the world [1]. It was first

discovered in 1989 from frogs collected in the University of Malaya campus. The species name is derived from the Malay word *raksasa*, meaning giant or largest. This trypanosome resembles *T. loricatum* but has an attenuated posterior end and the large form of the parasite measures 50-160 micrometres in body length.

Trypanosoma hosei, named after the frog host, was discovered in the blood of the frog *R. hosei* [4]. It is clearly distinguished from all known trypanosomes of Anura by the slender body and the posterior position of the nucleus. It is very similar morphologically but not identical to those known from freshwater fishes. This is a rather rare trypanosome, having been found so far only in three *R. hosei* and has not been found in other frogs and toads.

The present study and our unpublished results show that trypanosome parasites are rather common in the frogs and toads of Peninsular Malaysia. Of the four species reported here, *R. hosei* is a forest frog, while *R. cancrivora*, *R. erythraea* and *R. limnocharis* often live near human settlements. Furthermore, *R. cancrivora* and *R. limnocharis* as well as some other species are eaten by the non-Muslims. As such, the potential public health hazards posed by these and other parasites harboured by the frogs need to be addressed.

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