

RESEARCH NOTES

SMALL MAMMAL ECTOPARASITES FROM ANCOL, JAKARTA, INDONESIA*

Between September 1975 and May 1976, 210 rats, mice and shrews were collected from Ancol, a district of the northern-most part of the city of Jakarta situated along Jakarta Bay. Animals were live trapped at night in two kampungs (villages) immediately south of the

*This study was supported through funds provided by the Naval Medical Research and Development Command, Navy Department, for Work Unit MF 51.524.009-0037.

The opinions and assertions contained herein are those of the authors and are not to be construed as official or as reflecting the views of the Navy Department or the Naval Service at large.

Reprint requests to Publications Office, NAMRU-2, Box 14, APO San Francisco 96263 or 7-1 Kung Yuan Rd., Taipei, Taiwan.

Ancol canal and an empty field, overgrown with sedge (alang-alang) and scrub bushes, just north of the canal (Fig. 1). Trapped animals were returned to the laboratory in the morning, anesthetized with chloroform, combed and searched carefully for remaining ectoparasites. Some chiggers were pooled for attempts at rickettsial isolations. Other ectoparasites were preserved in 70% ethanol, cleared in chloral phenol solution and mounted in Hoyer's medium.

Five chiggers species, *Leptotrombidium* (*Leptotrombidium*) *arenicola*, *L. (L.) deliense*, *L. (L.) bodense*, *Ascoschoengastia* (*Laurentel-*

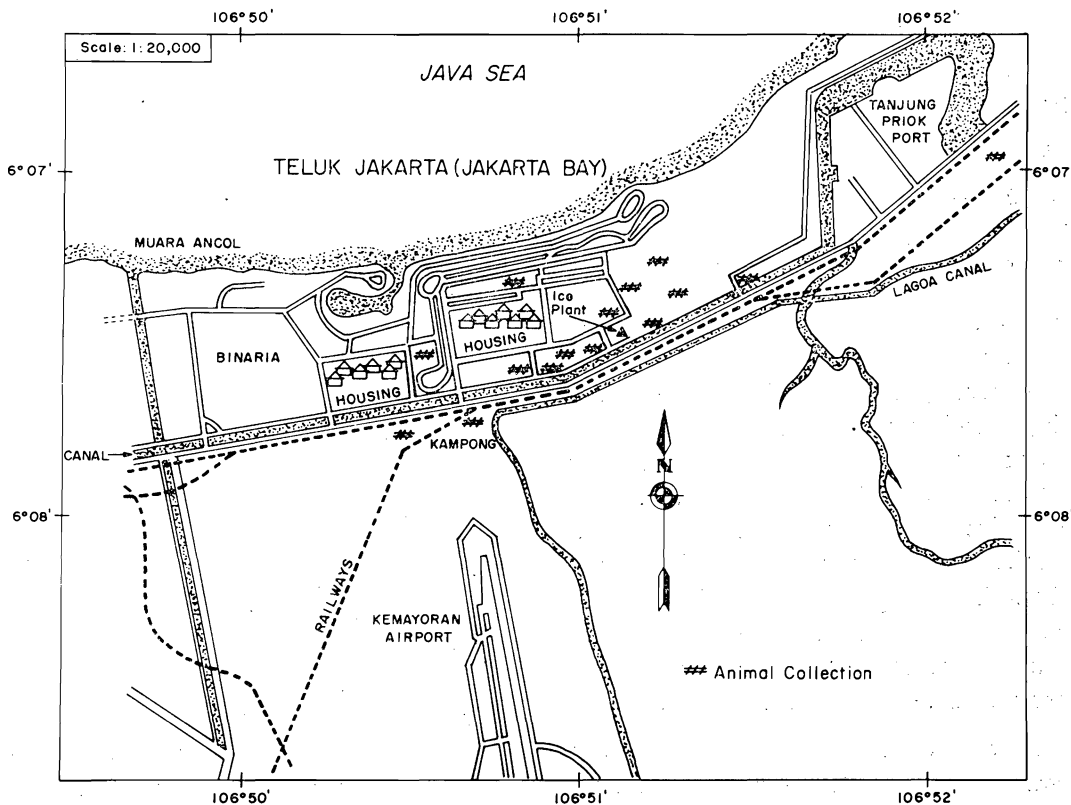


Fig. 1.—The Ancol area of Jakarta where small mammals were trapped.

Table 1

Number and species of hosts examined and ectoparasites recovered from Ancol, Jakarta.

Ectoparasites	Hosts						
	<i>Bandicota indica setifera</i>	<i>Rattus argentiventer</i>	<i>Rattus exulans</i>	<i>Rattus norvegicus</i>	<i>Rattus rattus diardii</i>	<i>Mus musculus</i>	<i>Suncus murinus</i>
	(91)	(15)	(2)	(8)	(29)	(6)	(59)
<i>Leptotrombidium (L.) arenicola</i>	0	78	0	0	762	0	1
<i>L. (L.) bodense</i>	0	0	0	0	5	0	0
<i>L. (L.) deliense</i>	0	0	0	0	1	0	0
<i>Ascoschoengastia (Laurentella) indica</i>	0	6	0	210	78	1	0
<i>Gahrliepia (Walchia) disparanguis pingue</i>	15	0	0	0	22	0	0
<i>Laelaps echidninus</i>	3	7	0	0	106	0	1
<i>L. nuttalli</i>	1452	84	1	731	90	0	21
<i>L. myonyssognathus</i>	2	0	0	1	7	0	37
<i>Liponyssoides</i> sp.	1	0	0	0	4	0	1
<i>Listrophoridae</i>	105	10	0	0	0	0	209
<i>Myobiidae</i>	1	0	0	0	0	11	0
<i>Xenopsylla cheopis</i>	4	0	1	1	26	0	5
Lice (undetermined species)	8	106	10	18	255	0	165

la) indica, and *Gahrliepia (Walchia) disparanguis pingue* were collected from seven species of mammals (Table 1). The most abundant chigger species was *L. (L.) arenicola*, a species previously reported only from Malaysia (Upham *et al.*, 1971. *J. Med. Entom.*, 8: 401). Species identification was confirmed by M.C. Nadchatram, Institute for Medical Research, Kuala Lumpur, Malaysia. *L. (L.) arenicola* was collected mostly from *Rattus rattus diardii* but was found also on *R. argentiventer* and the house shrew, *Suncus murinus*.

A total of 742 *L. (L.) arenicola* were collected from 29 *R. rattus diardii*; single pools of *L. (L.) arenicola* from one *R. rattus*

diardii and one *R. argentiventer* were positive for *Rickettsia tsutsugamushi* (details to be reported elsewhere).

One specimen of *L. (L.) deliense*, a known vector of scrub typhus in Indonesia (Gispén, 1949. *Med. Manndbl.*, 2: 238), was collected from a single *R. rattus diardii*. *A. (L.) indica* was collected in large numbers from *R. norvegicus*, *R. rattus diardii*. *R. argentiventer* and *Mus musculus*, most of which were trapped in the kampungs. Small numbers of *L. (L.) bodense* and *G. (W.) disparanguis pingue* were also identified. A single specimen of *Walchiella oudemansi* was recovered from the fields of scrub using the black plate method

of Hubert *et al.*, (1963. *Amer. J. Hyg.*, 78 : 131).

Mites from six taxonomic groups other than Trombiculidae were identified. *Bandicota indica setifera* were heavily infested with *Laelaps nuttalli*. The recovery of *L. myonyssognathus* is, to our knowledge, the first such from Indonesia. All fleas were *Xenopsylla cheopis*; lice were not identified.

ACKNOWLEDGEMENTS

The authors wish to thank Messrs. Sukaeri, Suryatman and Mrs. W. Riberu for their assistance in these studies.

J.R. HADI, E.E. STAFFORD, R. J. BROWN, D.T. DENNIS. U.S. Naval Medical Research Unit No. 2 Detachment, Jakarta, Indonesia.

THIRD CASE OF *SARCOCYSTIS* FROM MAN IN MALAYSIA

The two previous reports of *Sarcocystis* infection in man in Malaysia have prompted us to record another infection from this country. This was an infection noted by one of us (K.P.) as an incidental finding during routine examination of biopsy specimens of muscle in 1970.

The patient was a 20 year old student from Penang who had developed ischaemic contracture of the flexor muscles of the right foot following fracture of the right tibia and fibula, consistent with the clinical diagnosis of ischaemic contracture.

Although several pieces of muscle were sectioned, only a single section contained one cyst in a muscle fibre (Fig. 1). The cyst was ovoidal, 125 by 90 μm , in cross-section and had no evidence of compartments or cytophaneres, the wall being very thin as in the previous cysts reported by Kannan Kutty and Dissanaiké (1975. *Trans. Roy. Soc. Trop. Med. Hyg.*, 69: 503) and Kannan Kutty *et al.*, (1975. *Southeast Asian J. Trop. Med. Pub. Hlth.*, 6: 400). The zoites, as far as they could be measured from sections, were about 3 by 1 μm in size.

This cyst had a very strong resemblance to the cysts of *S. booliati* described by Dissanaiké

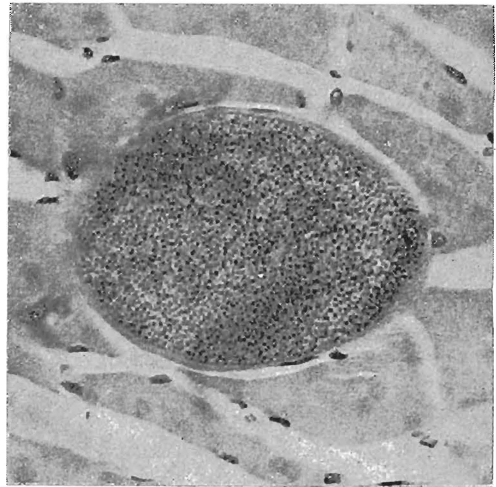


Fig. 1—Section of muscle showing cysts in cross section (Stained in Haematoxylin & Eosin. X 440).

and Poopalachelvam (1975. *Southeast Asian J. Trop. Med. Pub. Hlth.*, 6: 175) in the moon rat. This record further supports the suggestion made by Kannan Kutty *et al.*, (1975) that this infection is much more common in Malaysia.

K. PRATHAP and A.S. DISSANAIKE*. Departments of Pathology and *Parasitology, Faculty of Medicine, University of Malaya, Kuala Lumpur, Malaysia.

ANGIOSTRONGYLUS MALAYSIENSIS IN INDONESIA*

Bhaibulaya and Cross (1971. *Southeast Asian J. Trop. Med. Pub. Hlth.*, 2 : 527) described a new species of rat lung worm, *Angiostrongylus malaysiensis* from a Malayan *Rattus jaloriensis*. The parasite was subsequently reported from Thailand (Bhaibulaya and Techasoponmani, 1972. *Southeast Asian J. Trop. Med. Pub. Hlth.*, 3 : 451), widespread area of Malaysia (Lim, 1976. *Med. J. Malaya.*, 30 : 207) and from Indone-

sia (Carney *et al.*, 1974. *Third Int. Congr. Parasit. Munich, Germany*, 2 : 714). In the latter report third stage larvae from the giant African snail, *Achatina fulica*, collected in Samarang, North Central Java, were fed to laboratory rats and adult *A. malaysiensis* recovered from the pulmonary vessels.

*This study was supported through funds provided by the Naval Medical Research and Development Command, Navy Department, for Work Unit MF51. 524.009-0056.

The research described in this report involved animals maintained in animal care facilities fully accredited by the American Association for Accreditation of Laboratory Animal Care.

The opinions and assertions contained herein are those of the authors and are not to be construed as official or as reflecting the views of the Navy Department or the Naval Service at large.

Reprint requests to Publications Office, NAMRU-2, Box 14, APO San Francisco 96263 or 7-1 Kung Yuan Road, Taipei, Taiwan.

Further studies have confirmed the presence of *A. malaysiensis* from localities on the island of Sumatra, Indonesia, where adult worms have been recovered from the heart and lungs of 3 species of rats, *R. jaloriensis*, *R. diardii* and *R. exulans*. Adult worms have also been recovered from laboratory rats 50 days after being fed larvae digested (Wallace and Rosen, 1969. *Malacologia*, 7 : 427) from *A. fulica*.

Mixed infections of *A. malaysiensis* and *A. cantonensis* in both snails and rats were not unusual (Table 1). Adult male and female *A.*

Table 1

Angiostrongylus malaysiensis from selected areas of Sumatra, Indonesia.

Location	Host species	Number examined	Per cent infected	Per cent with mixed infection*
North Sumatra				
Medan	<i>Rattus diardii</i>	42	4.7	100
Central Sumatra				
Jambi	<i>Achatina fulica</i>	18	5.5	100
South Sumatra				
Lubuk Linggau	<i>Rattus diardii</i>	21	23.5	20
Baturaja	<i>Rattus diardii</i>	32	3.1	100
	<i>Rattus jaloriensis</i>	1	100	0
	<i>Achatina fulica</i>	43	33.5	27
Lampung	<i>Rattus diardii</i>	44	2.2	0
(Way Abung III)	<i>Rattus exulans</i>	13	15.3	50
	<i>Rattus jaloriensis</i>	9	55.5	80
	<i>Achatina fulica</i>	133	28.6	100

*Both *A. malaysiensis* and *A. cantonensis* occurring within the same host.

RESEARCH NOTES

malaysiensis and *A. cantonensis* were recovered from the same naturally infected rat host, and larvae of both sexes and both species were recovered from the same snail. In some instances of mixed infections, only one sex of one species was present with individuals of both sexes of the other species. Some host had only one species and adult worms of either sex or both sexes.

Although potential intermediate and definitive hosts from other Indonesian islands have been examined, *A. malaysiensis* has been found only on Java and Sumatra. This appears to be the first report from Sumatra.

Previous records for this parasite in Indonesian *R. diardii* and *R. exulans*, and records of mixed infections of *A. malaysiensis* and *A. cantonensis* in these rats are not known.

Confirmation of the adult worms to species was made by Dr. Manoon Bhaibulaya, Department of Helminthology, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand.

E.E. STAFFORD, S. TANUDJAJA, PURNOMO and W.P. CARNEY*. NAMRU-2, Jakarta Detachment, APO San Francisco 96356; *NAMRU-2, APO San Francisco 96263.

THE FIRST RECORD OF *HUNTERELLUS HOOKERI* PARASITIZING *RHIPICEPHALUS SANGUINEUS* IN INDONESIA

In the course of collecting study material in the field, specimen collected included 39 engorged nymphs of *Rhipicephalus sanguineus*, 9 specimens collected in April and 30 specimens in September 1975, respectively. These were hand-picked, collected from crevices in houses. Of the first collection, 3 (30%) and 6 of the second (20%) turned out be parasitized by chalcid wasps, *Hunterellus hookeri*.

The parasites emerged after the nymphs were placed in a controlled rearing chamber with humidity 70-80% under room temperature. This humidity was achieved by placing concentrated solution of KCl in the chamber. Each parasitized nymph gave rise to 9-13 wasps, which could only survive for 2-3 days in the chamber. The developmental periods of the wasps could not be ascertained.

Subsequent examination of the parasitized nymphs revealed that the internal part of the body was filled with hard black minute bodies of irregular sizes, seemingly representing faecal matter of the larvae. No sign of developed pupae could be seen. Of more interest were the portae from which the adult wasps emerged. These were located posterior or lateral to the anus, at places where the integuments were thin (Fig. 1). It was hard to trace whether this exit hole also represent the site of oviposition.

Although records of chalcid wasps parasitizing *Rh. sanguineus* are known from elsewhere in the world such as from Angola (Fiedler, 1953. *Onderstepoort J. Vet. Res.*, 26: 63), Florida (Bishop, 1934. *Proc. Entom. Soc. Wash.*, 36: 87), Kampala-Uganda (Steyn,

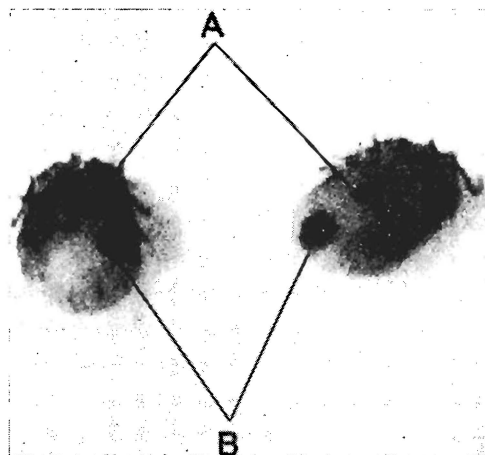


Fig. 1—Ventral view of parasitized nymphs of *Rh. sanguineus* showing: (A) anus, (B) portae from which hymenoptera parasites emerged.

1955. *E. Afr. Med. J.*, 32: 257), and Puerto Rico (Tate, 1941. *J. Agric. Univ. P. Rico*, 25: 1), this observation is the first of its nature reported from Indonesia.

ACKNOWLEDGEMENTS

The author wishes to thank Dr. E. Hardy from the University of Hawaii for his assistance and to Dr. B.R. Subha Rao, Head of Hymenoptera Section, Insect Identification Centre of the British Museum, for kindly identifying the chalcid wasps. Also thanks to the Director of the Museum Zoologicum Bogoriense, Dr. S. Kadarsan, for his full support in this study.

HASAN BASRI MUNAF. Museum Zoologicum Bogoriense, National Biological Institute, Bogor, Indonesia.

PREVALENCE OF HEMATOZOA IN SOME ANURANS FROM THE MALAYAN PENINSULA†

Anuran hematozoa previously reported from Southeast Asia belong primarily to the genera *Trypanosoma* Gruby, 1843, *Hemogregarina* Danilewsky, 1885, *Dactylosoma* Labbé, 1894, and *Lankesterella* Labbé, 1894. However, only the genera *Trypanosoma* and *Dactylosoma* have been reported from the Malayan Peninsula. In view of the limited published information with regard to the Malayan hematozoa, 344 anurans, representing four genera and eight species, were collected from various localities in Peninsular Malaysia and Singapore (Table 1) between August, 1974, and October, 1975. Examination of Giemsa-stained blood films showed that 13% of the 344 were infected with *Trypanosoma* spp., 10% with *Hemogregarina* spp., 9% with *Dactylosoma* spp., and 4% with *Lankesterella* spp. Mixed infections of two or three different genera in a single host were not uncommon. These findings represent the first report of the genera *Hemogregarina* and *Lankesterella* in anurans in the Malayan Peninsula.

Nabarro [1907. Cited by Bardsley and Harmsen, 1973. In *Advances in Parasitology* (Ed. Ben Dawes), Vol. 11, pp. 1-73, Academic Press, London and New York] described *Trypanosoma belli* in *Rana* sp. and a *Trypanosoma* sp. in *R. temporaria* in Hong Kong. Patton (1908. Cited by Bardsley and Harmsen, 1973. *Ibid.*) reported *T. hendersoni* in *R. hexadactyla* and *R. tigrina* in Vietnam. Mathis and Leger (1911. *Ann. Inst. Pasteur, Paris*, 25 : 671) reported *T. bocagei parva*, *T. b. magna*, *T. borelli*, *T. chattoni*, and *T. elegans* from *Bufo melanostictus* in Tonkin. *Trypano-*

soma rotatorium was found in *R. esculenta* and *R. plancyi* in Taiwan (Ogawa and Uezaki, 1917. *Arch. Protistenk.*, 57 : 14.), *R. esculenta* from Hong Kong (Hunter, 1908. Cited by Bardsley and Harmsen, 1973. *loc. cit.*) and *R. guentheri*, *R. limnocharis*, and *R. tigrina* from Tonkin (Mathis and Leger, 1911. *loc. cit.*). *Trypanosoma* spp. have also been recorded from *Rhacophorus leucomystax* (= *Polypedates l.*) from Java, Sumatra and Indochina, (Walton, 1950. *Anat. Rec.*, 108 : 626) and from *Kaloula pulchra* (= *Microhyla p.*) from Indochina (Walton, 1950. *J. Parasit.*, 36 : 40), and from *R. macrodon* in the Zoological Gardens, London, but which had been collected in Malaya (Wenyon, 1926. *Protozoology*. Vol. 2, pp. 779-1563, Baillière, Tindall and Cox, London).

Four morphologically distinct trypanosomes were found in five hosts in nine localities (Table 1). *Rana erythraea* showed a 41% infection rate, *R. hosei* 38%, *Bufo melanostictus* 6%, *R. limnocharis* 3%, and *R. macrodon* 2%. All five host species showed parasites morphologically resembling *T. chattoni*. All host species except *R. limnocharis* also exhibited infections with a *T. rotatorium*-like form. *Rana erythraea* and *B. melanostictus* both harbored parasites similar to *T. borelli*, and one *R. erythraea* was infected with a small unidentified C-shaped trypanosome. However, the identifications of these trypanosomes must be considered tentative since they are based solely on parasites observed in blood films without attempting to culture any of the forms; prevalence may also be higher than indicated because of limitations of solely relying on blood films (Bardsley and Harmsen, 1973. *loc. cit.*).

Mathis and Leger (1911. *loc. cit.*) observed a *Hemogregarina* sp. and *H. boueti* Franca, 1910, in *Bufo melanostictus* in Tonkin, and

†This study was supported by grant AI 10051 (UC ICMR) to the Department of International Health, School of Medicine, University of California, San Francisco, from the National Institute of Allergy and Infectious Diseases, National Institutes of Health, U.S. Public Health Service.

Table 1

Prevalence of infection with *Trypanosoma* (T), *Hemogregarina* (H), *Dactylosoma* (D), and *Lankesterella* (L) in some anurans (n = number examined from the Malayan Peninsula).

Localities	<i>Bufo</i>	<i>Rana</i>	<i>Rana</i>	<i>Rana</i>	<i>Rana</i>	<i>Rhacophorus</i>	Other	Total
	<i>melanostictas</i>	<i>cancrivora</i>	<i>erythraea</i>	<i>limnocharis</i>	<i>macrodon</i>	<i>leucomystax</i>		
	n T H D L	n T H D L	n T H D L	n T H D L	n T H D L	n T H D L	n T H D L	n T H D L
Johore:								
Johore Bahru	5 1 0 0 0				1 0 0 0 0			6 1 0 0 0
Kota Tinggi	11 2 0 0 0	1 0 0 1 1			1 0 1 1 1		13 ¹ 5 0 2 0	26 7 1 4 2
Negeri Sembilan:								
Ulu Jempol	2 0 0 0 0	15 0 1 0 0	23 8 7 8 0	9 1 1 5 0	47 1 1 2 0	10 0 0 0 0	9 ² 0 2 0 0	115 10 12 15 0
Pahang:								
Kuala Tahan						9 0 0 0 0		9 0 0 0 0
Tasek Bera	6 0 0 0 0		43 19 12 11 5		1 0 0 0 0	5 0 0 0 0		55 19 12 11 5
Tioman Island			1 1 0 1 1			9 0 1 0 0		10 1 1 1 1
Perak:								
Hilir District	11 0 1 0 0		6 2 4 0 0		1 0 0 0 0			18 2 5 0 0
Perlis:								
Kangar	1 0 0 0 0			25 0 0 0 0				26 0 0 0 0
Selangor:								
Kuala Lumpur	23 1 0 0 0				36 0 0 0 0			59 1 0 0 0
Singapore:								
		4 0 0 0 1	4 2 1 0 1	1 0 0 0 0				9 2 1 0 2
Other:								
	3 ³ 0 0 0 0		1 ⁴ 0 0 0 0		5 ⁵ 1 1 0 3	2 ⁶ 0 0 0 0		11 1 1 0 3
Total	62 4 1 0 0	20 0 1 1 2	78 32 24 20 7	35 1 1 5 0	92 2 3 3 4	35 0 1 0 0	22 5 2 2 0	344 44 33 31 13

¹*Rana hosei*, ²*Leptobranchium hasselti*, ³Kuala Pilah, Negeri Sembilan, ⁴Fraser's Hill, Pahang, ⁵Gombak and ⁶Bukit Lanjan, Selangor.

described *H. scheini*, previously reported as *Hemogregarina* sp. from *R. tigrina* in Annam (Schein, 1911. *C.R. Soc. Biol.*, 70:1000), from *R. guentheri*, *R. limnocharis*, and *R. tigrina* from Tonkin. Prowazek (1912. *Arch. Protistenk.*, 26:250) reported a hemogregarine in *B. melanostictus* in Sumatra, while Bergeron (1965. *FAO Report No. 2047*, pp. 1-95 Rome) listed *H. bengari* and *H. fasciatus* from "batracians" in Cambodia. Although no references to the two species of *Hemogregarina* listed by Bergeron (1965. *Ibid.*) could be found, it can be noted parenthetically that Mathis and Leger (1911. *loc. cit.*) reported the hemogregarine *Laverania bungari* Billet, 1898, from the snake *Bungarus fasciatus* in Tonkin. In view of the lack of supporting references and the correspondence between the names used by Mathis and Leger and by Bergeron, both *H. bengari* and *H. fasciatus* (*sensu* Bergeron, 1965) are designated *nomina dubia*.

Several distinct forms of hemogregarines were observed in seven hosts from seven localities (Table 1). *Rana erythraea* showed the highest prevalence of infection (31% of all examined), while 22% of the *Leptobranchium hasselti* examined were positive. Other hosts showed considerably lower rates of infection: *R. cancrivora* (5%), *R. limnocharis* (3%), *R. macrodon* (3%), *Rhacophorus leucomystax* (3%), and *Bufo melanostictus* (2%).

Dactylosoma ranarum (Kruse, 1890) (= *Hemogregarina splendens*) has been reported from *R. guentheri* in Tonkin (Mathis and Leger, 1911. *loc. cit.*) and Taiwan (Manwell, 1964. *J. Protozool.*, 11:526), and a *Dactylosoma* sp., tentatively identified as *D. ranarum* was found in *R. limnocharis* in Bukit Lanjan (Selangor) Malaysia (Landau *et al.*, 1974. *Southeast Asian J. Trop. Med. Pub. Hlth.*, 5:144). The only other representative of the genus in Southeast Asia, *D. taiwanensis*, was described by Manwell (1964. *loc. cit.*) from *R. limnocharis* in Taiwan.

Two *Dactylosoma* spp. occurred in five hosts in four localities (Table 1). *Rana erythraea* showed a 26% rate of infection. *Rana hosei*, *R. limnocharis*, *R. cancrivora*, and *R. macrodon* showed rates of infection with this genus of 15, 14, 5, and 3%, respectively. Preliminary studies indicate that while one of these species is *D. ranarum*, the other appears to be undescribed. A more detailed account of these two species will be given elsewhere.

Mathis and Leger (1911. *loc. cit.*) reported *Lankesterella minima* (Chaussat, 1850) (= *Hemogregarina m.*) from Tonkin; however, these authors did not adequately indicate whether they recovered *L. minima* from *R. guentheri*, *R. limnocharis*, and *R. tigrina* or from only *R. limnocharis*. Walton [1948. *J. Parasit.*, 34 (Suppl.):28] also records *L. minima* in *R. hexadactyla* from Burma, India and Ceylon.

Two, possibly three, *Lankesterella* spp. were found in three hosts in five localities (Table 1). *Rana cancrivora* exhibited 10% rate of infection, while *R. erythraea* and *R. macrodon* showed rates of 9 and 4%, respectively.

In addition, an as yet unidentified protozoan was found in the white blood cells of one *Leptobranchium hasselti*. Specific identification of this parasite is currently in progress.

ACKNOWLEDGEMENT

The authors thank Mr. Chong Kon Chu, University of California ICMR, for technical assistance, the Director, Institute for Medical Research, Kuala Lumpur, and his staff, and En. Mohd. Khan bin Momin Khan, Chief Game Warden, Kuala Lumpur, for their cooperation and support.

JOANN S. SULLIVAN and JAMES J. SULLIVAN*.
University of California ICMR, Institute for Medical Research, Kuala Lumpur, Malaysia.

*Present address: Central America Research Station, C/o U.S. Embassy, APO New York, N.Y. 09889.

DETERMINATION OF THE UNSATURATED VITAMIN B₁₂ BINDING CAPACITY IN NORMAL AND PHYSIOPATHOLOGICAL CONDITIONS

It has been well established that vitamin B₁₂ is attached to protein and most of it is stored in the liver in the human body. In liver disease and myeloproliferative group of disorders, the serum vitamin B₁₂ level is elevated while the vitamin B₁₂ binding capacity may be normal, elevated or decreased (Stevenson and Beard, 1959. *New Eng. J. Med.*, 260 : 206; Hift, 1966. *S. Afr. Med. J.*, 40 : 437; Retief *et al.*, 1967. *Blood*, 29: 501; Retief *et al.*, 1969. *Brit. J. Haemat.*, 16 : 231). Determination of the total vitamin B₁₂ binding capacity, i.e., serum vitamin B₁₂ level and the unsaturated vitamin B₁₂ binding capacity (UBBC), is therefore helpful in differential diagnosis and monitoring the treatment in these diseases. As the data of UBBC in Thai normal subjects and patients with diseases described above have not been reported, the present study was undertaken to assay the UBBC in the serum of Thai blood donors, pregnant women, patients with liver disease, chronic myelocytic leukemia and lymphoma.

The UBBC was assayed in serum of Thai blood donors of both sexes from the Thai Red

Cross Society. This assay was also done in pregnant women who attend the antenatal clinic at the Department of Obstetrics and Gynaecology, Siriraj Hospital; patients with infectious hepatitis, cirrhosis, amoebic liver abscess, chronic myelocytic leukemia, hepatoma, carcinoma of the liver and lymphoma from the Hospital for Tropical Diseases and Siriraj Hospital. The UBBC was determined by the radioisotope dilution and coated charcoal method of Gottlieb *et al.*, (1965. *Blood*, 25: 875) with a slight modification. An aliquot of serum was incubated with excess of ⁵⁷Co-vitamin B₁₂ and the excess of unbound radioactive vitamin was removed by the polyvinylpyrrolidone (PVP) coated charcoal. The UBBC was calculated from the radioactivity of the supernatant and expressed as pg vitamin B₁₂ per ml of serum.

The mean values of the UBBC in serum of 60 male Thai blood donors (960 ± 199 pg/ml) was not significantly different from that of 32 female Thai blood donors (959 ± 186 pg/ml). The values and the ranges in these subjects are shown in Table 1 in which data from the other authors are also included.

Table 1
Assay of UBBC of serum from Thai blood donors and normal subjects reported
by various authors.

No.	Mean ± S.D.	Range	References
3	1088	902 - 1226	Gottlieb <i>et al.</i> , (1965)
5	1149	850 - 1394	Retief <i>et al.</i> , (1967)
N.i.	1386	800 - 2100	Grassman and Retief (1969)
35	1778 ± 453	777 - 2967	Saraya <i>et al.</i> , (1973)
60 (M)	960 ± 199	703 - 1520	Present study.
32 (F)	959 ± 186	718 - 1401	

Table 2

Assay of UBBC of serum from normal subjects and patients with various diseases.

	Number examined	UBBC (pg/ml)		T-test Normal vs. Diseases
		Mean \pm S.D.	Range	
Normal - Male	60	960 \pm 199	703 - 1520	-
- Female	32	959 \pm 186	718 - 1401	-
Pregnant women	100	1088 \pm 321	548 - 2261	P < 0.05
Infectious hepatitis	44	577 \pm 401	89 - 2146	P < 0.001
Cirrhosis	10	471 \pm 445	3 - 1192	P < 0.001
Amoebic liver abscess	17	987 \pm 550	140 - 2056	P > 0.05
Chronic myelocytic leukemia	16	3337 \pm 830	993 - 8374	P < 0.001
Hepatoma and carcinoma of liver	9	467 \pm 403	29 - 991	P < 0.001
Lymphoma	7	1172 \pm 543	484 - 2093	P < 0.01

Results of the UBBC in serum of pregnant women, patients with various diseases are shown in Table 2. The mean value of the UBBC in pregnant women was significantly higher than that of the female Thai blood donors ($P < 0.05$). The values in patients with infectious hepatitis, cirrhosis, hepatoma and carcinoma of the liver were significantly lower ($P < 0.001$) than that of the Thai blood donors. These values were significantly higher ($P < 0.01$) in patients with chronic myelocytic leukemia and lymphoma but were normal in patients with amoebic liver abscess.

Findings that serum UBBC decreased in patients with liver disease, i.e., infectious hepatitis, cirrhosis and liver neoplasm were in accordance with results reported previously (Beard *et al.*, 1954. *Blood*, 9 : 789; Gottlieb *et al.*, 1965. *Blood*, 25 : 875; Retief *et al.*, 1969. *Brit. J. Haemat.*, 16 : 231). In acute liver disease, the liver released the store vitamin B₁₂ into the circulation, the serum vitamin B₁₂ was therefore very high which resulted in the low serum UBBC.

It has been well established that there are at least 2 vitamin B₁₂ binding proteins in the serum, i.e., transcobalamin I and transcobalamin II (TCI and TCII). TCI is nearly saturated with endogenous vitamin B₁₂ while TCII binds almost all vitamin B₁₂ administered *in vivo* or its addition *in vitro* (Hall and Finkler, 1966. *Blood*, 27 : 611). In chronic myelocytic leukemia, TCI increased while TCII decreased which caused a considerable increase both serum vitamin B₁₂ and UBBC levels (Areekul *et al.*, 1975. Unpublished data). These findings were in accordance with results reported by Retief *et al.*, (1969. *Brit. J. Haemat.*, 16 : 231). The finding in the present study of increased vitamin B₁₂ binding in pregnancy also confirmed the result reported by Laurence and Klipstein (1967. *Ann. Intern. Med.*, 66 : 25). Measurement of the amounts of the individual vitamin B₁₂ binding proteins (transcobalamins) in serum of these subjects are in progress.

ACKNOWLEDGEMENTS

The authors wish to thank Professor Supa Na-nakorn for the supply of serum from patients with chronic myelocytic leukemia and lymphoma, Dr. Denise C. Reynolds for her help in reading and criticizing the manu-

script and Professor Chamlong Harinasuta, Dean of the Faculty of Tropical Medicine for his support.

SUVIT AREEKUL and SRISUDA VONGTAPVANISH.
Department of Radioisotopes, Faculty of
Tropical Medicine, Mahidol University,
Bangkok 4, Thailand.