Studies on parasitic infections in Orang Asli (Aborigines) in Peninsular Malaysia

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Introduction

A REVIEW OF the literature on parasitic infections in Orang Asli revealed that much work has been done on various parasites. Among them, malaria (Polunin 1953; Wharton et al., 1963; Sandosham et al., 1966; Bolton, 1972), intestinal parasites (Polunin, 1963; Sandosham, 1953; Dunn and Bolton, 1963; Bolton, 1968; Gilman and Prathap, 1971; Dunn, 1972; Gilman et al., 1976a; 1976b), filarial infections (Wilson and Reid, 1951; Polunin, 1951, 1953; Laing and Wharton, 1960; Wharton et al., 1963; Ramachandran et al., 1964; Itam, 1967; Mak, 1974), and Pentastomid infection (Ramachandran and Prathap, 1967; Prathap et al., 1968, 1969) seem to be the commonest. However, very little information was available on other blood and tissue parasites. The food and other habits of Orang Asli suggest that they would be infected with some of the rarer parasites not usually seen in the rest of the local population. In the course of this study, it was necessary to examine faeces and other excretions and secretions of a number of Orang Asli, in addition to their blood. This communication therefore summarises the prevalence and distribution of all parasites detected in this study, which commenced in 1973. No attempt has been made to compare the prevalence of parasites according to tribe, age or sex. Similarly, no attempt was made to study the parasite density of any of the infections.

Materials and methods

A total of 140 thick and thin blood films was made around midnight from patients and their relatives in the Gombak Hospital as well as in a few Orang Asli villages, whenever possible. In addition, the millipore concentration technique was used on night blood to screen patients for filarial infections.

Each of 126 stool samples was examined by direct smear, brine flotation, formol ether and sedimentation techniques. In the latter technique, 5% glycerol was used as a routine to prevent hatching of any *Schistosoma japonicum* type eggs which may have been present. This was deemed necessary in view of the report of *S. japonicum* like infection of an Orang Asli (Murugasu and Dissanaike, 1973). Where indicated, sputum samples, after treatment with 2 percent KOH, were examined for eggs of *Paragonimus* and other parasites.

Intradermal skin tests were performed on a few people for schistosomiasis and paragonimiasis. S. japonicum and P. westermani antigens were kindly supplied by Professors M. Yokogawa, T. Oshima and Shigeo Hayashi.

Sera from 271 donors were tested for antibodies to various protozoan infections. The IFA technique was employed for all except for *Entamoeba histolytica* for which the IHA technique was employed. Antigens for the IFA tests were kindly supplied by Dr. A. Sulzer, Center for Disease Control, Atlanta, USA. The antigens and other reagents used for the IHA tests were commercial preparations bought from Hoechst (Amoebiasis IHA Reagent test kit (OTMO 11)).

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In all the tests where IFA techniques were used, only sera that showed reactivity at 1:64 or higher dilutions were considered positive, although reactivity at 1:16 dilutions was recorded for information. In the IHA tests for *E. histolytica* antibodies, those sera that were reactive at 1:128 or higher dilutions were considered positive.

Results

1. Intestinal Parasites.

a. Protozoa.

Table I gives the prevalence of intestinal protozoa as diagnosed by stool examination compared with results of Dunn (1972) and Bisseru and Aziz (1970). Entamoeba coli (21.4%) was the most common protozoan, followed by E. histolytica (8.7%) and Giardia (4.8%). No Balantidium coli or Isospora sp. were detected during this study.

b. Helminths.

The prevalence of common soil-transmitted nematodes compared with previous studies is given in Table II. The prevalence of all the common soil-transmitted helminths was high as shown in Table III. The most prevalent infection was hookworm (95.2%) followed by *Trichuris* (80.9%), *Ascaris* was present only in 47.6% people examined. A large number (23.0%) of them had mixed infection with all three soil-transmitted helminths. Double infections in all possible combinations were also detected. Single infections with these nematodes were rare (Table III). Although other intestinal nematodes like Strongyloides and Enterobius were present, these were understandably low due to the techniques used. It was interesting to note that out of the 126 stool samples examined, only one was completely free of helminthic infection.

Table I

Prevalence of Intestinal Protozoa among Orang Asli compared with Bisseru & Aziz (1970) and Dunn (1972)

Protozoa	Present authors (Total 126)	Bisseru & Aziz (1970) (Total 100)	Dunn (1972) (Total 1273)
Entamoeba histolytica	8.7	1	5.1
E. hartmanni	0.8		3.5
E. coli	21.4	4	28.1
Endolimax nana	6.3		6.2
Iodamoeba butschlii	2.4		2.8
Giardia lamblia	4.8	25	10.8
Chilomastix mesnili	1.6		2.1
Trichomonas hominis	1.6	7	0.4

Table II

Prevalence of commoner soil-transmitted nematodes among Orang Asli - comparison with previous studies

	2.0		Method		
Author (year)	No. examined -	Ascaris	Trichuris	Hookworm	Used
Nevin (1938)	104	70	14	16	?
Sandosham (1953)	117	79.6	24.0	48.7	DS, BF
Polunin (1953)	131	4.6	3.1	90.8	BF
Bisseru & Aziz (1970)	100	69	80	51	DS
Dunn (1972)	1273	39.1	57.2	68.7	DS, TIF
Dissanaike et al (present paper)	126	47.6	80.9	95.2	BS, FE, BF, Sed

DS = Direct smear; BF = Brine flotation; FE = Formol ether; Sed. = Sedimentation; TIF = Thiomersal-Iodine-Formol.

Table III

Prevalence of Intestinal helminths among Orang Asli

Helminths		sitive (%) tal of 126
Ascaris lumbricoides	60	(47.6)
Trichuris trichiura	102	(80.9)
Hookworm	120	(95.2)
Strongyloides stercoralis	1	(0.8)
Enterobius vermicularis	1	(0.8)
Hookworm Trichuris and Ascaris	29	(23)
Hookworm and Trichuris	19	(15.1)
Hookworm and Ascaris	4	(3.2)
Trichuris and Ascaris	1	(0.8)
Hookworm alone	4	(3.2)
Trichuris alone	3	(2.4)
Ascaris alone		nil
Persons negative for all intestinal helminths	1	(0.8)

2. Blood and Tissue Parasites.

a. Protozoa.

(i) Malaria.

Examination of 140 single thick and thin smears showed the presence of *P. falciparum* infection in 16 Orang Asli. Two of these had mixed infections with *P. vivax*. Nine showed infections with *P. malariae*. None of the slide-

positive cases were children below the age of 10. Only 1, a 2 year old child, showed clinical malaria at the time of blood examination.

(ii) Trypanosomiasis.

Low grade infections with trypanosomes have already been reported in 2 Orang Asli (Dissanaike *et al.*, 1974).

o. Helminths.

(i) Microfilariae.

Out of a total of 140 blood smears examined, 8 were positive for *Wuchereria bancrofti* and 12 for *Brugia*. There was no mixed infection. The distribution of the 2 species in various states of Peninsular Malaysia are given in Table IV: Orang Asli from Johore, Kelantan and Malacca did not show microfilariae. However, as the total numbers tested were below 10 for Selangor, Negri Sembilan, Johore, Kelantan, Trengganu and Malacca, this may not give a true picture of the microfilaria rates in Orang Asli populations in the various states. The prevalence of microfilaria is compared with the results of previous workers in Table V.

(ii) Other Helminths.

No other blood and tissue helminths were detected in this study, but skin tests carried out for Schistosoma japonicum and Paragonimus westermani have shown positive reactions in a few cases. The results of the tests are shown in Table VI.

3. Serological studies

The results of the serological studies on the protozoan infections are summarised in Table VII.

Table IV

Prevalence of microfilariae among Orang Asli from 8 states

	AND TO THE RESERVE AND ADDRESS OF THE RESERVE AN	positive with	Total	
State	Number examined	Brugia	Wuchereria	Positive
Selangor	7	1		1
Negri Sembilan	8	-	1	1
Johore	7	-	-	355
Perak	37	5	1	6
Pahang	75	6	6 5	
Kelantan	4	77	=	-
Trengganu	1	27	1	1
Malacca	1	112	15	-
TOTAL	140	12	8	20

Table V

Prevalence of microfilariae – comparison with previous published studies

Author (date)	Number examined	Number positive	infection	Brugia (of total	Wuchereria positives)	% mixed
Polunin (1953)	278	60	21.6	<u>@</u> }	Φ.	122
Wharton et al (1963)	1,099	212	19.3	62.3	30.2	7.5
Ramachandran et al (1964)	167	29	17.4	51.7	34.5	13.8
Itam (1967)	1,964	223	11.4	75.1	25.0	-
Mak (1974)	68	5	7.4	100	-	
Dissanaike et al (present paper)	140	20	14.3	60	40	-

a. Amoebiasis.

Of the 16 sera tested for *E. histolytica* by IHA technique 25% showed reactivity. However, the stools of those who were positive were not examined. In the general stool survey, 8.7% infection was revealed.

b. Malaria.

The results obtained on the serological tests on the Orang Asli donors have already been published elsewhere (Thomas and Dissanaike, 1977).

Table VI

Results of skin tests performed on Orang Asli with

Schistosoma japonicum and Paragonimus westermani
antigens

Skin test with	No. tested	Positive	Doubtful positive
S. japonicum	130	2	9
P. westermani	132	1	16

Table VII

Titres obtained for sera from Orang Asli donors with various blood and tissue protozoan parasite antigens

Antigen used	TD 6	Total No.	A contract of the contract of						Total No.	
	Type of test	of sera - tested	1:16	1:64	1:256	1:1024	1:4096	1:8192	at 1:6	6 + ve 64 and r titres
E. histolytica	IHA	16	342	42	-	3	L	1	4	25.0
Plasmodium falciparum*	IFA	271	-	80	90	67	4	=	241	88.9
P. malariae	IFA	271	675	114	34	17	570	-	165	60.9
Toxoplasma gondii	IFA	226	36	27	5	4	-	 0	36	16.0
Trypanosome gambiense	IFA	93	17	3	-	-	-	-	3	3.2
T rhodesiense	IFA	93	28	5	1	-	-	-	6	6.5
T. cruzi	IFA	93	8	_	_	_	-	==:	-	2.4
T. cyclops	IFA	48	0 <u>0</u>	122	_	2	27	-	-	844
T. lewisi*	IFA	29	12	7	3	-	_	= 2	10	34.5
Sarcocystis fusiformis	IFA	71	20	22	6		-	-	28	39.4

^{*} Tests at CDC, Atlanta, USA by the kind courtesy of Dr. A.J. Sulzer.

c. Trypanosomiasis.

Serological survey on 93 sera (Table VII) with 3 human *Trypanosoma* antigens showed that 6 of these sera reacted with *T. rhodesiense*, and 3 with *T. gambiense* antigen at significant titres. None of the sera reacted with *T. cruzi* at titres higher than 1:16. Similarly, none of the 48 sera that were tested with *T. cyclops* gave positive reaction, although 10 of 29 sera reacted with *T. lewisi* antigen at significant titres.

d. Toxoplasmosis.

Two hundred and twenty six sera were tested and the results are shown in Table VII. A total of 36 sera (16%) were positive at 1:64 and higher titres. An equal number reacted at 1:16 showing that a good percentage of the Orang Asli population of this country has experienced infection with *Toxoplasma gondii*.

c. Sarcosporidiosis.

The results of the serological tests using zoites isolated from *S. fusiformis* sarcocysts from the water-buffalo muscle showed the presence of *Sarcocystis* antibodies in 39.4% of Orang Asli donors. Further details of this work will be published in a separate communication.

Discussion

The present study revealed that the prevalence of parasitic infections like amoebiasis, soil-transmitted helminths, malaria and filariasis is quite high among Orang Asli. Many of them still lead a semi-nomadic life under poor sanitary conditions and are normally barefooted. These factors may account for such a high prevalence of these infections. A comparison of the prevalence of these parasites with those obtained by Dunn (1972) and other workers showed a higher prevalence of hookworm and Trichuris infections (Table II). This could be because several techniques (4) were employed for each stool sample in the present study. The previous workers have used only 1 or 2 techniques to detect the eggs. The brine flotation and sedimentation techniques which were used in the present study would have brought out even the scanty infections.

The high levels of malaria antibody in a large number of people tested indicate that the transmission rate is higher than what is apparent by slide examination. This has been discussed in an earlier paper (Thomas and Dissanaike, 1977) and is in agreement with the report of Bolton (1972).

In addition to parasites, which have already been recognised, 2 cases of trypanosome infection were detected during the study and have been reported (Dissanaike et al, 1974). The sera from these two persons were not very reactive with any of the trypanosome antigens tested (Else et al, 1976). Dr. A. J. Sulzer (personal communication) tested a number of Orang Asli sera at the Center for Disease Control, Atlanta, USA, with T. rhodesiense and T. cruzi antigens. He obtained very similar results. When he tested 29 of those sera with T. lewisi antigen (Table VII), 10 samples reacted at 1:64 or higher dilutions and 3 samples reacted at 1:286. It was clear from these results that there was reactivity in a few sera at lower titres showing possible infections among Orang Asli with some species of trypanosome. However, the sera were not too reactive with any antigens tested, and this may have been due to the absence of homologous antigens for testing. It was interesting to note that the sera were most reactive with T. lewisi antigen.

In an earlier survey done with sera collected from Southeast Asia, Sulzer (personal communication) noted that one serum sample reacted with an African trypanosome antigen at 1:256. All these findings suggest the possible existence of a small focus of trypanosome infection among Orang Asli.

Serological tests for *Toxoplasma* antibodies ndicated that infection was present although obvious clinical manifestations of the disease were not recognised. According to Tan and Zaman (1973); Cheah *et al.*, (1975); Thomas *et al.*, (unpublished data) the prevalence of *Toxoplasma* antibodies was highest among the Malays and lowest in Chinese. The present studies show that the prevalence of antibodies among Orang Asli is higher than that among Chinese (Table VIII) but lower than in Indians. This may be due to the fact that, being semi-nomadic, they do not have very close association with pet cats like the Malays. In fact their association is more with dogs.

Table VIII

Comparison of antibody titres of different ethnic groups (Toxoplasma)

	Tan & Zaman, 1973 IHA	Bisseru (1974) Dye Test	Cheah et al (1975) IFA %	Thomas et al* IFA %
Malays	25.4 (59)	:=	38.8 (431)	33.9 (118)
Indians	19.6 (51)	-	25.2 (373)	23.9 (138)
Chinese	5.0 (20)	-8	20.7 (635)	14.6 (212)
O. Asli	-	4.6 (44)	-	19 (268)
Others	122	_	12.5 (40)	_

Numbers of parasites - total number examined.

* Unpublished data.

Kutty et al (1975) reported a case of infection with Sarcocystis sp. in an Orang Asli girl at autopsy. Two other cases of Sarcocystis infection in Malaysia, one in a Malay (Kutty and Dissanaike 1975) and the other in a Chinese (Prathap and Dissanaike 1976) have also been reported from Peninsular Malaysia. The present serological findings show a number of Orang Asli donors (39.4%) have detectable levels of Sarcocystis antibodies. This indicates that the infection with Sarcocystis may be more common and only a more thorough study including muscle biopsy would reveal the true situation.

Thick film examination for filarial infection confirmed the earlier findings that filariasis is still prevalent among Orang Asli and that *Brugia* is the predominant species. However, mixed infections were not found in the present study.

Eggs of a Schistosoma japonicum-like parasite were earlier found in the liver and other tissues of the Orang Asli (Murugasu and Dissanaike, 1973 and Murugasu and Por, 1973). Subsequently, Leong et al. (1975) reported 8 additional cases at autopsy. The smaller size of the eggs suggests that this was probably a different strain to the classical S. japonicum and may be related to the Mekong strain. Recently, Murugasu (personal communication) detected eggs in a needle liver biopsy from an Orang Asli patient at the University Hospital, whose stools were negative. Through the courtesy of Professor Benjamin Cabrera, the Circum Oval Precipitation Test (COPT) was done on seven Orang Asli sera which included the case with the positive needle biopsy. Only the latter was positive. It would therefore be worthwhile carrying out this test and doing liver biopsies whenever the skin test and the COPT are found positives in future.

Paragonimus westermani is known to be a common parasite of the wild carnivores in Malaysia (Rohde, 1965 and Groves et al., 1967). It is also known in fresh water crabs (Rohde, 1966 and Lee and Miyazaki 1965).

In the skin tests, although a single positive case was detected, no eggs were present in the sputum or the faeces nor was there any radiological evidence of the infection in the lungs. A more thorough search might reveal this infection too in the Orang Asli.

In conclusion, the present study which confirms the high prevalence of malaria, soil-transmitted nematodiasis, amoebiasis and filariasis in the aborigines, focuses attention on the likelihood that blood and tissue infections like *Sarcocystis*, *Paragonimus* and perhaps other hitherto undetermined parasites would be detected with better and more thorough procedures.

Summary

During a brief study of the blood and tissue parasites of Orang Asli aborigines, the prevalence of intestinal infections, malaria and filariasis was noted and compared with results of previous authors. A higher prevalence of Hookworm and *Trichuris* infections in this study is attributed to the more comprehensive methods of examination used.

Reference is made to cases of trypanosomiasis and sarcosporidiosis reported earlier and serological studies have suggested a higher prevalence of these parasites, necessitating further detailed investigations. In view of reports of *Schistosoma japoncicum*-like infections in these aborigines, a detailed search for eggs of these parasites was made but with negative results although, of two skin test positives, one case was that of a patient with *Schistosoma* eggs seen in a needle biopsy.

Acknowledgements

We are grateful to the Director, Department of Orang Asli Affairs and the Medical Officers and laboratory staff at Gombak Hospital for their willing help and cooperation; to the laboratory staff of the Department of Parasitology especially to Mr Yap Pak Leng, Mr John Loganathan and Mr Perumal s/o Ramasamy for their assistance. Grateful thanks are due to Dr A Sulzer for his assistance with antigens and for some of the serological tests he so kindly performed for us; also to Professor Benjamin Cabrera whose assistance with the COPT test we greatly appreciate.

This work was assisted by a Research Grant from the University of Malaya.

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