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Review

PATHOGENS OF BLACKFLIES IN GUATEMALA AND THEIR INFLUENCE ON NATURAL POPULATIONS OF THE THREE ONCHOCERCIASIS VECTORS

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Abstract: Pathogens and parasites of larval and adult blackflies so far found in Guatemala were reviewed in relation to their host ranges, infection rates, and significance as regulatory factors on natural populations of the three onchocerciasis vectors.

INTRODUCTION

Diseases are often found in larval and adult populations of blackflies. Included as causal agents are viruses, bacteria, fungi, protozoa and helminths. Mermithids belonging to the last group are most commonly encountered in larval and adult blackflies from all zoogeographical regions. Pathogens and parasites of blackflies hitherto recorded from many parts of the world have already been reviewed (Jenkins, 1964; Strand *et al.*, 1977; Laird *et al.*, 1980; Lacey and Undeen, 1987).

Recently, special attention has been paid to research on biocontrol agents in relation to the future integrated control against simuliid vectors of onchocerciasis. For instance, in Africa, where WHO/UNOP/WB Onchocerciasis Control Programme is now under way, many studies have been carried out, and mermithid, fungal and protozoan parasites were reported from *Simulium damnosum* s. l., a West African onchocerciasis vector (Lewis, 1960; Briggs, 1970; Ezenwa *et al.*, 1974; Mondet *et al.*, 1977a, b). *Isomermis lairdi,* the most common parasite of this vector, has been extensively investigated with regard to its potential as a biocontrol agent (Mondet *et al.*, 1977b), although more studies must be carried out on its practical use. Before the Japan-Guatemala Onchocerciasis Pilot Project was commenced in 1976, there were a few sporadic reports of parasitism in larval and adult populations of Guatemalan simuliid vectors of onchocerciasis (Strong *et al.*, 1934; Dalmat, 1955; Garms, 1975).

During the project, mermithids, fungi and ciliates were observed in adults of Guatemalan blackflies (Ito *et al.*, 1980; Ochoa, 1982; Takaoka, 1982). Furthermore, pathogens and parasites of larval blackflies were surveyed, and their significance as regulatory factors upon natural larval populations of three onchocerciasis vectors (*S. ochraceum, S. metallicum* and *S. callidum*) were studied (Takaoka, 1980, 1981).

This paper chiefly reviews the results of these recent studies.

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Pathogens and Parasites of Adult Blackflies

To date, bacteria, fungi, protozoa, nematodes and trematode have been recorded from adult blackflies (Lewis and Wright, 1962; Jenkins, 1964; Strand *et al.*, 1977; Laird *et al.*, 1980). However, information is for the most part restricted to onchocerciasis vectors or related species. Parasites other than *Onchocerca volvulus* larvae are usually detected, while wild females are dissected for *O. volvulus* larvae in both natural and experimental infection studies. Thus, as already mentioned, fungi, protozoa and nematodes have been reported from adult *S. damnosum s. l.* in Africa. These parasite groups were also observed in adults of Guatemalan blackflies (Table 1).

Nematodes

Mermithids are frequently found in adult blackflies, since some proportions of infected blackfly larvae can pupate and emerge, parasite(s) within them being carried over up to the adult stage. Several species of mermithids belonging to *Isomermis, Gastromermis,* and *Mesomermis* have been reported from adults of *S. damnosum s. l.* in West Africa (Mondet *et al.*, 1977a). In Guatemala, mermithid larvae of adult blackflies were recorded for the first time by Strong *et al.* (1934) and later by several authors. Five blackfly species including three vector species are known to harbour mermithid nematodes (see Table 1). However, infection rates in each host were very low (0.09-3.85%). The identity of mermithids concerned remains unknown.

Next to mermithids, larvae of an unknown filaria are recognized in adults of *S. metallicum* and *S. horacioi* (Garms, 1975; Ito *et al.*, 1980; Takaoka, 1982). The third-stage larva of this filarial species was well described by Garms (1975). It is readily distinguished from that of *O. volvulus* by its length (0.92-1.29 mm) and by the presence of three caudal protuberances. Rates of infection by this unknown filaria were all at low level, with a single exception of 29.4% for *S. horacioi* reported by Ito *et al.* (1980).

Apart from the above-mentioned nematodes, there are one or more nematode parasites in adults of Guatemalan blackflies. One of these nematodes was first recorded by De Leŏn (1963) and later by Garms (1975) from the Malpighian tubes. According to De Leŏn and Duke (1966), it measures 0.9-1.1 mm in length, possesses a pointed tail, and can remain viable in normal saline or serum for up to 35 days. However, its infection rates appear to be low.

Protozoa

Microsporidans are very rarely recorded in adult blackflies (Shipitsina, 1963; Hunter and Moorhouse, 1976), although they are frequently observed in larval blackflies. In Guatemala, Strong *et al.* (1934) reported microsporidan infections in 34 *Simulium* females, while dissecting *S. ochraceum, S. metallicum* and *S. callidum* to search for *O. volvulus* larvae. However, no adult flies infected by this protozoan parasite have since been reported in Guatemala.

Tetrahymena-like ciliates have been recorded from adult *S. damnosum s. l.* (Lewis, 1960, 1965) and adult and larval *S. ruficorne* (Takaoka, unpublished data) in West Africa, and from *S. takahasii* in Japan (Takaoka, unpublished data). In Guatemala, ciliates parasitizing adult blackflies were recorded for the first time by Strong *et al.* (host species were, though, not mentioned—1934); similar protozoan parasites were again observed in three vector blackflies (Garms, 1975; Ochoa, 1982). Infection levels were, though, very low.

Table 1 Pathogens and parasites found in adults of Guatemalan blackflies

Pathogens and parasites	Host blackfly species	% infection (authors)
Mermithids	*S. ochraceum*	0.09 (Garms, 1975), 0.12 (Ochoa, 1982)
	S. metallicum	4.1 (Garms, 1975), 2.14 (Ochoa, 1982), 1.2 (Takaoka, 1982)
	S. callidum	3.85 (Ochoa, 1982)
	S. gonzalezi	1.3 (Garms, 1975)
	S. horacioi	0.3 (Takaoka, 1982)
Unknown filaria	*S. metallicum*	0.3 (Garms, 1975), 1.0 (Ito *et al.*, 1980), 0.2 (Takaoka, 1982)
	S. horacioi	29.4 (Ito *et al.*, 1980), 0.1 (Takaoka, 1982)
Other nematodes	*S. ochraceum*	0.4 (Garms, 1975), 0.06 (Takaoka, 1982)
	S. metallicum	0.1 (Garms, 1975)
	S. callidum	0.2 (Garms, 1975)
Ciliates	*S. ochraceum*	0.2 (Garms, 1975)
	S. metallicum	0.2 (Garms, 1975), 0.16 (Ochoa, 1982)
	S. callidum	0.4 (Garms, 1975)
Fungi	*S. ochraceum*	1.6 (Garms, 1975), 0.6 and 2.8 (Ochoa, 1982), 0.06 (Takaoka, 1982)
	S. metallicum	0.4, 0.5 and 1.2 (Garms, 1975)
	S. callidum	0.6, 0.9 and 1.3 (Garms, 1975)
	S. gonzalezi	3.5 (Garms, 1975)
	S. horacioi	0.1 (Takaoka, 1982)

Fungi

Infections caused by Phycomycetes have been recorded in ovaries of *S. damnosum s. l.* in Liberia (Briggs, 1970) and of three blackfly species in Canada (Undeen and Nolan, 1977). Garms (1975) reported fungi developing in ovaries of *S. ochraceum, S. metallicum, S. callidum* and *S. gonzalezi,* and thought these fungi as Phycomycetes. According to Ochoa (1982), these fungi may be classified into four types depending upon the shape of the parasitic forms (probably fungal thalli). Infections by these fungi were sporadic, with low rates (0.06-3.5%). No other fungal infections are recognized in Guatemalan blackflies, although *Coelomycidium* (Rubtsov, 1969), *Entomophthora* (Lewis, 1965) and *Coelomomyces* sp. (Garnham and Lewis, 1959) have been very rarely recorded elsewhere.

Pathogens and Parasites of Larval Blackflies

There are many reports on pathogens and parasites of larval blackflies from various parts of the world. However, until recently, no information was available in Guatemala except for a report by Dalmat (1955) who found microsporidans in undetermined *Simulium* larvae. In an investigation within and outside of the onchocerciasis endemic zones, Takaoka (1980, 1981) reported mermithid, microsporidan, fungal and viral infections in larval populations of blackflies, as summarized in Table 2. Here included are brief notes on each group of pathogens and parasites reported by the above author.

Mermithids

Mermithids including at least five species were found in 17 species of Guatemalan blackflies. Among these, four were already described as new species by Poinar and Takaoka (1979, 1981).

Isomermis benevolus Poinar and Takaoka was shown to be a common parasite of larval *S. metallicum* and *S. callidum,* and infections of both blackfly species with this nematode in a certain stream occurred throughout the year (averaging 8.5% and 14.1% respectively) with the higher rates during the early dry months, October to January, than in the remainder of the year. The worm burden was one or two in *S. metallicum* but varied from one to seven in *S. callidum.* Laboratory observations showed that at an incubation temperature of 18°C, this mermithid parasite moulted on the sixth day after emergence and copulated one week later; and eggs oviposited were seen two weeks after copulation.

Mesomermis guatemalae Poinar and Takaoka was described from *S. metallicum.* However, no further information is yet available.

Another *Mesomermis* species, though undescribed, was found parasitizing *S. callidum* in streams at Finca Nimayá (Takaoka, 1981). The number of parasites per infected simuliid larva averaged one, with a maximum of three. Laboratory rearings at 16°C revealed that moulting occurred at sixth to eighth days after emergence, copulation taking place five days later.

Table 2 Host ranges of pathogens or parasites in Guatemalan blackfly larvae

Host species	Mermithids	*T.bra.*	*T.var.*	*T.fib.*	*T.*sp-1	*T.*sp-2	*P.deb.*	*P.mul.*	*P.*sp-1	*P.*sp-2	*Py.*sp	*C.*sp	IV	CPV
Gigantodax wrighti	+[a]	+	+	−	+	−	−	−	−	−	−	+	−	−
Cnephia aguirrei	+	−	−	−	−	−	−	−	−	−	−	−	−	−
C. pacheco-lunai	+[b]	−	−	−	−	−	−	−	−	−	−	−	−	−
Simulium aureum	−	−	−	−	−	−	−	−	−	−	−	−	−	+
S. rubicundulum	+	−	−	−	+	−	−	+	−	−	−	+	+	−
S. paynei	+	+	+	−	+	−	−	−	−	−	−	+	−	−
S. earlei	+	−	−	−	−	−	−	−	−	−	−	−	+	−
S. pulverulentum	+	−	−	−	−	−	−	+	−	−	−	−	−	−
S. nigricornis	−	+	−	+	+	−	−	−	−	−	−	−	−	−
S. tricornis	+	+	+	−	−	+	−	−	−	−	+	+	−	−
S. veracruzanum	+	−	−	−	−	−	−	−	−	−	−	−	−	−
S. haematopotum	+	−	−	−	−	−	−	+	−	−	−	−	−	−
S. downsi	+	−	+	−	−	−	−	+	−	−	−	−	−	−
S. samboni	+	−	+	−	−	−	−	+	−	−	−	−	−	−
S. callidum	+[c]	−	+	−	−	−	+	+	+	−	−	−	+	−
S. ochraceum	−	+	−	−	−	+	−	+	−	−	−	+	−	−
S. metallicum	+[d]	+	+	+	+	+	−	+	−	−	−	+	+	−
S. puigi[e]	+	−	−	−	−	+	−	−	−	−	−	+	−	−
S. horacioi	+	−	−	−	−	−	−	−	−	+	−	+	−	−
S. patziciaense[f]	+	−	−	−	−	−	−	−	−	−	−	+	−	−

a, *Gastromermis cloacachilus*; b, *Isomermis vulvachila*; c, *I. benevolus* and *Mesomermis* sp.;
d, *I. benevolus* and *M. guatemalae*; e, misidentified as *S. jobbinsi* in the previous paper (Takaoka, 1981);
f, previously reported as *S.* sp-2 (Takaoka, 1981).

Gastromermis cloacachilus and *I. vulvachila,* also described by Poinar and Takaoka, are, each, a parasite of *Gigantodax wrighti* and *Cnephia pacheco-lunai* from upland streams (ca. 2,800-3,000 m above sea level) of the non-endemic area.

It is intriguing that no mermithid parasites were recovered in larval *S. ochraceum,* although mermithid infections were observed in adults of this species.

Microsporidans

Microsporidan protozoa are widespread in larval blackflies around the world wherever searched for. In Guatemala, 10 microsporidan species (*Thelohania,* 5 spp., *Pleistophora,* 4 spp., and *Pyrotheca* (?), 1 sp.) were reported from larvae of 15 blackfly species (Table 2). The genus *Thelohania* includes two undescribed species as well as three known and cosmopolitan ones (*T. bracteata, T. varians* and *T. fibrata*). All of these *Thelohania* species have a wide host range except for *T. fibrata* which was recognized in two blackfly species. On the other hand, two undescribed *Pleistophora* are highly host specific, *P.* sp-1 and *P.* sp-2 parasitizing *S. callidum* and *S. horacioi* respectively; whereas, as in other parts of the world, *P. multispora* is a common parasite, utilizing eight blackfly species as a host.

Three microsporidans (*T. bracteata, T.* sp-2 and *P. multispora*) were occasionally found in larval *S. ochraceum.* On the other hand, all the five *Thelohania* species and *P. multispora* were encountered in *S. metallicum,* and *T. varians, P. multispora, P.* sp-1 and *P. debaisieuxi* in *S. callidum.*

Fungi

The fungal infections found in nine Guatemalan blackfly species including *S. ochraceum* and *S. metallicum* were caused by *Coelomycidium* sp. (probably *C. simulii,* a common parasite of larval blackflies around the world). This fungus is widely distributed within and outside the onchocerciasis areas. However, its infection rates in two vector species as well as in other blackfly species were very low.

Viruses

There have been very few reports of viral infections among larval blackflies. Until now, three groups of baculoviruses have been reported: iridescent viruses (IVs) from Czechoslovakia (Weiser, 1968) and England (Batson *et al.,* 1976), cytoplasmic polyhedrosis viruses (CPVs) from Canada (Bailey *et al.,* 1975), Czechoslovakia (Weiser, 1978) and USA (Federici and Lacey, 1976), and densonucleosis viruses (DVs) from USA (Federici, 1976).

In Guatemala, infections caused by IVs were recognized in four blackfly species including *S. metallicum* and *S. callidum* from two streams in the onchocerciasis-endemic areas (Takaoka, 1980, 1981); and the CPV infection was also found in larval *S. aureum* from two upland streams (Takaoka, 1980).

Influences of Pathogens on Natural Larval Populations of Three Onchocerciasis Vectors

Influences of pathogens and parasites of various categories on natural larval populations of three vectors have been reported by Takaoka (1980, 1981).

Table 3 shows the incidence of mermithids, microsporidans, fungi and viruses in three

vector species. *Simulium ochraceum* exhibited only sporadic parasitaemia by comparison with *S. metallicum* and *S. callidum*. That is, infections due to pathogens of any kinds were found only in six of 44 streams where *S. ochraceum* were collected. Furthermore, this species is unique in lacking mermithid and viral infections. On the other hand, larvae of *S. metallicum* and *S. callidum* were most frequently found to harbour mermithids and microsporidans, fungal and viral infections being, though, sporadic.

Infection levels of these pathogens in the infected larval populations of three vector species are shown in Table 4. *Simulium ochraceum* showed the low infection rate (2.7%) by comparison with *S. metallicum* (13.9%) and *S. callidum* (7.9%). Average infection rates with mermithids in the larval populations of *S. metallicum* and *S. callidum* were 8.9% and 4.9%, respectively. However, infections with microsporidans and *Coelomycidium* were all at low levels.

In view of the various habitats utilized by three vector species, monthly or bi-weekly observations were made in different types of breeding streams in the onchocerciasis-endemic areas. The results showed that *S. ochraceum* never harboured mermithids, even in mid or lower parts of perennial streams where the other two secondary vectors were heavily parasitized by mermithids. It was also shown that mermithid infections in larval populations of *S. metallicum* and *S. callidum* were significantly higher in mid or lower parts of permanent streams than in upper parts of permanent streams or in temporary ones. Moreover, seasonal change in mermithid infection rates in perennial streams showed a distinct pattern, with increased infection rates during the dry season (November to April) and lowered rates

Table 3 Prevalence of pathogens in larval populations of three blackfly vectors of onchocerciasis in Guatemala

		Number (%) streams where:				
	Host species	Larvae were infected with:				
Host species	collected	Any parasites	Mermithids	Microsporida	*Coelomycidium*	Iridescent virus
S. ochraceum	44	6 (13.6)	0 (—)	5 (11.4)	3 (6.8)	0 (—)
S. metallicum	52	36 (69.2)	28 (53.9)	24 (46.2)	2 (3.9)	1 (1.9)
S. callidum	33	21 (63.6)	12 (36.4)	12 (36.4)	0 (—)	2 (6.1)

Table 4 Levels of infection with pathogens in infected larval populations of three blackfly vectors of onchocerciasis in Guatemala

Host species	Infection rate (%)				
	Any parasites	Mermithids	Microsporida	*Coelomycidium*	Iridescent virus
S. ochraceum	2.7[a] (0.3-7.1)[b]	0 (—)	3.6 (2.7-7.1)	2.2 (0.3-2.7)	0 (—)
S. metallicum	13.9 (1.3-75.0)	8.9 (1.5-75.0)	3.0 (0.4-26.0)	1.3 (0.1-2.5)	0.5 (0.5)
S. callidum	7.9 (0.3-50.5)	4.9 (0.7-50.0)	1.7 (0.3-50.0)	0 (—)	7.6 (5.1-10.1)

a, median; b, range

throughout the remainder of the year. The level of microsporidan infections of *S. ochraceum* also varied in relation to the different types of preimaginal habitats. This species exhibited very low infection rates in permanent streams but was free from microsporidan infection in temporary streams. In contrast, *S. metallicum* and *S. callidum* were equally parasitized in all types of streams at low infection levels. Fungal infections due to *Coelomycidium* (at a very low level) were found only in perennial streams.

Overall, it is summarized that mermithids play the chief role in suppressing natural larval populations of *S. metallicum* and *S. callidum* in the mid or lower parts of perennial streams, especially during the dry season; they hardly do so, however, in upper parts of permanent streams or in temporary ones. Other groups of pathogens are considered to be of negligible importance as natural population regulatory factors for the three vector species in all types of breeding streams.

Conclusion

Recent studies indicate that various kinds of pathogens and parasites including several new taxa are present in larval and adult populations of Guatemalan blackflies. As in other parts of the world, *Coelomycidium* fungi, microsporidans and mermithids are frequently observed in larval blackflies. However, infections by these pathogens are generally at low level.

It has been shown that influence of these pathogens on natural larval populations of three onchocerciasis vectors varies significantly. For instance, *S. ochraceum,* the principal vector, never harboured mermithid nematodes, while *S. metallicum* and *S. callidum* (the secondary vectors) were frequently parasitized by this parasite group.

Four mermithid nematodes have been newly described and their life history have been preliminarily studied in the laboratory. However, the potential of these mermithid parasites for simuliid control needs further study.

Finally, in view of exotic biocontrol agents against simuliid vectors, it should be mentioned that due to its pathogenicity, potential for mass-production and safety for man and nontarget organisms, *Bacillus thuringiensis* var. *israelensis* de Barjac, a pathogen of mosquitoes, is now recognized as one of the most promising biological agents for the control of larvae of blackflies as well as mosquitoes (Burges *et al.,* 1981). In fact, it has been proven to be highly pathogenic to larval *S. ochraceum,* yielding high mortality under both laboratory and field conditions (Undeen *et al.,* 1980).

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グアテマラにおけるブユの病原体およびそのオンコセルカ症媒介ブユ種に与える影響

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グアテマラにおけるブユの病原体または寄生虫の種類，その宿主特異性，感染率およびオンコセルカ症媒介ブユ3種の幼虫自然集団に与える影響に関して，これまで報告された知見を整理し，紹介した。

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Review

PHYLOGENETIC RELATIONSHIPS AMONG THE GENERA OF DIROFILARIINAE SANDGROUND, 1921 (NEMATODA: ONCHOCERCIDAE)

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Abstract: Phylogenetic relationships among the genera of the filarioid worm of subfamily Dirofilariinae were analysed based on cladistic. Fourteen characters among *Macacanema, Edesonfilaria, Dirofilaria, Loaina, Pelecitus, Foleyella, Loa* and *Bostrichodera* were compared resulting in a consensus phylogenetic tree. The last six genera form a group with at least one synapomorphy, and *Foleyella, Loaina* and *Pelecitus* are the most derived among the subfamily. *Edesonfilaria* and *Macacanema* most probably belong to a subfamily other than Dirofilariinae.

INTRODUCTION

The aim of modern systematics is not only to provide easy and accurate recognition of species but also an elucidation of their evolutionary history.

Previous classifications of Onchocercidae were presented by Yorke and Maplestone (1926), Wehr (1935), Lopez-Neyra (1956). Chabaud and Choquet (1953) and Anderson and Bain (1976). The currently accepted relationships within the family were developed by Chabaud and Anderson (1959) and later expanded by Anderson and Bain (1976).

The Onchocercidae may be a monophyletic group in the order Spirurida, as can be implied from the works of Chabaud and Bain (1976). They are medium to small sized nematodes with reduced buccal capsules, have arthropods as intermediate hosts, and occur in the connective tissues and heart of large blood vessels of terresterial vertebrates.

In systematics, considerable interest has been directed towards the concepts and methods of phylogenetic construction by numerical methods. One of the concepts and techniques for reconstructing the branching aspect of phylogeny have come to be known as cladistics or phylogenetic systematics (Hennig, 1965). Phylogenetic systematics attempt to recover the relationships among groups of organisms and produce classifications that reflect their geneological relationships (Wiley, 1981).

This study attempts to contruct hypotheses of relationships among the genera of Dirofilariinae. Morphological informations on the genera *Macacanema, Edesonfilaria, Bostrichodera, Loa, Foleyella, Loaina, Pelecitus,* and *Dirofilaria* are available in substantial detail. The literature on *Dirofilariaeformia* and *Skrjabinodera* is too scanty to be of use in the

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analysis. As such, these last two genera are not included in the analysis of Dirofilariinae.

Materials and Methods

The choice of genera in the subfamily is based on the works of Anderson and Bain (1976). *Skrjabinodera* and *Dirofilariaeformea* are excluded for lack of information and future works might clarify the true status of these two genera in the systematics of Dirofilariinae.

The characters of the following 8 genera were evaluated:

1. *Macacanema*
2. *Edesonfilaria*
3. *Dirofilaria*
4. *Loaina*
5. *Pelecitus*
6. *Foleyella*
7. *Loa*
8. *Bostrichodera*

Method of Phylogenetic Analysis

In this study, the writer has employed version 2.4 of PAUP (Phylogenetic Analysis Using Parsimony), a computer program developed by Dr. David L. Swafford of Illinois Natural History Survey, Illinois. The parsimony program chosen for this package is Wagner Parsimony. The logic of using Wagner Parsimony is well documented (See Maddison *et al.*, 1984; and others). It should be noted that parsimony has nothing to do with evolution but is more of a matter of explaining data as simply as possible. Wagner parsimony allows character state changes from plesiomorphic (ancestral) to the apomorphic (derived states), equally allows reversals to the plesiomorphic states, and attemps to construct a cladogram with the manimum total number of state transitions. One of the simplest parsimony models is that the preferred tree is the one of minimal length in Manhattan matrix with no *a priori* restrictions on the nature of permissible character state changes (Swafford, 1985).

In order to strengthen the outgroup process of character state transformation, *Skrjabinofilaria, Acanthocheilonema, Oswaldofilaria* and *Chabifilaria* were used as outgroup genera based on the works of Chabaud and Bain (1976), Bain (1981), and Bain and Chabaud (1986). Maddison *et al.* (1984) emphasized the importance of using more than one outgroup taxon simultaneously in order to determine correctly both the position and the direction of character state changes in the ingroup phylogeny. In this analysis the ingroup taxon are represented by the 8 genera being mentioned.

Explanation for Choice of Characters in Preliminary Analysis

Fourteen characters were used for the analysis. Primitive characters were listed as '0' and the derived characters as '1', '2', '3' and so on. The ordinal value of '9' was considered as a missing value due to lack of description or if more than one state (i.e. multistates) was found for that particular character among the members of the genus. The characters together with their character states and polarities are described in Table 1.

Table 1 Characters and their discrete states used in analysis

1. Shape of male tail
   0=coiled about 2-4 turns
   1=coiled 0.5-1.5 turns
   Polarity: 0 → 1
2. Length of tail
   0=long (length more than two and a half times width at anus)
   1=short (length less than two and a half times width at anus)
   Polarity: 0 → 1
3. Shape of preanal papillae
   0=small and non pedunculated
   1=pedunculated
   2=bulbous without stalk
   Polarity: 0 → 1 → 2
4. Caudal alae
   0=absent
   1=present but incospicuous
   2=highly conspicuous and without hyaline inclusions
   Polarity: 0 → 1 → 2
5. Arrangement of preanal papillae
   0=grouped near to each other but not in contact
   1=grouped very closely at terminal end and in contact with each other
   2=widely spaced from each other
   Polarity: 2 ← 0 → 1
6. Dissimilarity of paired spicules' shape
   0=highly dissimilar
   1=subequal
   Polarity: 0 → 1
7. Spicular ratio
   0=2-4
   1=less than 1.9
   2=more than 4
   Polarity: 2 ← 0 → 1
8. Shape of left spicule
   0=lamina highly filamentous and almost two times longer than calomus
   1=lamina filamentous or non-filamentous and is equal or slightly longer than calomus
   2=lamina highly sclerotized, non filamentous and very much shorter than calomus
   3=highly tubular and long, and without any apparent division into calomus and lamina
   Polarity: 2 ← 0 →1
   ↓
   3
9. Shape of right spicule
   0=not divided into calomus and lamina but has blunt end
   1=clearly divided into delicate calomus and sclerotised lamina with pointed end
   2=not clearly divided but with extra internal sclerotization; lightly sclerotized
   Polarity: 2 ← 0 → 1

Table 1 (continued)

10. Esophago-intestinal junction
    0=with valve
    1=without valve
    Polarity: 0 → 1
11. Division of esophagus
    0=esophagus with muscular and glandular parts; and the glandular portion wider than the muscular parts
    1=esophagus without division into muscular and glandular portions
    2=esophagus with muscular part and glandular part but glandular portion nucleated
    Polarity: 2 ← 0 → 1
12. Position of vulva
    0=at region of muscular esophagus
    1=at region of glandular esophagus or post-esophageal
    Polarity: 0 → 1
13. Shape of ovejector
    0=highly bulbous and heavily muscularised
    1=non-bulbous and lightly muscularised
    Polarity: 0 → 1
14. Microfilaria
    0=sheathed
    1=unsheathed
    Polarity: 0 → 1

Character 1. Shape of male tail

The male tail in most members of family Onchocercidae is coiled two to three turns. This character is found in the four genera of Onchocercinae used as distant outgroups and is also present in some members of study group Dirofilariinae. This character is regarded as a symplesiomorphy. *Foleyella, Loa, Loaina* and *Pelecitus* have tails that are coiled 0.5 to 1.5 turns and thereby noted as a synapomorphy.

Character 2. Length of male tail

The tail is considered being long if its length (distance between anus and posterior end of body) is more than two and a half times that of the width at anus. This character is considered as, primitive (indicated as '0'). *Acanthocheilonema, Skrjabinofilaria, Oswaldofilaria* and *Chabifilaria* and in fact majority of Onchocercidae, have this symplesiomorphy. A short tail is found among members of *Dirofilaria, Foleyella, Pelecitus, Loaina, Loa, Edesonfilaria* and *Macacanema* and is a synapomorphy for these genera. Another subfamily in the Onchocercidae which consistently has a short tail is the Lemdaninae.

Character 3. Shape of preanal papillae

In the majority of outgroups (*Acanthocheilonema* and *Oswaldofilaria* and *Chabifilaria*) the preanal papillae are small and not pedunculated. In the subfamily Dirofilariinae, the genera *Dirofilaria, Foleyella, Loa, Pelecitus, Loaina* and *Bostrichodera* have highly pedunculated preanal papillae. Having pedunculated papillae is proposed as a synapomorphic character.

Character 4. Caudal alae

The outgroups do not have this morphological feature except for *Skrjabinofilaria* which has a single caudal ala. Absence of caudal alae is considered a primitive state; however, no similar state could be found among the Dirofilariinae. It is postulated that having caudal alae is a synapomorphy for all members of Dirofilariinae. In order to resolve the relationships among the members of this subfamily, it is suggested that having small or not highly marked caudal alae as can be found in *Macacanema* and *Edesonfilaria* as the most recently evolved feature of caudal alae.

Character 5. Arrangement of papillae

The arrangement of the papillae can at times be variable even among the worms of same species. However, there is a consistency among the genera of the overall pattern of papillae groupings. In *Acanthocheilonema, Oswaldofilaria, Skrjabinofilaria* and *Chabifilaria,* the papillae are grouped near each other but not to the extent that they are in contact with each other. This feature is considered as primitive.

Character 6. Dissimilarity of paried spicules' shape

In outgroup genera and in a majority of Onchocercidae, the left and right spicules are highly dissimilar in shape. This feature is suggested as a plesiomorphic character.

Character 7. Spicular ratio

A majority of the spicules of the members of the outgroup genera have the ratio of left to right spicules ranging between two and four. Possession of this feature is considered as a symplesiomorphy. A ratio of higher than four or less than two is considered to be a derived character.

Character 8. Shape of left spicule

For *Acanthocheilonema, Skrjabinofilaria* and *Oswaldofilaria,* the left spicule is divided into a calomus and a lamina. Both portions are usually well-sclerotized. The lamina is usually between 1 and 2 times the length of the calomus and is not filamentous. A left spicule fitting this description is considered as primitive and other features are thereby treated as derived.

Character 9. Shape of right spicule

A right spicule that is not divided into a calomus and lamina, lacks an internal line of sclerotizations, and has a slightly or very blunt end, has the features common to the outgroups and is thereby considered to be primitive.

Character 10. Esophago-intestinal junction

A valve between the esophagus and the intestine is common among the species of the outgroup and the genera in the family Onchocercidae. Absence of this feature is considered as derived.

Character 11. Division of the esophagus

All members of the outgroup have the esophagus divided into muscular and glandular portions, with the latter being much wider and longer than the former. Absence of this

division is considered as an apomorphic feature.

Character 12. Position of vulva

The position of the vulva is variable among the species of the outgroup. The vulva in *Oswaldofilaria* is usually equatorial, while in *Skrjabinofilaria* it is commonly at the level of the muscular portion or the anterior part of the glandular portion of the esophagus. In contrast, in *Acanthocheilonema* the vulva is located near the end of the glandular portion of esophagus or is post-esophageal. An ontogenic criterion has to be invoked in this matter. In filarial worms whose development have been elucidated, it has been shown that the vulva in the immature female worm is usually situated in the anterior or middle region of the esophagus. There is a tendency for the vulva to be positioned more posteriorly in relation to its former position as the worm matures. It is suggested that the position of the vulva in anterior region of esophagus (muscular portion) is a primitive feature.

Character 13. Shape of ovejector

All the outgroups and some of the ingroups have bulbous and highly muscular ovejectors. Possession of this feature is proposed as a symlesiomorphy.

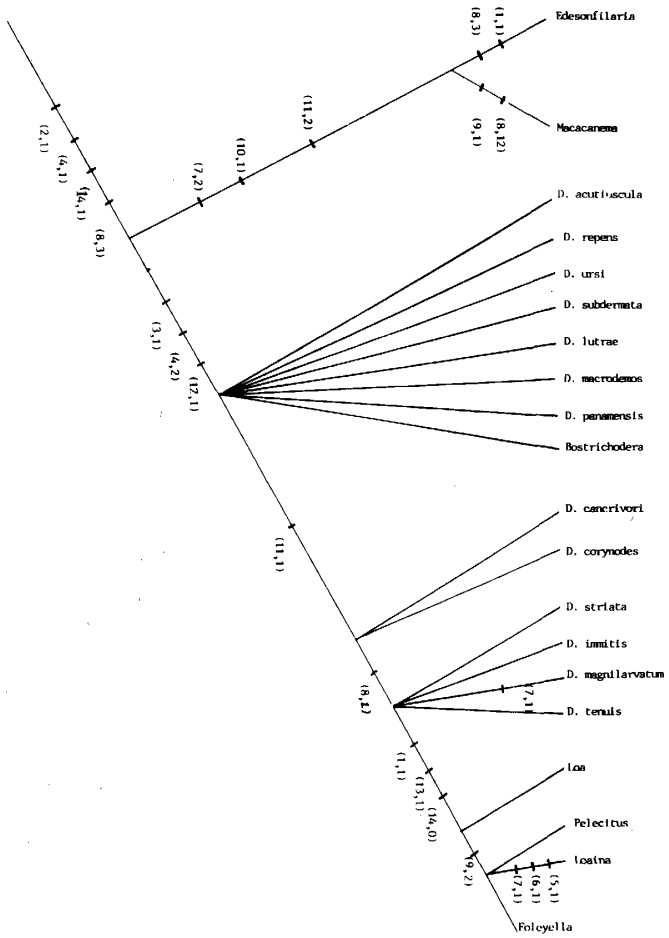

Figure 1 Consensus tree of phylogenetic relationships among genera of Dirofilariiae.

Character 14. Microfilaria

Having a sheathed microfilaria is considered as primitive since the sheath is an embryonic feature. Lack of a sheath is thereby a derived feature. Bain *et al.* (1982) also suggest that the sheathed microfilariae is a primitive feature.

Cladistic Analysis

Each character was transformed into a multistate character using ordinal values (Table 1). In this analysis characters 7, 8, 9 and 11 were entered as unordered. The 14 characters and 21 taxa was run 10 times, with a differing order of taxa each time, using David Swafford's PAUP Wagner Parsimony program.

Fifty trees were obtained by default for the maximum of trees that can be obtained from PAUP output. To compare the 50 trees and define areas of congruence, the CONTREE program version 1/3/86 available in PAUP was used.

RESULTS

The default maximum of 50 trees obtained had 26 steps and a consistency index of 0.808. The consensus tree is shown in Figure 1.

The phylogenetic tree shows that the subfamily shared the characters of short tails, presence of caudal alae, unsheathed microfilariae, and left spicule highly tubular, long and without any apparent division into calomus and lamina. *Edesonfilaria* and *Macacanema* have synapomorphies of having esophago-intestinal junction without valve; esophagus divided into muscular and, glandular portions, with the later being mucleated and a spicular ratio of more than 4. *Edesonfilaria,* meanwhile, is separated from *Macacanema* by having the left spicule highly tubular and long with no apparent division into calomus and lamina. Macacanema has the autapomorphic features of right spicule clearly divided into delicate calomus and sclerotised lamina with the later having pointed end; and the left spicule with the features of lamina highly sclerotised, non filamentous and very much shorter than calomus.

Bostrichodera, Dirofilaria, Foleyella, Pelecitus, Loaina and *Loa* are separated from *Edesonfilaria* and *Macacanema* by the synapomorphies of having pedunculated preanal papillae; lamina of left spicule filamentous and almost two times longer than calomus; highly conspicuous caudal alae; and esophagal valve at region of glandular esophagus or post-esophageal.

Dirofilaria cancrivori, D. corynodes, D. striata, D. immitis, D. magnilarvatum, D. tenuis, Loa, Pelecitus, Loaina and *Foleyella* are separated from *Edesonfilaria, Macacanema, Bostrichodera, D. acutiuscula, D. repens. D. ursi D. subdermata,* and *D. panamensis* by having the esophagus not conspicuously divided into muscular and glandular portions or the presence of both divided and undivided esophagus in the species of some genera (as in *Pelecitus* and *Foleyella* represented by ordinal value 9 in the data matrix).

Loa, Pelecitus, Loaina and *Foleyella* have two synapomorphies separating them from the other genera of Dirofilariinae whereby male tail coiled 0.5-1.5 turns, and non-bulbous and lightly muscullariased ovejector. *Pelecitus, Loaina* and *Foleyella* shared the apomorphic character of the right spicule lightly sclerotised, clearly divided into calomus and lamina, but with internal sclerotization.

Loaina has the autapormphies of subequal spicules and anal papillae grouped very

closely and in contact with each other at the terminal end.

Two reversals occured among the Dirofilariinae: 1) *Loa, Pelecitus, Loaina* and *Foleyella* having sheathed microfilaria 2) *D. striata, D. immitis, D. magnilarvatum, D. tenuis, Loa, Pelecitus, Loaina* and *Foleyella* having left spicule's lamina non-filamentous and are one to two times the length of calomus.

Discussion

All the genera in Dirofilariinae being examined were found by the analysis to share 4 apomorphic characters: tail being short, presence of caudal alae, unsheathed microfilariae, and left spicule being highly tubular, long and without any different division into calomus and lamina. Examination of all 50 trees revealed the presence of the last character. This feature is one of 3 states used as an 'unordered' in the analysis (character 8). It appears again as an autapomorphy for *Edesonfilaria.* The three other characters just mentioned are also not unique in Dirofilariinae since they are present in some genera in Onchocercinae and Lemdaninae. This finding is not surprising since the choice of characters for Dirofilariinae in the preliminary analysis depended heavily on descriptions of those worms from literature.

Macacanema and *Edesonfilaria* together with the above genera do not fulfill the requirements for being altogether monophyletic. Bartlett (1987) suggested that *Edesonfilaria* and *Macacanema* could possibly belong to a subfamily other than Dirofilariinae, i.e., Lemdaninae. This proposal appears valid based on the results of the present study. The characters that grouped them together were most probably homoplasies within Filarioidea. On the other hand, *Dirofilaria, Bostrichodera, Loa, Foleyella, Pelecitus* and *Loaina* form a group with at least one synapomorphy.

Loa, Pelecitus, Loaina and *Foleyella* are more dirived phylogenetically than *Dirofilaria.* They are characterised by the derived characters of the male tail coiled less than 2 turns, ovejector non-bulbous and lightly muscularised and a reversal character of the microfilariae (i.e., sheathed microfilariae). Among these four genera, *Loa* is more primitive due to the absence of internal sclerotization in the right spicule.

Pelecitus, Loaina and *Foleyella* share the same nested grouping among the Dirofilariinae. Bartlett (1983) postulated a close relationships between *Loaina uniformis* and *Pelecitus scapiceps.* The results of this phylogenetic analysis show that these two worms share sister group relationships.

Consensus cladogram of the subfamily (Figure 1) proposes that *Foleyella, Loaina* and *Pelecitus* are members of a monophyletic group and are the most derived among Dirofilariinae. The genera of Dirofilariinae were revealed by the cladogram as polyphyletic, and *Edesonfilaria* and *Macacanena* most probably belong to a different subfamily. Further studies are needed to examine all the genera in the subfamily, and find additional homologous characters to elucidate their phylogeny.

Decision on primitiveness of a particular organism can be complicated and difficult. The results of cladistic analysis rely heavily on choice of characters and their polarities. The more information available on a particular group of organisms especially that pertaining to their evolution, the less difficult is the task of postulating the directionality of evolution of characters. It must be recognised that information regarding each genus is only as good as the description provided by the investigators. Rodman *et al.* (1984) stated that the value of

an explicit, character supported phylogenetic tree is that it provides resolved geneologies for testing with new data and also new context for rethinking character homologies, particularly where they are controversial. Future studies might provide new data that are most probably improve the cladogram obtained in this study.

This study is among the first attempts to discover phylogenetic relationships among a taxon of Filarioidea based on the concepts of cladistics on phylogenetic systematics. The techniques utilized are procedurally defined and repeatable, and as such open to criticism and testing.

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BASIC STUDIES ON THE MONGOLIAN GERBIL AS A SUSCEPTIBLE HOST TO FILARIAL INFECTION; IgE-LIKE ANTIBODY FORMATION AGAINST *DIROFILARIA IMMITIS* ADULT WORM ANTIGEN

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Abstract: Antibody production in Mongolian gerbils (*Meriones unguiculatus*) immunized with crude extract of *Dirofilaria immitis* adult worm was tested with passive cutaneous anaphylaxis reaction. Immunized gerbils produced a long-term binding, heat-labile antigen-specific antibody which sensitized the rat skin. Those profiles were like an IgE antibody. In an intact gerbil, IgE-like substance was detected in an immediate intracutaneous reaction with anti-rat IgE antibody. Mast cells representing metachromasia were confirmed in the skin tissue section of Mongolian gerbils stained with Toluidine blue and were thought to be target cells for those anaphylactic reactions.

INTRODUCTION

The Mongolian gerbil (*Meriones unguiculatus*) is very frequently used for a host to maintain some filarial parasites and an experimental model of filariasis because of easy handling to care and high sensitivity to various filarial parasites such as *Brugia* spp., (Ash and Riley, 1970 a, b) *Dipetalonema viteae* (Dalesandro and Klei, 1976) and *Litomosoides carinii* (Matsuda *et al.*, 1976). Despite of utility in a research of parasitology, information on biological characteristics of the gerbil such as antibody response to parasites is limited (Tomisato *et al.*, 1983; Farrar *et al.*, 1991) and there is no report on antibody production induced by immunization with a parasitic antigen in the gerbil.

The purpose of this study was to examine antigen-specific antibody production in immunized gerbils and to identify presence of IgE-like antibody in an intact gerbil. We carried out an immunization of gerbils with crude extract derived from *Dirofilaria immitis* adult worm and an immediate intracutaneous reaction using sheep anti-rat IgE antiserum to detect IgE-like antibody in the gerbil.

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MATERIALS AND METHODS

Animals

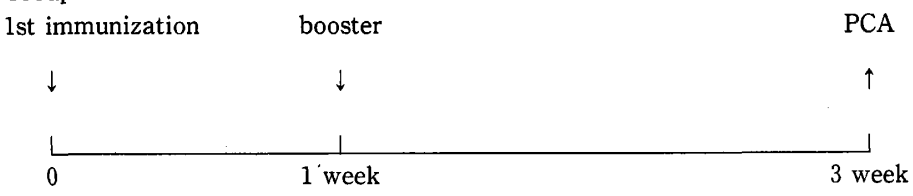
Three to 5 month-old Mongolian gerbils (JMS-strain) of both sexes were used in the following immunization with *D. immitis* (DI) antigen, passive cutaneous anaphylaxis (PCA) reactions for recipients and an immediate intracutaneous reaction for anti-rat IgE antiserum. These animals were supplied originally from Institute of Medical Science, Tokyo University in 1973 and were bred with sister-brother matings under a conventional condition in our laboratory (Shimizu *et al.*, 1990). Sprague-Dawley (SD) female rats aged 9 weeks were purchased from Saitama Experimental Animal Supply and were used as recipients of PCA reactions and a positive control of the immediate intracutaneous reaction for anti-rat IgE antiserum.

Immunization with D. immitis adult worm antigen in gerbils

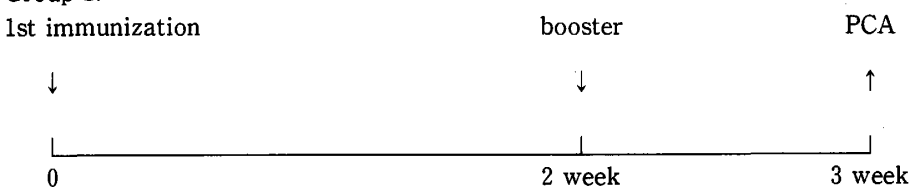
Crude extract derived from DI adult worm was prepared by the method as previously described (Fujita, 1975) and was used as DI antigen for immunization. Protein concentration in DI antigen was estimated by the method of Lowry *et al.* (1951). Experimental design of immunization is summarized in Fig. 1. Gerbils were randomly allocated to the 2 groups and were immunized with an intraperitoneal injection of various doses of antigen mixed with

Animal: male and female 3-5 month-old Mongolian gerbils (JMS strain)
Antigen: Crude extract from *Dirofilaria immitis* adult worm
Immunization: 10, 100, 500 or 1,000 μg antigen with 8 mg alum in 0.5 ml saline i.p. per an animal
Booster: an equal dose of antigen to 1st immunization with an equal way.
Interval:

Group 1.



Group 2.



Numbers of gerbils in each group:

Antigen dose (μg/animal)	10	100	500	1,000
Group 1	40	20	20	20
Group 2	60	60	—	—

Figure 1 Experimental design of immunization.

8 mg alum in 0.5 m*l* saline per an animal at different intervals. In group 1, 40 gerbils were immunized with 10 μg antigen and 20 animals each were immunized with 100, 500 or 1,000 μg antigen. One week later they were boosted with an intraperitoneal injection of an equal amount of antigen to the first immunization, and two weeks after the boost they were bled from the heart. In group 2, 60 gerbils each were immunized with 10 or 100 μg antigen. Two weeks later they were boosted with an intraperitoneal injection of an equal amount of antigen to the first immunization, and one week after the boost they were bled from the heart. Blood samples from immunized gerbils were centrifugalized separately (not pool) and each serum was stored at -20°C until PCA reaction.

Passive cutaneous anaphylaxis (PCA) reaction

Antibody in serum samples from gerbils immunized with DI antigen were tested with homologous and heterologous PCA reactions using gerbils and SD rats for recipients. Dermal sites of gerbils and rats were sensitized with sera from immunized gerbils in a volume of 0.1 m*l* at 10-fold-dilution. They were challenged 4 or 72 hr later with intravenous injections of 1 mg DI antigen in 0.5 m*l* 1% Evans blue in saline into the saphenous vein in gerbils and 1.0 m*l* into the tail vein in rats. Thirty minutes after antigen administrations, recipients were sacrificed under ether-anesthetization and were skinned to measure diameters of the reactions on the inner side of the skin. A blue spot larger than 3 mm in diameter was regarded as positive reaction. Positive sera were tested PCA reaction again after heat treatment at 56°C for 30 min.

Immediate intracutaneous reaction in gerbils for anti-rat IgE antiserum

To investigate presence of an IgE-like antibody in intact gerbils, the immediate intracutaneous reaction for sheep anti-rat IgE antiserum was carried out. Sheep anti-rat IgE antisera (Miles Laboratory, Inc.) in 2-fold serial dilutions in a volume of 0.1 m*l* were inoculated intracutaneously to sites on the back skins of rats and gerbils. One mililiter and 0.5 m*l* 1% Evans blue solution were injected intravenously into the tail vein in the rat and into the saphenous vein in the gerbil, respectively, immediately after inoculation with antisera. After 30 min, areas of the blue spots were examined. The reaction in the rat was regarded as a positive control and was compared to that in the gerbil.

Preparation of skin tissue sections of gerbils

The dorsal skins of gerbils were shaved and were quickly removed at the time of sacrifice. Blocks of the skin tissues were fixed with 10% buffered formalin and embedded in paraffin. Skin tissue sections were stained with Toluidine blue and examined under a light microscope.

RESULTS

PCA reaction

Table 1 gives numbers of positive samples from immunized gerbils of group 1 in PCA reactions. 0-23% and 5-15% of serum samples showed positive reactions in heterologous PCA reactions using rats for recipients after a 4-hr- and a 72-hr-sensitization period, respectively. There was no tendency to correlate between antigen doses and positive rates

Table 1 Positive rates of sera from immunized Mongolian gerbils of group 1 in PCA reactions

Antigen dose (μg/animal)	Sensitization period (hr)	PCA recipient					
		SD rat			Mongolian gerbil		
		Numbers of samples		Positive rate (%)	Numbers of samples		Positive rate (%)
		positive	total		positive	total	
10	4	9	40	23	0	40	0
	72	6	40	15	0	40	0
100	4	3	20	15	0	20	0
	72	1	20	5	0	20	0
500	4	0	20	0	0	20	0
	72	2	20	10	1	20	5
1,000	4	1	20	5	0	20	0
	72	3	20	15	1	20	5

Table 2 Positive rates of sera from immunized Mongolian gerbils of group 2 in PCA reactions

Antigen dose (μg/animal)	Sensitization period (hr)	PCA recipient					
		SD rat			Mongolian gerbil		
		Numbers of samples		Positive rate (%)	Numbers of samples		Positive rate (%)
		positive	total		positive	total	
10	4	7	60	12	0	60	0
	72	1	60	2	0	60	0
100	4	5	60	8	0	60	0
	72	13	60	22	0	60	0

in samples from group 1 gerbils. In homologous PCA reactions using gerbils for recipients, two samples represented positive reactions after a 72-hr-sensitization period.

Table 2 shows positive rates of samples from group 2 gerbils in PCA reactions. In heterologous PCA reactions after a 4-hr-sensitization period, positive rates of samples from group 2 gerbils immunized with 10 μg and of those immunized with 100 μg were 12% and 8%, respectively. Positive PCA reactions with 72 hr of sensitization period were obtained in 2% and 22% of each 60 samples from gerbils immunized with 10 and 100 μg antigen, respectively. In homologous PCA reactions, positive reaction could not be observed.

Those positive samples in PCA reactions after a 72-hr-sensitization period were heated at 56°C for 30 min and were assayed again. PCA positive reactions completely abolished in all samples after heat treatment (Fig. 2).

Immediate intracutaneous reaction for anti-rat IgE serum

Fig. 3a shows the immediate intracutaneous reaction for sheep anti-rat IgE antiserum in a positive control of a rat. A 1,600-fold dilution of anti-rat IgE gave a positive reaction in a rat skin, whereas saline and normal sheep serum did not.

Fig. 3b represents a reaction for that experiment in an intact gerbil. In an intact gerbil, also a 1,600-fold dilution gave a positive reaction with a distinct blue spot, but saline and

(a) (b) after heat inactivation

No. 69 No. 69

No. 14 No. 14

Figure 2 Passive cutaneous anaphylaxis with sera from Mongolian gerbils immunized with crude extract of *Dirofilaria immitis* (DI) worm in a rat skin. A 1:10 dilution of sera were inoculated intracutaneously. Animals were challenged by an intravenous injection of DI antigen together with Evans blue dye 72 hr after the sensitization. Samples (No. 69 and 14) gave positive reactions (a). After heat inactivation at 56°C for 30 min, the same samples failed to induce the reactions (b).

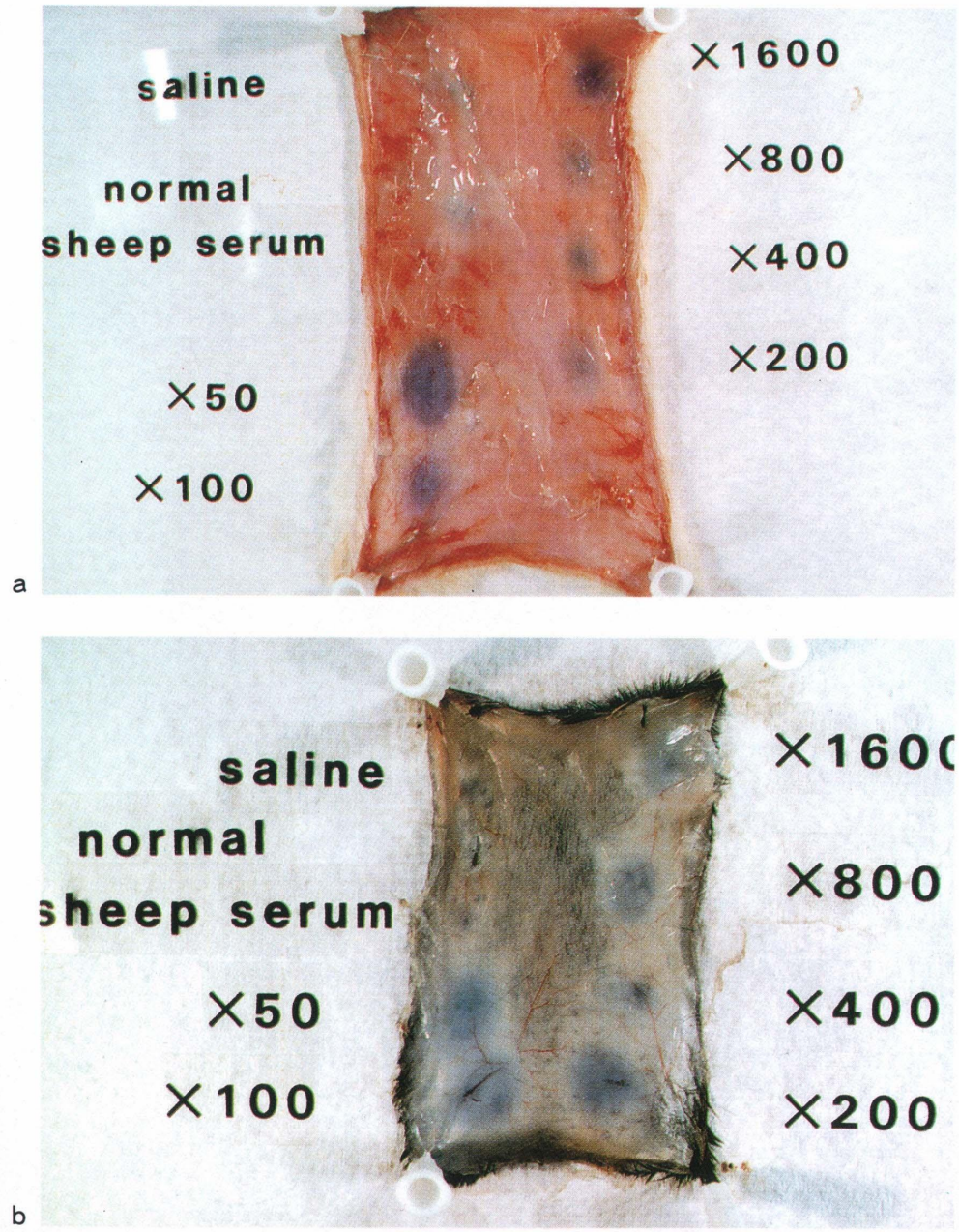


Figure 3 Immediate intracutaneous reactions induced by sheep anti-rat IgE antiserum. Serial 2-fold dilutions of antiserum preparation, saline and normal sheep serum were inoculated intracutaneously into a rat (a) or into a normal Mongolian gerbil (b), followed by an intravenous injection of Evans blue dye. Even a 1,600-fold dilution of antiserum gave positive reactions both in a rat (a) and in a Mongolian gerbil (b), whereas saline and normal sheep serum did not.

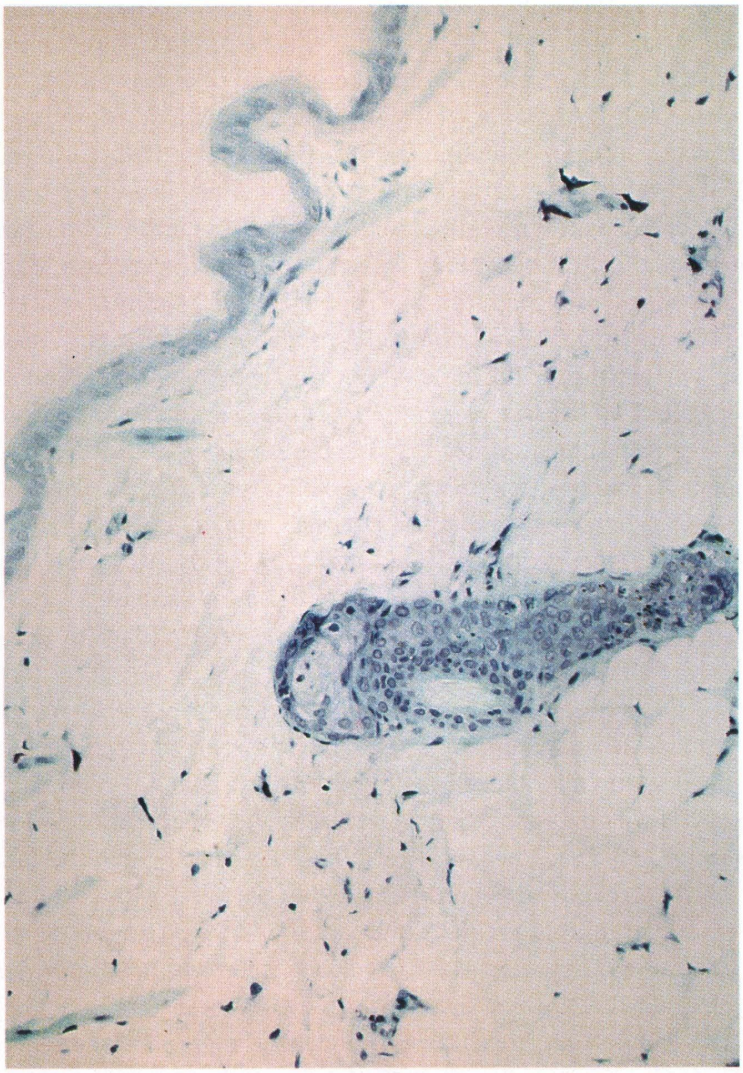


Figure 4 Histological findings in the skin tissue section of a Mongolian gerbil stained with Toludine blue. Mast cells representing peculiar metachromasia were idenfied.

normal sheep serum failed to induce the reactions.

Histological findings of the skin tissue of gerbils

Fig. 4 gives a photograph of the skin tissue section of the gerbil stained with Toluidine blue. Mast cells representing peculiar metachromasia were identified in the skin tissue of the gerbil.

Discussion

This is a preliminary report on antigen specific antibody induced by immunization with DI worm antigen in the Mongolian gerbil. Immunized gerbil produced a long-term binding heat-labile antibody that sensitized the rat skin. These profiles were similar to murine IgE (Mota and Wong, 1969; Ovary *et al.*, 1975; Watanabe and Ovary, 1977). But the antibody from gerbil was less homocytotropic which was one of peculiar characteristics of IgE and only 2 samples from a total 220 immunized gerbils represented positive in a long-term homologous PCA reaction. The cause may be that the gerbil is hard to produce IgE-like antibody by nature or it has a large individual difference because it has not been controlled genetic characteristics of this species for a laboratory animal such as a inbred strain. Hosts produce an antigen-specific antibody during the course of helminth infection (Mota *et al.*, 1969; Kojima and Ovary, 1975; Watanabe and Ovary, 1978; Rousseaux-Prevost *et al.*, 1979; Oikawa *et al.*, 1981). On the gerbil infected with *Brugia* spp., production (Tomisato *et al.*, 1983) and down regulation (Farrar *et al.*, 1991) of an IgG antibody, a protective resistance to reinfection (Yates and Higashi, 1985; Kazura *et al.*, 1986; Klei *et al.*, 1990) and reduction of cellular responses (Lammie and Kats, 1983; Klei *et al.*, 1981, 1988, 1990) have been reported. Those studies indicated that chronic *Brugia* spp. infection induced the gerbil a state of immunosuppression. One of the cause of high sensitivity of the gerbil to some filarial parasites may be attributed to the immunosuppression. Difficulty of IgE-like antibody production in the gerbil in this study may also support susceptibility of the gerbil to various parasites.

In an intact gerbil, a present study strongly suggested a presence of IgE-like substance that induce the immediate intracutaneous reaction with anti-rat IgE antibody. It has been unknown how much homology of IgE between the rat and the gerbil is. But IgE-like substance of the gerbil was thought to have cross reactivity with rat IgE and to be specifically bound to anti-rat IgE antibody because normal sheep serum and saline failed to induce hypersensitivity of the skin. It is generally accepted on mice, rats and human that IgE has high affinity for mast cells and basophil granulocytes and the reaction of cell-bound IgE with either antigen or anti-IgE triggers the release of chemical mediators from those cells (Kulczycki and Metzger, 1974; Ishizaka and Ishizaka, 1975; Austen, 1979; Sterk and Ishizaka, 1982). We could speculate that IgE-like substance of the gerbil also bound to mast cells in the skin, induced the release of chemical mediators from mast cells through almost same mechanisms as those of mice or rats, and as a result of those anaphylactic reactions, we could observed positive reactions on the skin. It is now well established that mast cells and basophils also carry on their surface receptors capable of interacting with IgG. For example, IgG_1 in mice (Tigellar *et al.*, 1971) or in guinea pigs (Ovary, 1986), IgG_{2a} in rats (Morse *et al.*, 1968) or IgG_2 in human (Assem and Turner-Warwick, 1976) sensitizes target cells for anaphylactic reactions. In gerbils, we could not detect IgG which was implicated in anaphylactic reactions in this study.

In the present study, we could induce antigen-specific antibody in the gerbil by immunization with DI worm antigen and confirmed a presence of IgE-like substance and mast cells in an intact gerbil. We previously reported that the gerbil has also basophilic granulocytes (Shimizu *et al.*, 1991) which were known to be IgE-binding targets as well as mast cells in mice or rats in peripheral blood. We showed that the gerbil had enoumous potentialities of

a model to analyse host-parasite relationship including IgE production and anaphylactic reactions.

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フィラリア感染好適宿主としてのスナネズミの基礎的検討
—犬フィラリア虫体粗抗原によって誘導されたIgE様抗体—

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フィラリア感染の実験室内好適宿主であるスナネズミ（Molgolian gerbil; *Meriones unguiculatus*）を，犬フィラリア（*Dirofilaria immitis*）虫体より抽出した粗抗原によって免疫し，抗体産生について受身皮膚アナフィラキシー（PCA）反応を用いて検索した。免疫したスナネズミの血清中には，ラットを被検動物とした72時間PCA反応で陽性を呈し，56°C30分間の熱処理によって不活化される抗原特異的抗体，すなわちIgE様抗体が検出された。

正常スナネズミのIgE様抗体を検出するために，抗ラットIgEヒツジ抗体に対する，即時型皮内反応を行った。スナネズミの皮膚もラットと同様に，1,600倍希釈抗体に対して明瞭な陽性反応を示したことから，正常スナネズミにもIgE様物質の存在が示唆された。

正常スナネズミの皮膚に，トルイジン・ブルー染色でメタクロマジーを呈する肥満細胞の存在が確認され，上記の抗原抗体反応の場であると考えられた。

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BACTERIAL DIARRHOEAS IN JOS, NIGERIA

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Abstract: In a 4 year study from October 1983 to March 1987, in Jos, Nigeria, bacterial enteric pathogens were isolated from 27.0% of 1,137 children aged 0-5 years who were clinically diagnosed for acute diarrhoea. A similar investigation was also carried out on apparently healthy children of primary school age (7-16 years), who were on regular school attendance. Eight different primary schools located in the urban and rural areas of Jos, were selected for this phase of the study. The 1,468 children examined in the latter group had a total infection rate of 11.9%. Though the children were considered to be apparently healthy, 25.6% of them reported a history of diarrhoea, lasting between 1-21 days; 12.7% of these were bacterial positive as opposed to 11.6% for the non diarrhoeal group.

In yet a third phase of the study, a total of 39 different households, predominantly of adult population were investigated for the presence of common organisms among members of the same family. Enteropathogenic *Escherichia coli* (EPEC) serovar O128:K67 was isolated from 4 of 14 members of one family, while EPEC serovar O28:K73 was isolated from 3 of 7 members of another family including 2 cases of multiple infections.

Some of the 48 strains of EPEC out of 130 isolates, although H antigen was not examined, could possibly belong to EHEC (VTEC: verocyto-toxin producing *E. coli*) which are thought to be one of the causative agents of haemorrhagic diarrhoeae as well as hemolytic uremic syndrome which are severe illnessess.

INTRODUCTION

Diarrhoeal diseases of bacterial aetiology continue to be a global concern. High rates of infantile morbidity and mortality have been reported, particularly in developing countries (Ronde and Nerthrup, 1976; Barma, 1981).

The reason why infants, and not adults, remain the high risk group could readily be attributed to poor hygenic standards of the adult caretakers in addition to the immature

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immune status of the children.

The present study reports on symptomatic and asymptomatic subjects with total infection rate higher in the asymptomatic adult group.

Reports indicating some adult Nigerian's staple meals as having antidiarrhoeal properties were suggestive to us as a contributing factor for the higher youth-adult asymptomatic carriage rates.

Material and Methods

Subject: Children of 0-5 years age group were attended to in the wards, O.P.D and the Child Health Clinic of Jos University Teaching Hospital, while the school children and adults were attended to in their schools and homes respectively.

Materials: Stool samples were collected in each case, into Cary-Blair's transport medium. Transportation to the laboratory and analysis were done within 24 hr of collection.

Culture: All stool specimens were inoculated on to; SS, DHL, TCBS and Skirrow's Campylobacter selective solid media for primary isolations. Selenite broth (for Salmonella spp.) and alkaline peptone water (for *Vibrio* spp.) were also used as enrichment broth. Cultures were incubated at 37°C for 18-24 hr, while *Campylobacter* cultures were incubated at 42°C for 48-72 hr.

Biochemical identifications: Suspect colonies were inoculated on to; triple sugar iron agar (TSI), lysine indole motility agar (LIM), Simmons' citrate agar and Christensen's urea, for tentative identification of; Salmonella, *Shigella, E. coli, Vibrio, Aeromonas* and *Yersinia* (Lennette *et al.*, 1983).

Oxidase positive colonies from the *Campylobacter* culture, showing typical Gram negative spira or coccoid forms on microscopic examination were biotyped according to standard methods (Lennette *et al.*, 1983).

Serotyping: Salmonella, *Shigella,* EPEC, *Yersinia* and *Vibrio* species were examined by slide agglutination procedure, using overnight pure growth of respective organisms on non-selective solid media. Antisera were obtained from Denka Seiken, Japan.

ETEC LT assay: The method is described briefly.

The Biken LT Assay Kit (Biken, Osaka, Japan) was used for identification of LT producing strains of *E. coli.* Biken Agar was melted and placed in 15 m*l* amounts into 90 mm plastic petri-dishes. Each Biken Agar plate was appropriately inoculated with four different cultures of *E. coli,* and incubated at 37°C for 48 hr.

Polymyxin B disc of 200 IU was carefully placed on each colony to improve intracellular release of LT. A hole was punched in the centre of the plates and incubation repeated for 5-6 hr. 20 μl of LT antiserum was placed in the central well. Observation was made for precipitin lines after incubation at 37°C for 24, 48 and 72 hr.

Results

Twenty-seven per cent of children aged 0-5 years with clinically diagnosed diarrhoeal disease were found bacteria positive, as against 11.9% for primary school children of ages 7-16 years and 12.7% for family units including adults respectively (Tables 1, 2, 3). All the known conventional diarrhoea-associated bacteria isolated from the clinically diagnosed

Table 1 Infection rate of enteropathogenic bacteria in children 0-5 yrs of Jos, Nigeria

Source	No. of cases examined	No. of positive cases	No. of strains isolated/total case (%)					
			Shig.	EPEC	Sal.	*Vibrio*	*Campy.*	Others
Pediatric Wards	220	75 (34.1)	24 (10.9)	13 (5.9)	14 (6.4)	2 (0.9)	10* (4.5)	12 (5.4)
G.O.P.D.	525	146 (27.8)	48 (9.1)	40 (7.6)	12 (2.3)	3 (0.6)	40* (7.6)	3 (0.5)
C.H.C.	392	86 (17.6)	23 (5.9)	25 (6.4)	7 (1.8)	0 (0)	31* (7.9)	0 (0)
Total	1,137	307 (27.0)	95 (8.4)	78 (6.9)	33 (2.9)	5 (0.4)	81* (7.1)	15 (1.3)

*: Estimate No. Actual No. are 8/172, 34/439, 17/214.
Shig.: Shigella, Sal.: Salmonella, *Campy.: Campylobacter*
Others: *Klebsiella pneumoniae, Proteus morganii, Staphylococus aureus* etc.

Table 2 Bacterial enteric pathogens isolated from children of 8 different primary schools located at the urban and rural areas of Jos, Nigeria

Bacteria	No. of isolates (%)		Total
	with diarrhoea 376* (25.6)	without diarrhoea 1,092* (74.4)	1,468*
EPEC	10 (2.7)	45 (4.1)	55 (6.8)
C. jejuni	18 (4.8)	40 (3.7)	58 (8.4)
Shigella	5 (1.3)	19 (1.7)	24 (3.1)
Salmonella	8 (2.1)	17 (1.6)	25 (3.7)
ETEC (LT)	5 (1.3)		5 (1.3)
A. hydrophila	2 (0.5)	4 (0.4)	6 (0.9)
Y. enterocolitica	0	1 (0.1)	1 (0.1)
Total	48 (12.7)	126 (11.6)	174 (11.9)

*: No. of children examined

Table 3 Bacterial enteric pathogens isolated from 39 family units from 2 villages in the rural area of Jos, Nigeria

Bacteria	No. of isolates (%)		Total
	with diarrhoea 35*	without diarrhoea 186*	221*
EPEC	2 (5.7)	8 (4.3)	10 (4.7)
C. jejuni	—	3 (1.6)	3 (1.3)
Shigella	—	10 (5.4)	10 (4.6)
Salmonella	—	2 (1.0)	2 (0.9)
ETEC (LT)	—	3 (1.6)	3 (1.4)
A. hydrophilla	—	—	—
Y. enterocolitica	—	—	—
Total	2 (5.7)	26 (14.1)	28 (12.7)

*: No. of cases examined.

Table 4 Cases of multiple isolations from primary school children and family units of Jos, Nigeria

From primary school		From family units	
with diarrhoea	without diarrhoea	with diarrhoea	without diarrhoea
EPEC O27:K+ *C. jejuni*	EPEC O114:K90 *C. jejuni*	*Shigella flexneri* Salmonella spp.	EPEC O28:K73* Salmonella spp.
	EPEC O127:K63 EPEC O148:K+		EPEC O28:K73* *Shigella flexneri* LT (+)
	EPEC O26:K60 *C. jejuni*		*Shigella boydii* 14 LT (+)
	EPEC O127:K63 *C. jejuni*		
	C. jejuni Salmonella O4		
	EPEC O25:K1 Salmonella O3, 10		

*: Isolated from members of the same family unit.

Table 5 Serovars of enteropathogenic *E. coli* isolated

OK Serovar		0-5 yrs	Primary school children	Adults
O1:K51	(H)	3	5(1)*	—
O111:K58	(H)	7	2	1
O114:K90	(I)	5	1	—
O124:K72		—	1	—
O124:K91		1		
O125:K70		2	2	—
O126:K71		5		—
O127:K63		5	5(1)*	1
O128:K67	(H)	5	2	6(1)*
O142:K+		3		
O148:K+	(T)	4	14(1)*	1(1)*
O159:K+	(T)	—	—	1(1)*
O25:K1	(T)	2	—	1
O26:K60	(H)	6	6	—
O27:K+		1	4(1)*	—
O28:K73	(I)	1	0	3
O44:K74		4		
O44:K77		—	1	—
O55:K59		4		
O6:K15	(H)	1	—	—
O86:K61		7	6(1)	—
				Total 130

(T):(ETEC); (I):(EIEC); (H):(EHEC)
(1)*: No. of isolates from diarrhoeal cases.

cases were also found in the apparently healthy population studied except for *Vibrio cholerae* O1 (*V. cholerae*), of which a total of 5 (0.4%) cases were isolated and were all identified as non-agglutinable serovar. *Aeromonas hydrophila* and *Yersinia enterocolitica* were isolated from some of the primary school children and not from the adults and infants groups (Tables 1, 2, 3).

Multiple isolations were also indicated (Table 4), EPEC seems to be the predominating pair in each of the cases (8 of 11 cases were paired with EPEC). Tables 5 and 6 presents the different bacterial serovars and serogroups isolated from the study.

Table 6 Serovars or serogroup of Salmonella and *Shigella* isolated

Serovar or serogroup		0-5 yrs	Primary school children	Adults
Shigella flexneri	1a	2		
	1b	18	2	—
	2a	8	—	1
	2b	4		
	3a	6	2	—
	4	5		
	5	2		
	6	14	3	—
Shigella dysenteriae	2	2		
	3	4	2	—
	4	—	1	—
	7	2		
	8	1	—	1
	5	—	—	—
	?	2		
Shigella boydii	2	1		
	5	1	1	—
	7	1		
	10	4	1	—
	14	6		
	15	2		
Shigella sonnei		9	5	1
Shigella spp.		1		
Salmonella typhi		2	1	—
Salmonella	O4	13	3	—
	O7	1		5
	O8	3		
	O9	2		
	O1, 3, 19	1	4	—
	O3, 10	—	9	—
Salmonella spp.		12	19	6

Discussion

The present study suggests that enteric infections of bacterial aetiology may often present with mild to asymptomatic conditions in the youth and adult populations in Jos, Nigeria (Tables 2, 3). Our suggestion is further strengthened in our study by the finding of the primary school children who were still able to carry out their routine school activities in spite of the fact that 25.6% of them were found to be diarrhoeal positive. The figure was derived from questionaire report only; the stool samples were, however, formed in consistency with little or no pathological findings on macroscopical examination. 12.7% of these were bacterial positive. It could not be ascertained within the scope of this study as to possibility of any associated immune conditions. However, some Nigerian adult staple meals, such as Gari, Cassava and some grains have been reported to have antidiarrhoeal properties similar in action to kaolin (Abosede, 1987; Ukunwe, 1987). It is possible that the Nigerian food quality may be largely responsible for the asymptomatic excretions of organisms. An infection rate of 27.0% in the 0-5 age group was of great concern to us. The adults who take care of these children may be found among asymptomatic pathogen carriers and their role in contributing to such a high infection rate is noteworthy. A number of vectors and mechanisms may be involved but the poor standard of hygiene in handling children and their feeding appear to be predominant.

Serovars of *E. coli,* reported to be associated with: enterotoxogenic (ETEC), enteroinvasive (EIEC), and enterohaemorrhagic (EHEC) properties (Levine, 1987) were also isolated, although we have conventionally grouped all as EPEC (Table 5). As presented on the Table the cases of; serovars O1:K51 (EHEC); O148:K^+ (ETEC) and O127:K63; O128:K67 (EHEC); O27:K^+ and O159:K^+ (ETEC) where only 1/5, 2/15, 1/6, 1/8, 1/4 and 1/1 were respectively isolated from diarrhoeal cases of the primary school children and adults but infants who were clinically diagnosed for acute diarrhoeae are noteworthy.

While we could not evaluate the pathogenic role of *E. coli* serovars isolated from this study to ascertain whether they were in each case responsible for the diarrhoea, particularly in the non-infant population, we feel obliged to consider their isolation from the adults with or without diarrhoea as a potential source of hazzard for the infants, particularly in areas of low socio-economic standards as evidenced in the present study area.

A recent massive outbreak of the kindergarden infantile diarrhoeae caused by EHEC (VTEC: verocytotoxin producing *E. coli*) O157:H7 in Japan in 1990 (Ito, 1991) resulting in the hospitalization of 34 pupils and 2 deaths has led us to reappraise our data on the isolation of EPEC in Nigeria. As shown in Table 5, although we did not examine H antigen, some of the 48 strains out of 130 isolates (36%) could possibly belong to EHEC (VTEC). The majority of them except O128:K67 were derived from 0-5 year old infants and primary school children.

O'Brien *et al.* (1983) reported that VT (VTI) produced by *E. coli* O26:H11 as well as O157:H7 was the same as Shiga-toxin produced by *S. dysenteriae* 1, thus making clear the homogeniety between VT of *E. coli* and Shiga-toxin. It is well known in North America that the toxin produced by O157:H7 degenerates and kills Verocells and clinically causes haemorrhagic diarrhoeae developing often haemorrhagic uremic syndrome thus becoming a severe illness.

These facts remind us of the complaints of the pupils as reported by school teachers while engaging in the field surveys of the infantile diarrhoeae in Nigeria. Some school teachers had

reported that some children were suffering from pains at the lower back (around kidney area), and also passed red urine. Unfortunately we had no antiserum to O157:H7 at that time and we were unable to pursue the issue further. The pupils from whom no bacterial pathogens were isolated inspite of heavy hemorrhagic diarrhoeae and hemolytic uremia may be considered to be candidates infected with O157:H7. It should be noted, however, that apart from causing diarrhoea, often a common and serious disease in Nigeria, EHEC (VTEC) might cause many other diseases. When viewed from these angles, it seems very important and also urgent that the study of the aetiology of infantile diarrhoea be resumed in Nigeria. Findings from our study lend support to this recommendation.

Vibrio species was not isolated from the apparently healthy group, but from the 0-5 age group (0.4%) who presented clinically diagnosed acute diarrhoea, all 5 strains isolated were non agglutinable (NAG).

Of interest to note that *V. cholerae* Ogawa serovars are currently being isolated from clinical cases reported between April-July, 1991, at the Jos University Teaching Hospital. A number of deaths have been reported in Jos and environs (unpublished data).

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ナイジェリア国ジョスに於ける細菌性下痢症について

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1983-1987年の4年間にわたる，ナイジェリア国ジョスにおける腸内病原菌を対象とした調査において，臨床的に急性下痢症と診断された0－5歳児1,137症例から，307株（27.0%）の病原菌が分離された。同様な調査は，8つの農村，および市内の小学校における健康通学児童（7－16歳，1,468名）についても行われた。申告により下痢病歴のある児童は25.6%で，菌の検出率は12.7%，下痢病歴のない者からの検出率は11.6%であった。

この様にナイジェリア児童の保菌者率は，先進国に比較すると大変に高い。一方，成人を含む39家族については，家族内感染を想定して同じような調査が行われた。1家族14人中4人からO128:K67（EHECの可能性あり）が分離され，他の家族では7人中3人がO28:K73が分離され，うち2人は多重感染をしていた。最近(1990年)の日本における出血性下痢性大腸菌EHEC, O157:H7に因る幼稚園児の集団下痢発生は，34人の入院患者と2人の死者を生じた。このことは，我々のナイジェリアにおけるEPEC分離データの再考慮を促した。その結果，たとえH抗原は調べなかったにせよ，分離されたEPEC130株中48株(36.9%)のうち，いくつかはEHECの可能性がある。残念なことに，当時我々はO157:H7に対する抗血清を入手出来なかったので，相当数のこの菌を取り逃がしたことが考えられる。当時，小学校の教師から子供達の中には，背中（腎臓部領域）の痛みを訴え，赤い小便をする者がいて，健康状態も良くないと言われたことを思いだすと，今になって了解できるものがある。即ちナイジェリアにおいては，相当数の児童がEHEC（VTEC）に感染し，健康が阻害されていると予想され，この新しい観点にたっての再調査が，緊急に必要と考えられる。

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大阪空港検疫所におけるインド，ネパール長期旅行者下痢症患者からのランブル鞭毛虫検出成績

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はじめに

近年のわが国と諸外国との交流の増加により，海外旅行者数も年々増加しつつあり，1991年における全国の空港検疫所における検疫機数および検疫人数は，厚生省生活衛生局食品保健課検疫所業務管理室資料によれば，8万機，1,700万人にも達している。このような状況を背景に，コレラや赤痢をはじめとする腸管感染症の輸入症例の増加が指摘されてきた(阿部ら，1981；山田ら，1983；宮田ら，1990)。

著者等も同様の観点から，1983年から3年間に，大阪空港より入国した海外旅行者下痢症患者についての腸管原虫感染の実態調査を行い，特にインド，ネパールへの長期旅行の下痢症患者の約14%にランブル鞭毛虫感染が見られることを明らかにした（木村ら，1987)。

そこで今回は，インド，ネパール長期旅行者のランブル鞭毛虫感染状況について，1986年より1991年までの6年間に，同地域への長期旅行者下痢症患者を対象とした継続調査を実施すると共に，その成績を解析し若干の疫学的考察を加えた。

材料と方法

入国時に，過去2週間以内の下痢，嘔吐，腹痛，発熱等を申告した旅行者のうち，医師の診察を受け，病原細菌検出を目的として検便を実施した者の中で，インドまたはネパールのいずれか，あるいは両国での滞在期間の合計が10日以上の旅行者を，ランブル鞭毛虫感染検査の対象者とし，以下の方法で検便を実施した。

患者糞便約1 gを10 mlのMIF固定液に投入して固定・染色後，少量をスライドグラスに塗抹，鏡検した。また糞便の残量をMGL法により処理した後，沈渣をヨード・ヨードカリ液で染色し鏡検した。なお本報告で用いたランブル鞭毛虫陽性者について，その発症時および検疫時の症状を調べると共に，細菌学的検査を実施した。

成　績

1．調査対象者

1986年1月から1991年12月までの6年間における，ランブル鞭毛虫検出を目的とした調査対象者は692名で，うち男性は554名(80.1%)，女性は138名（19.9%)，平均年齢は27.4歳であった。各年の病原細菌検出を目的とした検便数(総検便数)とランブル鞭毛虫調査対象者数の割合（検査率）を表1に示した。この表で明らかなように，総検便数の中での調査対象者の占める割合は，年々減少する傾向が見られた。

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Table 1 Total number of examinees, No. examined for *G. lambldia* (*G.l.*) and No. of *G.l.* positive cases at Osaka Airport Quarantine Station in 1986-1991

	1986	1987	1988	1989	1990	1991	Total
Total No. of examinees	1,373	1,871	1,801	1,583	1,546	1,583	9,757
No. examined for *G.l.*	132	205	148	107	69	31	692
Examination rate (%)	9.6	11.0	8.2	6.8	4.5	2.0	7.1
No. of *G.l.* positive cases	18	19	4	8	4	6	59
Positivity rate (%)	13.6	9.3	2.7	7.5	5.8	19.4	8.5

2．検出成績

検査の結果，692名の8.5%にあたる59名から，ランブル鞭毛虫の嚢子あるいは栄養体を検出した。各年の陽性者数と陽性率は，1986年は132名中18名（陽性率13.6%），1987年は205名中19名（9.3%），1988年は148名中4名(2.7%)，1989年は107名中8名（7.5%），1990年は69名中4名（5.8%），そして1991年には31名中6名(19.4%)であった(表1)。全期間を通しての，各月ごとの合計の陽性率を表2に示したが，3月（9.2%），4月（13.6%），5月（13.6%），9月（11.3%），10月（14.8%）の春季，秋季に高率であった。これに対して1月には1.8%，また7月，12月には全く検出されないなど，夏季，冬季には低率であった。

Table 2 Seasonal variation in positivity rate of *G. lamblia* infection in 1986-1991

Month	No. examined for *G. lamblia*	No. of *G. lamblia* positive cases	Positivety rate (%)
Jan.	55	1	1.8
Feb.	52	4	7.7
Mar.	185	17	9.2
Apr.	81	11	13.6
May	44	6	13.6
Jun.	15	1	6.7
Jul.	22	0	0
Aug.	98	6	6.1
Sep.	53	6	11.3
Oct.	27	4	14.8
Nov.	34	3	8.8
Dec.	26	0	0
Total	692	59	8.5

3．陽性者の旅行期間

59名のランブル鞭毛虫陽性者の旅行期間の内訳は，11—14日が6名，15—29日までが26名，30日以上が27名となり，2週間以上の者が53名と全体の89.8%を占めた。各年の旅行期間毎の陽性者数を表3に示した。

4．対象者および陽性者の旅行先

検査対象者の中には，インド，ネパール以外に経由国あるいは滞在地として，タイ，香港，シンガポール，中国などの諸地域へも旅行した者が37%あり，その滞在期間も長短さまざまであった。そこで，他地域への旅行の有無は今回考慮外とし，対象者をインド旅行者（インドおよびインドと他地域への旅行者），同様にネパール旅行者およびインド，ネパール両国旅行者の3群に区分し，各群毎の検査対象者と陽性者を集計した。その結果，インド旅行者群における陽性率は8.4%（35/419）となり，ネパール旅行者群の3.2%（2/62）より

Table 3 Days of oversea travel of *G. lamblia* positive cases

Year	No. of positive	Days of travel		
		11-14	15-29	30≦
1986	18	0	11	7
1987	19	4	9	6
1988	4	0	1	3
1989	8	1	0	7
1990	4	0	2	2
1991	6	1	3	2
Total	59	6	26	27
(%)		(10.2)	(44.1)	(45.7)

Table 4 Desination of the cases examined for *G. lamblia* and *G. lamblia* positive cases

Destination*	No. of examined	No. of positive	(%)
India	419	35	8.4
Nepal	62	2	3.2
India+Nepal	211	22	10.4

*Including other areas for example Thailand, Hongkong, China etc.

高率であった。またインド，ネパール旅行者群の陽性率は10.4% (22/211) と最も高かった（表4）。

5．陽性者の年齢および性別

陽性者の平均年齢は25.2歳で，その内訳は20歳代が49名(83%)，10，30，40歳代が各3名，50歳代が1名であった。また男女比では，男性が47名と全体の79.7%を占めていた（表5）。

6．病原細菌との重複感染例

ランブル鞭毛虫の他に，1種類以上の病原細菌が検出された者が，陽性者の28.8% (17名) に見られた。このうちでは赤痢菌感染者が15名と最も多く，重複感染例の70%以上を占めていた（表6）。

Table 5 Age and sex distribution of the cases with *G. lamblia*

Sex	Age						Total
	0-9	10-	20-	30-	40-	50-	
Male	0	2	40	2	2	1	47
Female	0	1	9	1	1	0	12
Total	0	3	49	3	3	1	59

Table 6 Results of bacteriological examination in 59 cases with *G. lamblia*

Pathogen isolated*					Total
SHI+SAL	SHI+Cj	SHI	SAL	ETEC	
3	1	11	2	1	17

*SHI: *Shigella* spp.
SAL: Salmonella spp.
Cj: *Campylobactor jejuni*
ETEC: Enterotoxigenic *Escherichia coli*

Table 7 Clinical sings in the cases with *G. lamblia*

	No. of single infection*		No. of multiple infection†		Total cases	
Clinical signs	41		18		59	
(on onset)	No. of cases				Total	
Watery diarrhoea	29	(70.7%)	14	(77.8%)	43	(72.9%)
Loose passage	12	(29.3%)	4	(22.2%)	16	(27.1%)
Combined with						
・Abdominal pain	6		4		10	
・Fever	5		3		8	
・Abdominal pain and fever	2	15 (36.6%)	2	10 (55.6%)	4	25 (42.4%)
・Fever and vomiting	1		0		1	
・Abdominal pain, fever and vomiting	1		1		2	
(at quarantine)						
Diarrhaea	3	(7.3%)	1	(5.6%)	4	(6.8%)
Loose passage	34	(82.9%)	16	(88.9%)	50	(84.7%)
Cured	4	(9.8%)	1	(5.6%)	5	(8.5%)

*Single infection: Cases infected with *G. lamblia* only.
†Multiple infection: Cases infected with *G. lamblia* and other pathogen(s).

7．陽性者の臨床症状

ランブル鞭毛虫陽性者の発病時，および検疫時の症状をまとめたものが表7である。一日数回から数十回の水様性下痢をもって発病した者が最も多く43名(72.9%)，ついで一日数回の軟便が16名（27.1%）であった。また水様性下痢あるいは軟便に，腹痛，嘔吐，発熱のいずれかの症状を単独に，または複数の症状を伴い発症していた者が25名（42.4%）を占めていた。発病時の症状では，腹痛や発熱を伴っていた者の割合が，ランブル鞭毛虫単一感染者の36.6%と比較して，細菌との重複感染者においては55.6%と高率であった。また検疫時における臨床症状では，軟便が継続していた者が50名と全体の84.7%を占めていた。また検疫時すでに症状が回復していた者は，単一感染者に多く見られた。

考　察

今回の調査で問題となる点として，年々のランブル鞭毛虫検査対象者数の減少が挙げられる。特に1990年以降の検査対象者数の低下は著しく，1991年では1986年の1/4以下となっている（表1）。この期間中，大阪空港における総検便数には大きな変動がないことから，インド亜大陸への長期旅行者の減少が，低下の直接の原因と考えられる。この点の詳細は明らかにしえなかったが，この期間はインド，ネパール両国間の緊張や，インド国内情勢の不安などがあり，これが長期旅行者減少に少なからず影響があったものと推測される。

前回，著者等が実施した海外旅行者下痢症患者の腸管原虫調査では(木村ら，1987)，下痢症患者の中で，特にインド，ネパール長期旅行者からのランブル鞭毛虫陽性率が14%に達した。今回の調査では，陽性率に実施年により2.7%から19.4%と大きな差が見られたが，全期間を通じては8.5%であった(表1)。国内各地の総合病院におけるランブル鞭毛虫感染状況については，鈴木（1982)，角ら（1986)，関戸ら（1988）および市澤ら（1990）が報告しており，その陽性率はそれぞれ，0.56%（9,473件中53例)，0.33%（14,756件中49例)，0.16%（6,168件中10例）および0.6%（4,363件中28例）となっている。これらの報告における調査対象者は一般入院・外来患者や人間ドック入院患者であり，著者等が対象とした下痢症患者とは異なることから，これらの成績をそのまま今回の陽性率と比較することは難しい。しかし両成績間に大差が認められることは，インド，ネパール長期旅行者が，ランブル鞭毛虫感染のハイリスクグループとなり得ることを改めて示唆する結果となった。

ランブル鞭毛虫の推定感染国としてインド，ネパールが重要であることは，前報(木村ら，1987)および金（1988）の報告によっても指摘されている。今回の旅行先群別での集計では，インド旅行者群とインド，ネパール両国旅行者群での陽性率（8.4%および10.4%)が，ネパール旅行群者での陽性率（3.2%）と比較し高率であった。これは推定感染国として，インドがネパールと比較してより重要であることを推測しうる成績であると考えられる（表4)。

今回の調査でも，20歳代青年が陽性者の83%を占め(表5)，また各年とも陽性者の80%から90%が2週間以上の旅行期間を有していた(表3)。この結果は，トレッキングやボランティア活動でこの地域に長期滞在することが，腸管寄生原虫や蠕虫感染の機会を増大させることをさらに裏付けるものとなった。

陽性率の季節的変動については，前報では春季および夏期に高かったが，今回は春季および秋季に高率であった(表2)。いずれも休暇中，あるいは休暇明けの帰国者が増加する時期であり，この時期の旅行者下痢症患者には，充分に注意を払う必要があろう。

陽性者の28.8%にあたる17%からは，赤痢菌その他の下痢起因性細菌が同時に検出された（表6)。これら病原細菌重複感染者の発症時の臨床症状では，ランブル鞭毛虫単一感染者より，発熱や腹痛，嘔吐を伴うケースの割合が多く見られ（表7)，病原細菌の影響を推測させるものであった。しかし，発症時およびその後の継続的な水様下痢や軟便を示す下痢症患者には，病原細菌検出の有無に関わらず，ランブル鞭毛虫感染を疑い，検査を実施する必要があると思われる。またランブル

鞭毛虫陽性者からは，胆嚢炎や肝炎様の激しい症状の者は見られなかった。しかし，本原虫の小腸上部のみならず，胆道内への寄生例も報告されていることから（金子ら，1979；津嶋ら，1985；山田ら，1980；奥野ら，1976），今後帰国者の中にも胆嚢炎等の症状を呈する感染者も見いだされる可能性がある。

海外，特に熱帯地域発展途上国への旅行者は年々増加の傾向にあり，それとともにランブル鞭毛虫症の国内への持ち込みの増加も懸念される。このような状況のもと，今後は腸管細菌のみならず，ランブル鞭毛虫を始めとする腸管寄生性原虫や蠕虫に対しても，検疫体制を強化，拡大すべきではないかと考える。

結　語

インド・ネパール長期旅行者のランブル鞭毛虫感染状況の解析を目的として，大阪空港検疫所から入国した海外旅行者下痢症患者について，1986年より1991年まで6年間の継続した調査を行い，以下の成績を得た。

1）インド・ネパール長期旅行の下痢症患者692名中の59名（陽性率8.5%）にランブル鞭毛虫感染が見られた。陽性率の最も高い年は1991年で19.8%，最も低い年は1988年で2.7%であった。また春季，秋季には，夏期，冬期と比較して陽性者が高率に見られた。

2）ランブル鞭毛虫陽性者59名のうち，2週間以上の長期旅行者が53名（89.8%）を占めていた。

3）旅行先別による検討では，インド，ネパール両国への旅行者群中の陽性率が10.4%と最も高く，ついでインド旅行者群の8.4%，ネパール旅行者群の3.2%であった。

4）陽性者の平均年齢は25.2歳，男女比は8：2であった。また陽性者の68%が20歳代の男性であった。

5）陽性者の28.8%にランブル鞭毛虫の他に，赤痢菌を始めとする他の病原細菌が検出された。

6）陽性者の臨床症状の特徴として，発病時に頻回の水様性下痢と，以後の軟便の継続が見られた。

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SURVEY OF *GIARDIA LAMBLIA* INFECTION IN RETURNING TRAVELERS WITH DIARRHOEA FROM INDIA AND NEPAL AT OSAKA AIRPORT QUARANTINE STATION

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Patients with traveler's diarrhoea who returned from traveling India and/or Nepal more than 10 days were subjected to stool examination for *Giardia lamblia* at Osaka International Air Port Quarantine Station in 1986 to 1991. Results obtained were summarized as follows:

1) *G. lamblia* was detected in 59 of 692 cases examined (positivity rate, 8.7%). The positivity rate in each year was: 13.6% in 1986, 9.3% in 1987, 2.7% in 1988, 7.5% in 1989, 5.8% in 1990 and 19.4% in 1991, respectively. The seasonal variation in the piositivity rate was recognized as it was higher in March (9.2%), April and May (13.6% each), September (11.3%), and October (14.8%) than other months. No positive case was found in July and December.
2) 89.8% of the positive cases were the travellers over 14 days.
3) Travellers who visited both India and Nepal showed high positivity rate (10.4%). Those who visited only India or Nepal showed lower rate (8.4% and 3.2%, respectively).
4) More than 68% of positive cases were male at the age of 20s.
5) In 28.8% of the positive cases, some species of pathogenic bacteria (e.g. *Shigella* spp.) were concomitantely detected.
6) Major complaints of the positive cases were watery diarrhoea, lastig and loose passage. It was pointed out from the results that stool examination for *G. lamblia* to returning travelers with dearrhoea from India and Nepal should be made at qurantine as they were at high risk of *G. lamblia* infection.

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症例報告

ハロファントリンにより治癒した輸入三日熱マラリアの1例

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はじめに

1991年の日本人年間海外渡航者数は10,633,777人，また外国人の日本への入国者数は3,855,952人であり（法務省入国管理局統計），その数は年々増加し，それに伴って日本国内における輸入マラリアの症例数も増えてきている。現在年間罹患者数は，届け出られるだけでも100名を越え，マラリアはもはやめずらしい病気ではなくなってきている。しかし，日本国内で入手可能な抗マラリア薬は限られており，有効かつ安全な治療薬の導入に特別な関心が払われてしかるべきである。我々は，輸入三日熱マラリアを経験し，治療薬としてハルファン（HALFAN™: halofantrine hydrochloride）を試みた。ハルファンは1984年に臨床治験が開始され現在に至るが，本邦においては薬剤の入手が困難である事もあって，これまでに治療報告はない。

症　例

患者：39歳，パキスタン人男性。1989年5月より日本に滞在。

主訴：発熱，悪寒戦慄，嘔気。

既往歴：1984年にパキスタンにおいてマラリアの既往がある。

現病歴：1991年4月初旬より発熱を繰り返し，4月15日堀江病院を受診した。受診時，悪寒戦慄，嘔気，下痢を認め，急性胃腸炎の疑いで入院となった。

入院時現症：体温40.3℃，脈拍102/分整，血圧120/60。結膜に黄疸，貧血を認めず，胸部聴診所見に異常はなかった。下腹部に圧痛を認めたが，筋性防御反応は無かった。腹部聴診上，腸蠕動の亢進を認めた。腹部超音波検査では，肝脾腫を認めなかった。

入院時検査成績：CRPが陽性を示したが，白血球増加は認めず，また貧血も無かった（図1）。生化学検査では，軽度低K血症以外ほぼ異常を認めなかった。

入院後経過：上記所見よりウイルス性の腸炎が疑われ，入院後直ちに抗生物質の投与ならびに，発熱，嘔吐，下痢による脱水・低カリウムのため補液を開始した。しかし治療に抵抗して，体温は4月15日より1日おきに39—40℃の発熱を認めた（図1）。4月23日，血液培養検査を行ったが菌は検出されなかった。熱型より三日熱マラリアが疑われたため，4月24日採取した薄層塗抹標本を調べたところ，赤血球内に三日熱マラリア原虫が認められた。寄生赤血球率は0.05%であった（写真1，図1）。また，間接蛍光抗体法による三日熱マラリア原虫抗原，熱帯熱マラリア原虫抗原に対する抗体価は，それぞれ1：1,024，1：256であり，血清学的にも三日熱マラリアと診断された。治療には，新しい抗マラリア薬であるハルファンを用いた。ハルファン（1錠中ハロファントリン塩基

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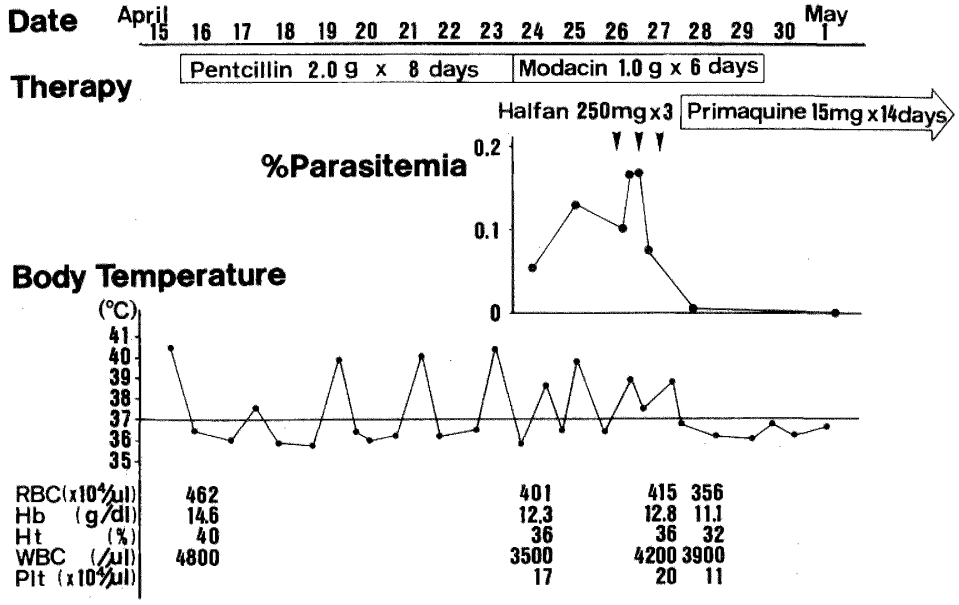

Figure 1 Clinical course of the patient.

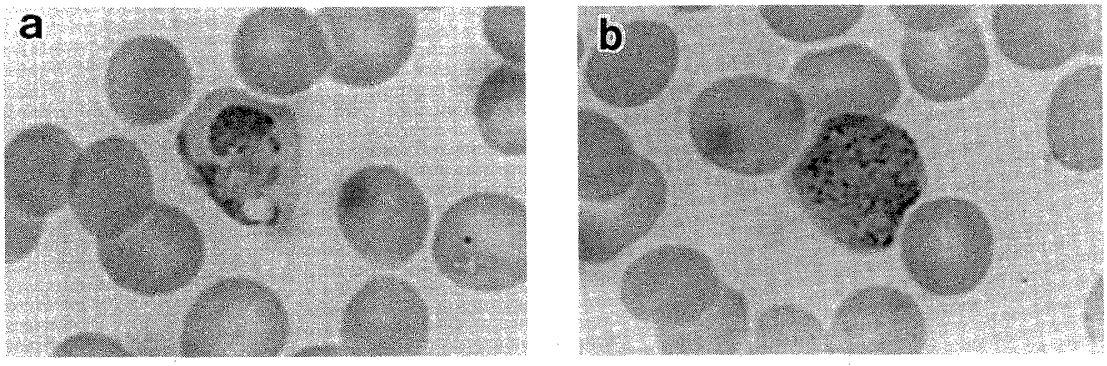

Photo. 1 Giemsa-stained blood smear of the patient showing a) a trophozoite, b) a gametocyte of *Plasmodium vivax* in enlarged erythrocytes.

233 mg)は，4月26日18：00，27日0：00，6：00に2錠ずつ合計6錠経口投与し，薬剤投与直前に，耳朶血にて塗抹標本を作成した。また，4月28日より肝内型の抗マラリア薬であるプリマキンを1錠（15 mg）ずつ14日間，毎朝一回経口投与した。ハルファン投与後の体温は4月27日15：00に36.9℃の平熱に回復した。また，寄生赤血球率も徐々に低下し，28日には，0.01%以下となった(図1)。4月27日9：00頃に，ハルファンの副作用と思われる嘔気，嘔吐が軽度認められたが，それ以外の症状は認められなかった。4月30日退院となり，その後再発を認めていない。

考　察

現在，本邦において市販されている抗マラリア薬は，Fansidar™だけである。血漿中最高濃度も2－6時間で得られ，クロロキン耐性熱帯熱マラリアにも有効であるなど，一般輸入例の治療には適しているものと考えられる。しかし，消化器症状を始めとする副作用と，1,000人に1人程度に重篤な皮膚・粘膜症状が報告されており，その点

において投与時に注意を要する（WHO, 1990)。また，Fansidar耐性熱帯熱マラリアの流行も既に拡散しつつあり（WHO expert committee on malaria, 1992)，新しい抗マラリア剤の開発が常に必要となる。比較的新しい治療薬として，最近日本において著者らにより試された薬にmefloquineとartemetherがある。前者はキノリンメタノールで構造上キニーネに似ており，特に多剤耐性マラリアに有効と考えられている（WHO, 1990)。嘔気・嘔吐，平衡感覚障害を始めとする副作用が問題となっているが，我々の経験では，軽い消化器症状を呈した以外副作用は認めていない（大西ら，1991; Yamaguchi *et al.*, 1992)。後者は重症熱帯熱マラリアの治療に特に有効で，副作用も軽微と考えられている。本邦における治療例では，ほとんど手後れに近い重症マラリアもすみやかな治癒に至り，副作用も特に認めていない(狩野ら，1988)。しかし再燃の高いことにより，一般輸入例に対する治療適応は無いと考える。

本報告で使用したハルファンは，特に薬剤耐性マラリアに抗する治療薬として開発され，1984年に臨床治験が開始された。治験報告例はすでに1,000例を越え，治癒率は95%以上である(Smith Kline and French Laboratories Limited, 1988)。構造は図2に示すようなフェナントレンメタノールで，従来の抗マラリア薬と構造を異にし，その差異がマラリア原虫のハルファンに対する交差耐性の低いひとつの理由と考えられている。血中最高濃度は一回量投与後6時間で得られ，半減期はおよそ1－2日と比較的短い（S.K. and F. Lab. Ltd., 1988)。Boudreauら（1988）は，ハルファン治療による平均解熱時間は60時間，平均原虫消失時間76時間と報告している。またChitchang and Wongteptien（1989）は，三日熱マラリア例のハルファン治療による平均解熱時間は42時間，平均原虫消失時間62時間と報告している。本例では前者が21時間，後者は確定できなかったが38時間後に0.01%以下となり，治療において著効を示したと考えられた。経時的に原虫形態を観察したところ，4月27日6：00，即ち3回目のハルファン投与時に，既に三日熱マラリア原虫の細胞質はアメーバ状を呈さずむしろ濃縮傾向にあり，28日8：00には空胞の形成が顕著であった。ハルファンの副作用は表1に示すように下痢，腹痛，嘔気，嘔吐等の消化器症状を中心に上げられるが，頻度としては特に低く，安全に投与できる薬剤で

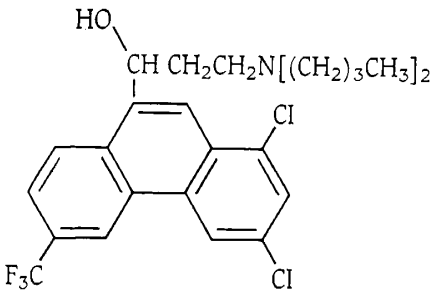

Figure 2 Structure of halofantrine.

Table 1 Clinical events after treatment with Halfan*

Clinical events	Post-treatment events Number (%)
Diarrhoea	69 (6.8)
Cough	64 (6.3)
Abdominal pain	57 (5.6)
Nausea	39 (3.8)
Vomiting	39 (3.8)
Dizziness	31 (3.0)
Headache	23 (2.3)
Fever	22 (2.2)
Pruritus	21 (2.1)
Chills	20 (2.0)
Backache	18 (1.8)
Myalgia	17 (1.7)
Pallor	14 (1.4)
Palpitations	14 (1.4)
Jaundice	8 (0.8)

* The study involved 975 naturally infected subjects and 36 artificially infected volunteers (Source: Smith Kline and French Laboratories Limited).

あると考えられている。本症例における軽度の嘔気・嘔吐は薬剤投与前からも認められていたが，ハルファンによる副作用とも考えられた。

本症例は日本滞在後およそ11カ月で発症した，一日おきの熱型とそれに伴う症状を示す典型的な三日熱マラリアの再発例と考えられた。三日熱マラリア治療薬としてはクロロキンが妥当で，現在のところハルファンは第一選択薬とは考えられないが，本邦においては，これまでにハルファンによる治療の報告例はなく，その有効性をまず例証，報告するための治験として，患者に説明をし同意を得た後に治療を行った。臨床諸家が抗マラリア薬を選択する時，その薬剤による速やかな原虫消失効果を特に期待する。我々はさらに多剤耐性マラリアを含む熱帯熱マラリア数例を，同様にハルファンで治療しているが(投稿準備中)，原虫消失時間など，治療効果においてハルファンは従来の抗マラリア薬に匹敵していると考える。現在本邦において，熱帯熱マラリアによる重症例や薬剤耐性マラリア症例が増えてきており，ハルファンは今後注目されるべき抗マラリア薬と考えられる。

おわりに

パキスタン人の三日熱マラリアの再発例を経験し，その治療薬としてハルファンを試み著効を示した。またハルファン投与後の患者副作用は，軽微であった。輸入マラリアの例数も増えるにつれ，クロロキン耐性マラリアに遭遇する機会も多くなると考えられるので，その新しい治療薬として，今後ハルファンの適用例を検討して行く必要があろう。

本症例の要旨は第33回日本熱帯医学会総会（平成3年11月，京都）で発表した。

謝　辞

本報告の作成にあたり，鈴木幹雄医師に助言をいただいたことを付記する。

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AN IMPORTED CASE OF VIVAX MALARIA TREATED WITH HALOFANTRINE

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[Case] The patient was a Pakistani male, 26 years of age, who came to Japan in May, 1989. The last malaria episode was in Pakistan about 5 years prior to this date. On admission to Horie Hospital on April 15, 1992, he had a body temperature of 40.3°C, which did not respond to antibiotics. Vivax malaria parasites were first detected from thin blood smears taken on the 24th at the density of 0.05%. Antibody titers against *Plasmodium vivax* and *Plasmodium falciparum* antigens were shown at 1:1,024 and 1:256 respectively by the indirect fluorescent antibody test. Two tablets each of Halfan (halofantrine hydrochloride, 233 mg base/tablet, Smith Kline and French Laboratories Limited) were administered orally at 18:00 on 26th, 0:00 and 6:00 on 27th, followed by primaquine at 15 mg per day for 14 days. Body temperature fell to normal at 15:00 on the 27th (36.9°C) and parasites were reduced to below 0.01% parasitemia on the 28th. No adverse reactions were recognized except slight nausea and vomiting.

Halfan has been used for the treatment of human malaria since 1984, and reported to be a effective against all parasite species. The drug is a phenanthrene derivative which does not share chemical structure with any other antimalarials, and is therefore particularly effective in the treatment of drug resistant malaria. Our report is the first in Japan of successful treatment of malaria with Halfan. Clinical side effects of the drug on this patient seem to have been very mild. Parasite clearance was rapid and fever dropped dramatically. Fansidar is the only antimalarial drug available in Japan so far, and imported drug-resistant malaria not only against chloroquine but Fansidar has been on the increase. The general use of Halfan is thus expected.

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症例報告

熱帯熱，三日熱マラリア混合重症感染の1例

—artemetherによる治療経験—

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近革　啓文[1]・小林　豊[1]・中村　健[2]・伊藤　洋一[2]・
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平成4年9月10日受付/平成4年10月14日受理

緒　言

熱帯熱，三日熱マラリア混合重症感染の1例を報告する。患者は31歳男性。インドネシアより帰国後，発熱と下痢を来し，発病7日目に本院入院。脳症，急性腎不全，ショックとDICを合併し，熱帯熱マラリア原虫寄生赤血球率は最高29%に達した。種々の抗マラリア剤を投与したが臨床症状は急速に進行し，腎不全は血液透析を必要とした。最終的にartemetherによる治療が奏功し救命し得た。また，腎生検で急性尿細管壊死を示唆する所見を得た。

本邦における，重症マラリア治療の貴重な症例と考え報告する。

症　例

患　者：31歳，男性。会社員。

主　訴：発熱，全身倦怠感，下痢。

既往歴：特記すべきことなし。

家族歴：特記すべきことなし。

現病歴：1991年4月17日から25日まで，実験動物の調査目的でインドネシアのジャワ島南西海岸に位置するMuarabinuavgeおよび同所から約50 km離れたDeli島に合わせて3日間滞在した。現地ではクロロキンの週一回の予防内服を行っていた。4月25日帰国。5月2日，38°Cの発熱が出現。軽度の悪寒戦慄を伴った。4日近医を受診し，風邪といわれ投薬を受けたが，症状の改善はなく，39—40°C台の発熱が持続した。7日より1日5行以上に及ぶ水様性下痢を認めた。8日北里大学病院内科を受診。受診時血圧低下と黄疸が認められ緊急入院となった。

入院時現症：身長165 cm，体重60 kg，体温40°C，血圧80/60 mmHg，脈拍数80/分・整。意識清明。皮膚：四肢末梢は湿潤で冷感あり。軀幹に虫刺され様の丘疹が散在。出血傾向はなし。眼球結膜に黄疸を認めた。眼瞼結膜に貧血はなし。リンパ節：触知せず。胸部：異常所見なし。腹部：心窩部から右季肋部に圧痛と反跳痛を認めた。心窩部に肝を3横指触知した。脾臓は触れなかったが，トラウベ半月の縮小を認めた。下肢に浮腫なし。神経学的に異常所見なし。

入院時検査成績(表1)：検尿で蛋白尿と沈渣に白血球，赤血球，顆粒円柱を認めた。便潜血が陽性であった。末梢血では白血球，赤血球ともに増加していたが，高度の血小板数減少，aPTTの延長とFDPの増加を認めた。血沈は1時間値3 mmであった。血液生化学検査では溶血と腎機能障害の所見が認められた。総コレステロールが低値で

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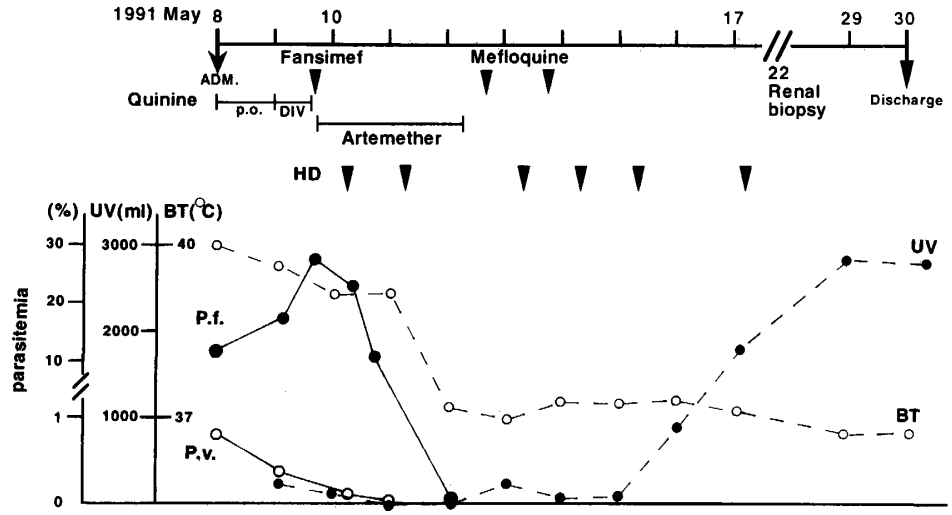
Table 1 Laboratory findings

Urinalysis:		Chemistry:		Immunology:	
gravity	1.034	TP	6.1 g/d*l*	CRP	26,900 μg/d*l*
protein	(3+)	Alb	54.5%	RA test	(−)
blood	(2+)	α_1−glb	7.2	FANA	20×
RBC, WBC	5−8/HPF	α_2−glb	9.2	Anti-ENA Ab	(+)
granular cast	(+)	β-glb	10.8	Anti-DNA Ab	14 U/m*l*
NAG	78.4 U/*l*	γ-glb	18.3	CA*	(+)
Stool:		T. Bil	3.0 mg/d*l*	Anti-cardiolipin Ab	(+)
occult blood	(2+)	D. Bil	1.8	CH_{50}	16 U/m*l*
Peripheral blood:		GOT	80 IU/*l*	C3	42 mg/d*l*
WBC	12,000/mm^3	GPT	55	C4	10
myelo	1.0%	ALP	182	IC**	1.2 μg/m*l*
meta	8.0	γ-GPT	54	IgG	1,200 mg/d*l*
stab	43.0	LDH	1,592	IgA	249
seg	35.0	CPK	44	IgM	338
eosino	0.0	T. cho	90 mg/d*l*	Coomb's test	(−)
lympho	4.0	TG	387	STS	(+)
mono	2.0	BUN	42	TPHA	(−)
baso	0.0	Creatinine	3.7	BGA:	
atypical lym	7.0	UA	8.0	PH	7.42
RBC	508×10^4/mm^3	Na	136 mEq/*l*	PCO_2	31.3 Torr
Hb	17.2 g/d*l*	K	4.9	PO_2	95.7
Ht	49.2%	Cl	95	HCO_3	20.7 mEq/*l*
Plt	1.3×10^4/mm^3	Serum Hb.	46 mg/d*l*	BE	−1.9
PT	12.1 (11.5) sec	Haptoglobin	14	SO_2	97.5%
aPTT	57.0 (32.6)				
Fibrinogen	205.9 mg/d*l*				
ESR	3 mm/hr				
FDP	80<160 μg/m*l*				
ATIII	70%				
Plasminogen	49				

*CA: circulating anticoagulant
**IC: immune complex

あった。免疫学的検査ではCRPの高値と血清補体価の低値を認めた。免疫複合体は陰性であった。血清梅毒反応は生物学的偽陽性を示し，循環抗凝血素が陽性であった。腹部超音波検査では，肝脾腫を認めた。

入院後経過(図1)：入院時直ちに末梢血薄層塗抹標本を検鏡したところ，膨大し，シュフナー斑点を有する典型的な三日熱マラリア原虫寄生赤血球と，熱帯熱マラリア原虫の輪状体をもち，大きさは正常で斑点のない赤血球が認められ（図2A)，その後の標本からは生殖母体も認められた（図2B)。熱帯熱マラリア原虫寄生赤血球率は12%で，三日熱マラリア原虫寄生赤血球率は0.7%であった。以上より三日熱，熱帯熱マラリア混合感染で急性腎不全とDICを示す重症マラリアと診断した。直ちに塩酸キニーネ600 mg経口投与とドキシサイクリン100 mg滴静注を開始，計2回投与した。十分な補液，昇圧剤およびFOYとハプトグロビン製剤も併用した。しかし9日に熱帯熱マラリア原虫寄生赤血球率は29%に達した。高熱が持続し，下痢が頻回であった。塩酸キニーネを250 mg点滴静注に変更し，ファンシメフ1錠を投与し



WBC	(/mm³)	12000	12300	8300	6900	5800	6700
Hb	(g/dl)	17.2	10.8	11.4	10.2	9.5	10.8
Plt	(x10⁴/mm³)	1.3	2.7	5.9	11.1	22.8	37.6
BUN	(mg/dl)	42	86	90	53	48	11
Cr	(mg/dl)	3.7	6.4	9.3	7.7	6.4	1.2

Figure 1 Summary of the patient's clinical course. ADM: admission to our hospital, UV: urine volume, BT: body temperature, HD: hemodialysis, P.f.: *Plasmodium falciparum*, P.v.: *Plasmodium vivax*.

たが経口薬は消化器症状のため継続は困難であった。9日の深夜から無尿に陥った。こちらからの質問に対する応答が乏しくなり，意識レベルの低下を認めた。脳波では全誘導にかけてlow voltage and slow waveを呈し，汎性異常を認め，脳マラリアの合併が疑われた。10日より血液透析を導入し，抗マラリア剤をartemether (Kunming Pharmaceutical Factory, Yunnan China) に変更した。投与量は初回に200 mg，以後6時間毎に100 mgずつ追加し，総量600 mgを筋肉内注射した。原虫寄生率は急激に低下し，12日には原虫の消失を確認した。発熱，下痢も消失し，意識清明となり全身状態は速やかに回復した。Artemetherによる治療後，再燃防止のためmefloquine 500 mgを12日，13日に投与した。腎機能も回復，利尿が得られ，17日には透析を離脱し得た。血小板数は16日には正常値に復した。22日経皮的腎生検を施行した。30日退院。退院時BUN 11mg/dl，creatinine 1.2mg/dl，24時間クレアチニンクリアランスは105.11/dayであった。6月1日よりプリマキン15 mg/dayを2週間投与した。7月18日発熱出現。三日熱マラリアの再発を認め，クロロキンとプリマキンを2クール投与にて改善した。11月23日38℃の発熱が出現。再度三日熱マラリアの再発を来した。26日ハロファントリン投与後，プリマキン15 mgを2週間，引き続きクロロキン300 mgとプリマキン45 mgを週1回，8週間投与したところ現在にいたるまで再発は認めていない。

腎生検所見（図3）：光顕レベルでは糸球体はほぼ正常で，尿細管の中等度萎縮と間質へのび漫性の炎症細胞，好酸球の浸潤を認めた。間質，尿細管の変化が主体で，急性尿細管壊死の再生像と考えられた。

血清マラリア抗体価（図4）：間接蛍光抗体法（IFAT）により測定した。5月10日に熱帯熱マラリア原虫抗原に対し256倍，三日熱マラリア原虫抗原に対し1,024倍の高値を示した。その後，経時的に抗体価の測定を続けたところ，患者の回復に

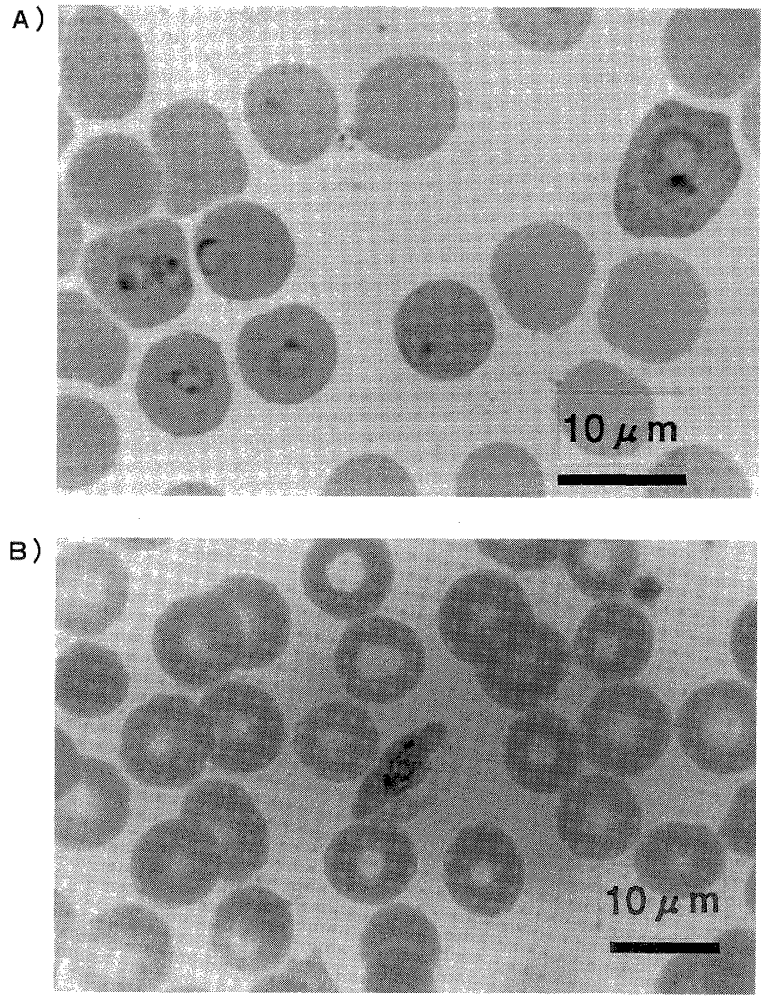


Figure 2 Giemsa stained blood smears, A) taken on the day of admission, showing ring forms of *P. falciparum* and *P. vivax* infected red blood cell with marked Schuffner's dots. B) taken on the fifth day, showing a gametocyte of *P. falciparum*.

伴って下降する抗体価が，7月18日，11月23日の2回の三日熱マラリアの再発毎に三日熱マラリア抗体価が熱帯熱のそれに比較して，有意に上昇していることが認められた。

考　察

重症熱帯熱マラリアは，急速な進行を示し，致死的合併症を引き起こすことから，早期診断と共に適切な抗マラリア薬の選択が必須である。WHOの重症マラリアの基準では，臨床症状として脳症，重度貧血，低血糖，出血傾向，DIC，黄疸，腎不全，肺水腫，ショックなどが挙げられ，赤血球内原虫寄生率5％以上となっている（WHO, 1990）。本症例は入院時発熱，下痢，黄疸，ショック状態を呈し，急性腎不全とDIC，経過中に脳マラリアを合併した。熱帯熱マラリア原虫寄生率は入院時12%，最高29%と高率で，極めて重症のマラリアといえる。本邦ではマラリアはほとんどが輸入例として遭遇するため，医者の認識が乏しく見逃されることも少なくない。本症例は他医で風邪と診断され，本院への受診は発症7日

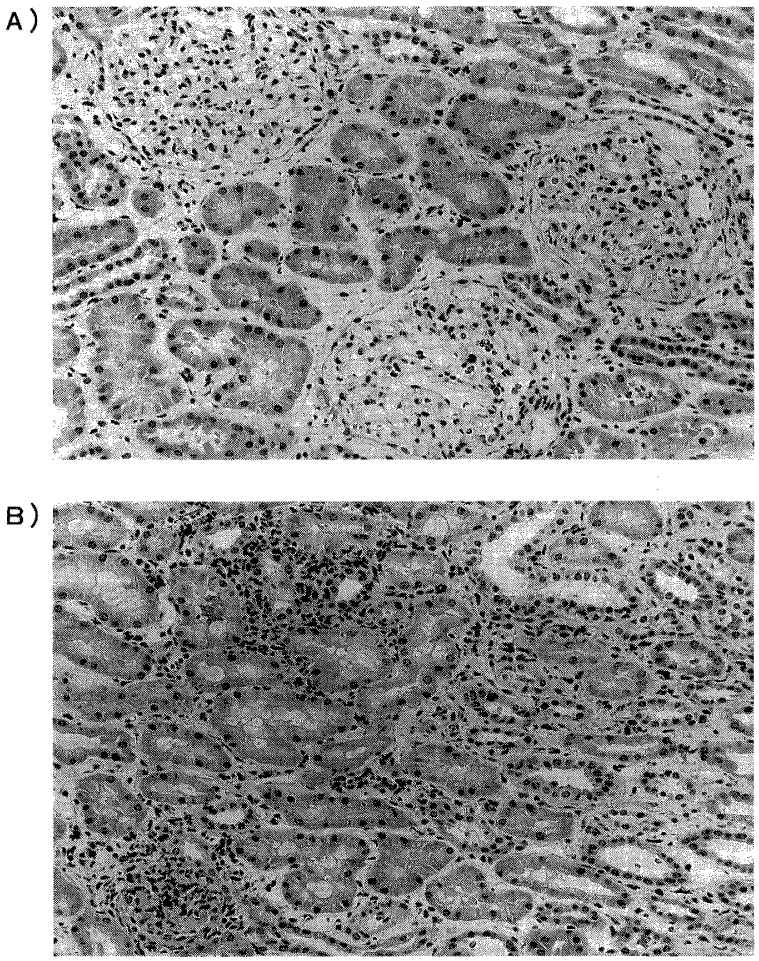


Figure 3 Renal-biopsy specimen. A) Changes in the glomerulus and the proximal tubules are minor. B) The interstitial tissue is infiltrated by plasma cells and lymphocytes (Hematoxylin and eosin; ×100).

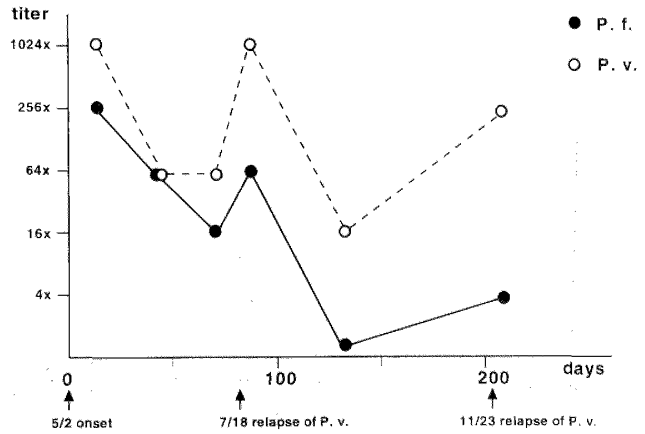


Figure 4 IFAT antibody profile of the patient.
P.f.: *Plasmodium falciparum*, P.v.: *Plasmodium vivax.*

目であった。熱帯熱マラリアは，発病後治療開始遅延日数が5日を過ぎると，生命の危険が高くなることが指摘されている(海老沢ら，1991)。熱帯熱マラリアではマラリアの古典的な症状とも言える周期的な発熱は特徴的ではなく，多彩な症状を呈する（Scully *et al.*, 1989)。海外渡航者に発熱を見た際，マラリアを念頭におくことが強調される所以である。今後，重症マラリアに遭遇した場合，よりよい対処をするべく，今回行った治療にあたっての問題と反省すべき点を挙げてみたい。まず重症マラリアの治療は非経口的に行うことが原則で，塩酸キニーネ点滴静注が第一選択とされとている(WHO, 1990)。しかし，我々は注射薬の入手が間に合わず，塩酸キニーネの経口投与で開始せざるを得なかった。塩酸キニーネ点滴は第2病日より開始し得たが，寄生原虫率の上昇と臨床症状の悪化を認め，キニーネの治療をそれ以上継続することは危険であると判断された。

Artemetherは，中国において古くからマラリア治療に用いられてきた*Artemisia annua*より抽出されたqinghaosuの誘導体である。原虫消失時間，平均解熱時間共に極めて速効性で，耐性マラリアに対する有効性，軽微な副作用，また非経口投与可能から重症マラリアに対する薬剤として注目されている（Klayman, 1985; 狩野ら，1988)。本症例においてはArtemether投与1時間後には原虫の膨化，細胞質内の空胞形成などの変化が見られ，2時間半後にはほとんどすべての原虫の染色性に変化が起こり，細胞質濃縮に陥った原虫も多数観察された。38時間後には末梢血中の原虫は消失し，臨床症状も速やかに改善した。Artemetherの速効性が，臨床所見と良く一致して確認できた。

今回のような重症マラリアでは，補助療法も欠くことが出来ない。ショック，DICに対する処置，適切な透析導入も今回救命し得た大きな要素である。原虫寄生赤血球の破壊によるヘモグロビン血症，ヘモグロビン尿症が腎不全の一因に挙げられている。ハプトグロビン製剤の使用は，この病態に対して有効かもしれない。

熱帯熱マラリアに伴う腎障害には急性腎不全と，稀ではあるが免疫学的機序を介する腎炎，ネフローゼ症候群の2つが報告されている（Boonpucknaving and Sitprija, 1979)。急性腎不全は，高度の原虫血症と溶血を呈する症例に合併する。その腎組織所見に関しての報告では，糸球体は保たれるが，尿細管の変性と萎縮，尿細管上皮細胞内および尿細管腔内の色素顆粒の存在と，間質への小円形細胞浸潤，線維化といった尿細管，間質の病変が主体とされ，いわゆる急性尿細管壊死の所見である（Agrawal *et al.*, 1984; Stone *et al.*, 1972)。本症例でも糸球体の形態はほぼ正常に保たれていたが，尿細管，間質の変化があり，いままでの報告にほぼ一致する所見であった。

おわりに

熱帯熱，三日熱マラリア混合感染で，急性腎不全，溶血，DIC，ショック，脳症状を合併した重症例を経験した。重症マラリアは，短時間で症状が進行する。輸入マラリアの増加と共に，重症マラリアに遭遇する機会も多くなることが予測され，その認識と迅速な対応のできる医療環境の整備が望まれる。

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A CASE OF SEVERE MIXED *PLASMODIUM FALCIPARUM* AND *P. VIVAX* INFECTION SUCCESSFULLY TREATED WITH ARTEMETHER

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As the number of individuals traveling to malaria endemic countries increases, many patients with malaria are increasingly being observed in Japan. Nevertheless malaria is liable to be misdiagnosed, incorrectly treated, and a severe course is thus not unusual. However there are hardly any reports describing severe malaria in a Japanese patient due to mixed *P.f.* and *P.v.* infection. We report a case of severe malaria due to mixed *P.f.* and *P. v.* infection. The effectiveness of the new antimalarial drug, artemether, in the treatment of this case of severe malaria is also documented.

A Japanese 31-year-old male was admitted to Kitasato University Hospital because of fever, diarrhea, and circulatory collapse. The history of this patient is that one week after his return from a 9 day visit to Indonesia, during which he had weekly chloroquine prophyraxis, developed fever and chills. He visited a hospital where he was treated for a common cold. High fever was sustained and accompanied by watery diarrhea. On admission to our hospital, his temparature was 40°C. The patient was lethargic but fully oriented and responsive. He was dehydrated and perfusing poorly. Physical examination revealed jaundice, hepatomegaly, and diffuse tenderness from the right costal margin to the epigastrium. *P.f.* and *P.v.* were detected from a blood smear (12% and 0.7% parasitemia, respectively). Oral

therapy with 600 mg quinine hydrochloride and 100 mg doxycycline was initiated. The next day, his fever was unalterd, diarrhea and severe oliguria were evident. *P.f.* parasitemia increased to 29%. Antimaralial therapy was changed to 250 mg quinine hydrochloride administered intravenously and fansimef, one tablet. Within 24 hr, he developed anuria and drowsiness. Cerebral malaria was suspected and hemodialysis was started. Treatment with quinine was replaced by artemether. Two hundred mg of artemether was administered as first dose intramusculary followed by 100 mg at interval of 12 hr, up to a total of 600 mg. Examination of blood smear indicated complete elimination of asexual stages of the parasite, within 38 hr after the onset of treatment with artemether. Thereafter, diuretic phase began and the patient made a complete recovery. Histopathology of a renal biopsy specimen showed tubular atrophy with interstitial lymphocytic infiltration, mild fibrosis and pigmented casts, all suggestive of tubular necrosis but no glomerular involvement. Thus acute tubular necrosis was considered as a cause of acute renal failure in this patient. To consolidate artemether therapy and eliminate possible recrudescence, treatment with mefloquine was initiated, at the dose of 500 mg a day orally for 2 days. On the day the patient was discharged, he was put on primaquine at the dose of 15 mg a day for 2 weeks. Forty-eight days later, despite the treatment given, he had fever and was diagnosed as a *P.v.* relapse case. Treatment with chloroquine and twice as much primaquine cleared parasitemia. Four months following this last treatment, the patient again had the symptom of fever and was treated this last treatment, the patient was again had the symptom of fever and was treated this time with halofantrine and primaquine. Treatment with primaquine for 2 weeks was followed by primaquine (45 mg) together with chloroquine (300 mg) once a week for 8 months. Serum anti-*P.f.* and *P.v.* IFAT titer profile of the patient over the entire course of his treatment confirmed the clinical course of the disease.

The goal of the initial therapy of malaria is lowering the level of parasitemia as rapidly as possible under strict intensive care with monitoring of vital organ functions. Artemether was effective in achieving this goal even in this case of severe and complicated malaria due to mixed *P.f.* and *P.v.* infection.

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Research Note

HEALTH, SOCIAL JUSTICE AND THE THIRD REPUBLIC

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Abstract: It is time worn to state that health is wealth. Yet this assertion is no less true and significant today as it was yesterday. Indeed, a healthy nation is contigent on healthy citizenry (1st NDP). This perhaps explains why no nation had ever attempted to go to sleep while its 'health house' is on fire. In fact, the health of a nation defines its potentials and actual deeds. The continual featuring of health in all the national development plans so far made, underscores the importance of the health of this nation's citizens to our social planners. Be that as it may available evidence shows a vaccum between rhetorics and action. While no two analysts agree on the modalities of making health care delivery services accessible to all (Steiner, 1966; Navarro, 1976; Titmus, 1966; Field, 1960). This paper is an attempt to contribute to this debate. Essentially, it warns that unless we re-order our priorities and achieve social justice in the health sector first, we may never be in a comfortable position to discuss the issue of integration less alone meaningful progress in the third republic; come 1992, when the present military government hands over power to a democratically elected government. Here lies the thrust.

INTRODUCTION

In his summation of the development of formal welfare scheme in Nigeria, Sanda (1987) observed that health care in the period 1870 to 1910 was largely in the hands of the Missionaries even though the state coped with the health needs of the British soldiers through the Military hospitals in Zungeru, Lokoja and Zaria. The first world war period saw the establishment of bush hospital especially around the war zones, while the depression of 1930s and the discontent among the ex-service men and the nationalist agitations all combined to direct the attention of the colonial government to the society's welfare needs. It was in this context the laws of 1940 were made to cope with the labour and children welfare needs, the 1945 ordinaces were directed at solving the problems of disabled soldiers and the 1948 law designed to enforce the obligations of families to under 14 children. By the same token, workmen's compensation ordinance of 1941, 1945 and 1959 laws of the Federal republic of Nigeria all tried to protect workers on matters of contract, injuries at work compesation, forced labour, employment of young persons, wages, maternity leave and so forth. He then concluded by saying that the welfare structure that emerged became a combination of the

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traditional pre-colonial heritages with equal dependence upon the efforts of the voluntary organisations and government as well as the extreme regionalization before the advent of the military. It goes without saying that the various Nigerian governments since independence have been aware of the 'welfare-plight' of Nigerians (1st-4th NDP). Be that as it may, the extent to which precepts have been substituted for action remains largely questionable. Aside the fact of official default in pursuing the orthodox approach to health care delivery services with all its curative emphasis, despite epidemiological reports which amply demonstrate that the bulk of the prevailing diseases in the tropics are preventable, reports concerning the state of disrepair to which Nigerian health services have continued to be taken lightly by the government of the day, while coup leaders now use such abysmal state of health as excuses for taking over power. This is further exacerbated by a number of factors, among which are the recent increase in brain drain which apperars to have hit the health sector hardest, the fact of a steady rise in population and most of all. The WHO's call for health for all by the year 2000 (WHO 1978).

It is right to acknowledge that the Nigerian government is aware of this situation and have made a proposal for a health insurance scheme. However, the proposal as it stands now, will not actualise social justice in health distribution as it is tailored to cater for only the government 6 million work force and their 30 million dependants (The Guardian, Oct. 1, 1989). What this means is that the injustices of not catering for the remaining 94 million will continue to be perpetrated. Besides, with a ceiling of 12,000 patients currently proposed and an average of 32 patients per day; to be attended by an unassisted doctor, the entire package seems a panic measure designed to stem the tide of brain drain, by putting more money in the way of of the the medical doctor rather than improve the well being of the community. Besides, if patients are to be wholly responsible for consultation and drug fees, the the question one would like to ask is whether the 'old and stale wine' has not managed to find it's way into 'new wine skin'. It is with a deep sense of correcting this unjust situation that we are proposing a patially free and comprehensive package with universal coverage.

National Health Insurance Scheme: a Proposal

Epidemiological studies have made it possible for us to predict the incidences of diseases in human populations. In taking advantage of this, various countries like Canada, Britain, Denmark, Zimbabwe and a host of others have designed and implemented various forms of insurance schemes, not the least, health; for their people. It is our firm belief that Nigeria can do likewise.

Health insurance scheme, like all other social policies is designed to act not only as checks in times of need, but also to conserve, protect and improve human responses (Friedlander, 1968; Rein, 1970). For clarity, we shall discuss it under the following 3 headings, namely: Scope, Finance and Method of provision.

Scope

The issue of what scope or range of people within a population should be covered by health policy is a fundamental one, with its consequences real. In addressing this issue, various countries have adopted various styles. For example, in Britain, a series of social

schemes existed until the Beveridge proposal of 1946 that led to the national health insurance scheme they have today. The same can be said for Canada, Denmark, New Zealand and a host of others. Today however, most of these countries run a centralized insurance scheme. The truth in the statement that the well-being of a nation is dependent on the state of health of its inhabitants suggests that we guarantee a minimum amount of health to every Nigerian. It is in the light of this that we pestulate a compulsory, comprehensive and patially free insurance scheme with a universal coverage for this nation.

FINANCE

The issue of how to finance insurance scheme is no less complex (Steiner, 1966). Again in going about it, countries have always found it convinient to classify the population into employers of labour, employees, the self employed and dependants. We subscribe to this but would insist on the following modalities.

EMPLOYERS: A proportion of the total profits of all registered companies and those of unregistered employers of labour be taxed. All newly established firms should however be given three years tax-felief period to enable them stabilize. The payment should be made at the beginning or end of every financial year.

EMPLOYEES: All workers should be taxed to support the scheme. Such deductions should be graded and should take the form of pay-as-you-earn.

SELF EMPLOYED: All self employed people should be asked to pay a stipulated annual flat-rate to support the scheme. Like the employers, all newly self-employed people should be given a period of three years to stabilize. Besides, such rates should not be beyond the affordable reach of this category and should not be too low to make others feel cheated.

DEPENDANTS: As the name implies, this category has no means of livelihood. At least not as yet, and so cannot be taxed. All these payments should be subsidized by the Federal government which like the employer should reserve a percentage of the total revenue accruing from the sales of all natural resources of this nation to support the scheme. This can be further supported by the Federal, state and local governments by donating a part of their internally generated revenue to further fund the scheme.

PENSIONERS: By the same token, all pensioners are to pay a part of their earned income as contributions to the scheme. Again, we recommend that such contributions should be graded. This way, the scheme can be successfully financed, with the 'never-sick' carrying the 'ever-sick'.

METHOD OF PROVISION

The fact that the bulk of the prevailing diseases in the tropics are preventable is no longer in doubt. If anything, epidemiological researches have made it abundantly clear. It is therefore to be expected that any proposal for health should take into account this salient fact. We are therefore proposing a two-headed prong: namely, preventive and curative measures.

PREVENTIVE MEASURES: Here the Federal Ministry of Health is to pruchase drugs and are expected to organise periodic innoculations against the various contigencies of diseases as it currently done uncer the expanded programme on immunization. It should devote a part

of its buget to setting up schools for training public health educators and other paramedical staff, who will inturn facilitate the implementation of its programmes,.

Since more than a tenuous relation exists between cultural practices and the occurence of certain diseases in some areas (Meck, 1971; Oke, 1982), it will be the duty of such public educators to identify this relations and to point out why such people should invest in health by discontinuing such practices. If this preventive measure is sufficienly carried out, it should lessen the cases of ill-health for curative attention.

CURATIVE: There are diverse methods of operating insurance schemes, as there are countries. However, such method as may exist in a country, is usually dictated by the historical background, the needs, resources available and the acceptibility of the common use of such resources. Nigeria is currently into the era of privitazation. However, the herculean nature of the task of maintaining a comprehensive health insurance policy with universal coverage goes beyond that of utilizing a private firm (Brieland, 1980). It is in recognition of this that we recommend reliance on existing structures.

We propose that all government health structures in all three levels of government be made the live-wire of this programme. The assignment of the Federal as well as the state governments should be limited to supervisory role. In this, the Federal Ministry should take precedence over the state, which should be seen as an organ for carrying out its directions in the various localities.

All structures are to be regarded as existing in the local government where they are located, and steps should be taken to see that all local governments have at least a 200-500 bed space hospital, that is adequately staffed. We suggest that the bulk of the cases should be handled on out-patient basis. Moreso when preventive dosage will be a part of the focus. The actual running of the hospitals should be left in the hands of the governing councils or management committees whose duty it should be to oversee and determine the budget of each of such hospitals. Such persons as will be appointed should compose of staff of the Federal or state ministry of health, in addition to limited members of staff of the hospitals and those of the public.

This should be followed by a direct call by the Federal Ministry of Health to its organs in states, to register and maintain a record of people in their localities. Such registration should be comprehensive enough to show the demographic arrtibutes of the people. More importantly, it should show their ages, occupation and addresses. The duplicate of such records should be sent to the Federal Ministry of Health which ideally should maintain a central bank data unit on all localities.

Knowing the number of people by their social characteristics will further lend hand to ascertaining the number of persons who fall into the categories of people to the levied and the number of dependents. This will faclitate planning. By the same token, a list of all doctors who are interested in participating in the scheme should also be kept. All doctors already working for the various governments can either continue to work and receive their usual salaries or can withdraw their appointments and take-up contract like all other private doctors interested in the scheme. Based on the list collected, both the doctors and patients should be divided on local government basis. After this, doctors should be given the list of people they are to be ministering to. Such doctors should however have the powers of referral or even to consult other doctors in cases that need specialized treatment. This should be at no extra cost to the patient.

Again, the number of doctors to patient ratio should be determined by the size and bed-space of their hospital. Doctors will be expected to be paid on item for service basis. This principle should apply whether the service is that of a general practitioner, dentist, opthalmologist or surgeon. However, services by specialist should attract better pay than those of general practitioners. Another way to go about this is to utilize only specialists and remunerate commensurately. The advantage of item for service payment is that it will not only make practice in this setting lucrative, but also stem the tide of brain drain. Such payments and ceiling will be pegged by the governing council at the Federal Ministry of Health, so that a uniform fee is adopted by all local government councils. All those who are working for the effectiveness of this scheme, be they doctors, paramedical staff, monitoring unit, or part-part governing council, should be remunerated from funds generated for the scheme. For effective monitoring of operation and prevention of false claims, it is expected that only the health centres or hospitals to be cited at the local government headquarters should be utilized for this scheme.

Patients may be allowed to change their doctors, but the right should be minimised as it can be abused, and used only at discretion, as where lack of confidence is established, or where a consenting note is issued by his/her personal doctor. In addition, such a person should give four weeks notice giving reasons and preferences. All patients are expected to show proof of eligibility. Only those who pay should be entertained. People out of their stations can utilize any hospital of their choice on the production of an up-to-date proof of subsctrption. What this means is that doctors may be allowed to treat patients outside their lists, but this should not be more than a predetermined number and such cases should be reconcilled with his personal doctor in the locality.

Consultation with doctors for all patients should be free, but patiets should be made to pay 75 per cent the cost of prescribed drugs. Hospitalised patients should pay 33.33 per cent hospital bill and 25 per cent the cost of diagnostic tests and in cases where patients foot all the bills, receipts should be submitted to the audit department of the hospital concerned, for reimbursement.

In order not to strangulate patients, government should produce and/or import most of the drugs. This will help to keep the lid on the gain-margins made by pharmaceutical companies and hence reduce costs of health at the end of the day. Since doctors will form the corner stone of this scheme, the least the supervisory body can do to guarantee fair play is for it to set up in all the states in Nigeria, committee of insurance doctors to discipline erring doctors or to refer the matter to the Nigeria Medical Association, which duty it is to exercise this role. Finally, all this should be given the necessary legal backing.

Discussion

The range of people to be covered by any National Health Insurance Scheme is something that all nations intending to establish such program must grapple with. Generally, the scope of any national insurance scheme is limited by a number of factors, foremost of which is the nature of the scheme. For example, is it private or public? Under private insurance, you have self-help, which in the beginning of the insurance industry, involves a group of people pooling resources together against the contigencies of diseases, ravages of fire and other forms of natural disaster. Private insurance could also involve the individual taking out

insurance policy on whatever contigency he wants to and lastly it could mean employers or unions making private arrangement to cater for the welfare of their employees or members. One common strand that runs through all such arrangements is that it covers only those who take them out or on whose behalf they are taken.

Private arrangement constitutes the principal form of insurance in Denmark, France and Japan. It is also the practice in most of the states in the United States, which in addition to Switzerland remains the only two industrialized countries without resident compulsory insurance (Enc. Britannica, 1987).

The other method of making provision for health insurance scheme involves the public or government. The government of any nation can take steps to provide a range of social security measures like housing, sanitation, transport, pension, old age, medicare and so on. Coverage under public tutelage is limited by the aim of establishing the programme and the resource available to the state. The latter is further predicated upon the magnitude and general perception of the wealth. For example, a nation where all are agreed that no man can ge self sufficient in the face of the unpredictable forms or dimensions of life's hazards, and does have the resources, are more likely to impress it on their government to make social security measures a matter of priority.

Consequently, several countries have adopted modes, ranging from limited coverage of the population to full coverage. For example, 100 per cent coverage exists in the U.K. and in Cuba. Eighty per cent in Argentina, Brazil and Costa Rica. Fifty per cent coverage exists in Uruguay, Mexico and Panama; while more than 25 per cent exists in Bolivia and Venezuela whereas, others and in particular, African countries have less than 10 per cent (Enc. Brit., 1987).

Differing approaches to coverage also exists. For example New Zealand extended medical coverage to all residents in a number of stages. Beginning with free in-patient treatment in 1939, through out-patient treatment and free pharmaceutical to part payment of general practitioners in 1941. Further steps were taken later on. And while India Government extends full coverage only to states that can maintain them, Korea extended health insurance only to her urban employed citizens (Enc. Brit., 1987). Korean example is akin to the one currently proposed by the Federal Government of Nigeria, where only the government six million work firce and their thirty million dependants are to be covered. The injustices of this proposal is seen when this computed sum of 36 million is removed from the current estimated population of 130 million (Sanda, 1986). This has far reaching consequences for health services in the rural areas where at least 90 million Nigerians live and where less than 10 per cent government establishments are located (1st-4th NDP) and for the realization of WHO's call for health for all by the year 2000 (WHO, 1978). This perpetuated social injustice as seen in the concentration of social amenities in the urban area to the neglect of the rural population, who in part provide the bulk of the nation's wealth, is also noticable in Kenya, Zimbabwe, South Africa, Ethiopia and a host of other African states (Dixon, 1987). A further consequence of the above imbalance in the allocation of resources between the rural and urban sectors, is that the greater part of the health vote is spent in the urban area. Sadder still is the fact that available records show that the latter is not without a share of its injustice as most of the supposed urban vote end up under such ambiguous labels like 'hospital programmes' which are completely devoid of all services, be it preventive, palliative or curative (3rd NDP).

In our opinion, what is needed is a comprehensive preventive and curative man-centred national health insurance scheme, that will promote the health of everyman, not as found in one occupational group or social class, but man as devoid of all social taxonomic concepts and deserving a baseline guarantee by the society against the contigencies of diseases and other hazards of life that can debar him from living a fuller life.

Social insurance schemes anywher found are usually financed from contributions, derived from the government, employers and employees. What makes the difference among countries is the proportion in which these services are utilized. Chambers' Enc. (1973) reported that when compared to Britain, which has universal coveage, the national scheme and pension schemes in West Germany, France, and Italy, are characterised by their high contribution and benefit rates and the smaller percentage contribution of the state. Whereas, in the unemployement riddled Western Europe of the 1970s, the trend in Denmark, Ireland, Italy, The United Kingdom, Portugal and The Netherlands, was that of shifting costs away from employers unto taxes. By the same token, Enc. Brit. (1987) reported that no cost falls on the limited schemes in operation in places like Burundi and Ethiopia, and on the wider scheme in Malaysia, Philippines and Singapore. Whereas, contributions play a very small role in Austria, Czechoslovaka, Denmark, New Zealand and the USSR, where the bulk of costs is covered by taxation. National health insurance scheme in the UK is generally financed 50 per cent from taxes and 50 per cent from contributions.

What we have established is that all societies are dynamic and at all times try to make social security measures as relevant and amenable as possible to the prevailing circumstances and needs of their people.

Aside all the above listed sources of funds for sponsoring national insurance schemes, assitance by agencies also play a vital role in this exercise. In fact such programmes as those of the needy, aged, survivors and so forth, are supplemented with assistance payments in Austria, Czechoslovaka, France, West Germany, The Netherlands, United Kingdom and the US. Yet the US and Switzerland, can be lumped together in another extreme as archtype countries where tripartite financing as defined above is not practised. In fact, in the United States, such schemes are resisted by various organised bodies like the American Medical Association which argue that it is unwise to socialize medical practice in a free enterprise oriented country (Soc. sc. enc., 1968, Erinosho, 1981).

All systems sponsoring national insurance schemes are faced by problems of actuarial principles. They have to entertain such questions as to whether to make benefits related to prior earnings or whether needs should not take precedence. While this issue cannot be completely resolved, countries have adopted differing measures which themselves have influenced the state of health in such nations. For example, the United States adopted the earning-related benefit, whereas the United Kingdom in addition to adopting similar measure has introduced a floor to ensure that the common man is guaranteed some substainance level or minimum standard of living.

Benefits under the scheme we are proposing are essentially medical and of service. Not cash. We are contented to allow existing firms handle company injury as dictated by existing union laws. What we are concerned with in this work, is laying a foundation for the take-off of a national health insurance scheme with a universal coverage.

Experience has shown that contributions to national health insurance schemes and other forms of social security designs, are strictly taxes on earned incomes. In our proposals, we

stated that the Federal Government, state, local, employers, employees and pensioners are to bear the brunt of financing the scheme in Nigeria. This is different from the practice of asking the employers and employees to carry the greater part of the burden if what the government is proposing comes through.

The advantage of asking the various arms of the government to contribute is obvious. Nigeria lacks accurate census figure. The last estimate was 130 million. If we remove government 6 million work force and 30 million dependants, we shall a staggering sum of 94 million uncartered for. Therefore, it would help to adopt a method that will involve everybody.

The Federal Government like the state and local government councils, is to donate a certain precentage of all their internally generated revenue and should in addition, give a part of the proceeds from the sale of all natural resources of this country. This is the only way the wealth can be held commonly.

In addition to the practice of taxing the employer and employees as individuals, we proposed that both be taxed accordingly and for the company to be taxed as a corporate body. The benefit of giving tax relief to newly established companies is seen in the fact that not many large corporations exist in Nigeria, and while many are still out of jobs, making employers pay part of the insurance for each employee, may be good in the short run but might prove reptogressive in the long run as it will probably lead to utilizing short cuts to contibution liability like giving jobs out on contract for a money wage, introducing overtime or even retrenchment. A phenomenon that will further worsen the already soared labour market. As for the argument that it is socially unjust to tax the employers as well as the profit of their corporation, we feel it is the least they can do to keep the system going. In any case, reports exist to show that employers' contributions are not always paid at the expense of profit (Enc. Brit., 1987). Besides Romanysyn (1971) bas argued that all welfare programme does is to maintain the discipline of work and in real term ensures that somebody will be around to do the dirty job and by so doing, prevent people from probing into the more fundamental question of the causes of dependency. And while such thoughts are also inherent in the work of Filgerald (1977), De Schweinitz is of the opinion that the question of inequality would not have arisen if all it takes is the ability to contribute to society. This reason also underlay and serves to highlight the sharp difference in our proposal as against existing ones where governments set up special funds to run insurance schemes rather than contribute from its main revenue as we advoated.

We also proposed that a proportion of all pensioners' allowance be taxed to support the scheme. The tax should be graded and should take th form of pay as you go. It is common knowledge that the aged in all societies spend more on health than workens. Again, in a situation where we are proposing a universal coverage, it is only right to include all beneficiaries with known sources of income. Steiner (1971), has also made this call.

Enc. Brit. (1987) lists three modes of provision of health insurance benefits, namely; direct, indirect and reimbursement. Under direct service approach, the government or sponsoring body owns the facilities which include hospitals and clinics. It also pays for the supply of drugs and remunerates all staff. As the name implies, this is a direct contrast of the second method which entails the funding body making contract with people who provide and get remunerated accordingly. Whereas patients foot the entire bills and later submit them for claims in the third category.

A comparative study will show a preponderance with any of these methods. Reimbursement for example is largely used in France and to some extent in Australia and Sweden. The indirect method is heavily utilized for all services in such countries as Belgium, West Germany, Luxembourg and The Netherlands. Whereas the direct mode is employed in The UK, Scandinavia, Greece, Spain, Portugal and a host of others.

At a glance, this forms represent a tendency towards regidity. Habit has however shown the contrary. Indeed countries use a combination of these at any point in time, with the emphasis on any particular method dictated by the global configuration, the socio-economic and political order of the day. For example, aside employing the direct method, the national insurance scheme in Britain utilizes indirect contract for general practitioner, community pharmacist, opticians and most dentists. The same phenonmenon is said to be at work in Greece, Italy and most latin American countries (Enc. Brit., 1987). This observation can be made for our proposal wihch utilizes the direct method in the main but also insists on the third category where patients end up footing the bills.

In writing this paper, we made a number of assumptions:

1. That since men live in an organised community, the society owes it as a duty to provide a basic social security measure especially in the light of the contigencies of diseases and other forms of hazards the individual is ill-equiped to grapple with.
2. Inherent in the above is the assumption that health is an inalienable right of all citizens and should remain so if human societies are to make meaningful progress.
3. Bearing in mind the concept of social justice, we have implicitly argued that the wealth of Nigeria belongs to all Nigerians and should be maximally used for the benefit of all if we are to develop.
4. That when once we identify health as an active ingredient of development, the advocacy that it be accessible, compulsory and comprehensive is not too difficult to follow.
5. That to undermine these assertions is to support arbitrary and continuous duplicated health policies, to undermine the very health of the uninsured 94 million Nigerians with each of them automatically turned into a melancholic agent of development, to threaten the survival and well-being of the nation as well as render the principles of integration and meaningful progress in the third republic redundant.

The days of the much celebrated Daniel Defoe's hero-Robbinson Cruso has come to an end in most countries of the world (Crampton, 1972; Smelser, 1966; Titmus, 1966). Today, men are no longer thought to be masters of their fates who must, following the principles of social Darwinism which pervaded western societies of the 19th century, engage in stiff competition for existence and reap the full consequences for his action or inaction. Indeed, it would appear that the definition of society which emphasis interaction has made increasingly impact on the leaders of men. Whether this phenomenon has its roots in the discovering of the fact that poverty has a direct relation with the social institutions that determine the allocation of personal and social resources needed for existence and competing for positions and power as Romanyshyn (1976) has hypothesised, or the benevolence act of a mover-unmoved, suffice to say that developing nations are aware of this and are atuning themselves more to the well-being of their peoples. Sadly however, a great gap still exists between precepts and deeds in most of these countries. The problems of implementing a national health insurance scheme are diversified, and range from the presence of resourses, manpower to planning. We strongly believe that Nigeria has the resources to set up such a scheme if

properly managed. Tawney (1966) must have had Nigeria in mind when he argued:

Health . . . is a purchasable commondity, of which a community can possess, within limits as much as it cares to pay for. It can turn its resources in one direction and 50,000 of its members will live who would otherwise have died. It can turn them in another and 50,000 will die who would otherwise have lived.

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Research Note

THE INTEGRATION OF TRADITIONAL HEALES INTO THE MAIN-STREAM OF OUR HEALTH CARE DELIVARY SERVICES: A RE-APRAISAL

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Abstract: This paper is essentially theoretical and attempts to re-appraise the issues involved in the call for the integration of traditional healers by the WHO. In order to discuss in perspective, it focusses on the Nigerian situation and demonstrates why integration represents a more viable avenue to the realization of 'Health for all by the year 2000 AD'. The paper winds up suggesting a modality for achieving this.

When the day shall dawn upon us from on high to give light to those who sit in darkness and in the shadow of death . . . Luke 1:79.

HEALTH PLANNING

Erinosho (1981) has argued that health planning for developing nations: . . . is aimed at ensuring for the average citizen in the population, access to medical care and other basic facilities which would enable him/her to attain a reasonably high health status and life long expectancy.

It is given therefore, that the efforts of health personnel will be geared towards achieving this goal. Even then, such methods as employed have however, never been divorced from the differing state ideologies and consequently, their political economy. For Unscheuld (1976), the medical system of any society has to do with the distrubution of the health resources in that society. A look at the pattern of health care delivery services in the USA, and USSR will epitomise this.

THE US MODEL

The capitalistic economies of the United States and nay, of all Western World is predicated on a regulated free enterprise. Although evidences abound to show strong element of welfarism: in the United States health care delivery service is left at the whims and caprises of enterpreneurs who act as fronts to the various insurance companies that exist to fill the vacuum of the provision of health care (Anderson, 1963). Under such an arrangement, Erinosho (1981) noted that "the citizenry is at the mercy of those companies which invariably derive substantial profits from the sale of health policies".

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THE USSR MODEL

Health care delivery services in the Socialist Soviet Union and indeed all Eastern bloc, seems to operate on the premise that building a healthy nation is contigent on having healthy citizenry. The state -as typical of this bloc- therefore, sees it as obligatory on her, to provide health needs for all her citzens. Little wonder she directly intervenes in all ramifications of the provision of health services; in order to meet this goal (Field, 1966). This therefore, explains the phenomenon of relatively low cost of sophisticated health shopping enjoyed by the people of this bloc.

THE NIGERIAN MODEL

In Nigeria, medical system is organised along Euro-america model. Orthodox medicine exists side by side with traditional medicine, even though, the latter and older is yet to be given official recognition by the government (Oyebola, 1981).

As a medical system that was borne out of colonial experience, orthodox medicine emphasizes curative rather than preventive health services: whereas, social epidemiological research reports have made it abundantly clear that by far greater proportion of the diseases prevalent in the tropical Africa are preventable. The implication of this is that reliance on orthodox medicine alone will not actualize 'health for all by the year 2000 AD'.

Orthodox medicine is founded on science and as a varifiable knowledge limits its explanation only to what it can empirically varify. As a practice that is guided by scientific ethics, modern medicine restricts its explanation to germ theory which explains disease causation in terms of physical or biological discontinuity resulting from such things as insect bites, bad odour, habitation in unhygenic environment and so forth (Erinosho, 1981; Oke, 1982). But while it is true to say that the reflection of scientific ethics in modern medicine is in consonant with the world view of western societies, it is right too to say it goes beyond this for the African whose explanation, in addition to taking cognisance of the physical world also incorporates the psyche realm (Oke, 1982; Erinosho, 1981; Gluckman, 1963; Armntrong, 1971; Macleans, 1971; Impareto 1975; Chilivumbo, 1976; Unschuld, 1976). This insufficiency is most noticably in the illness behaviour of Nigerians. Cases have been heard where hospitalized patients secretly have their relations bring them traditional medicine (Asuni, 1979; Osuntokun, 1975; Tory, Sunday Guardian, Feb. 10, 1986).

Other problems centre around the urban based nature of orthodox medicine which encourages the scanty number of personnel she parades to cluster around urban centres, brain drain, high cost of training paramedical pernonnel and that involved in purcahsing neccessary equipment. The list is endless. It can be argued that it is the recognition of the flaws in the orthodox system; particularly its variant position with epidemiological reports, that have made the Federal Government of Nigeria to engage in the Expanded Programme on Immunization (EPI).

Again, while it is true to say that Nigeria has everything to going by engaging in this programme, it is relevant too to mention that analysts see it as another 'urban' project. These facts if coupled with the knowledge that over 2/3 of Nigerians live in the rural area and are ill provided with medical facilities (WHO, 1975; Ademuwagun, 1969. 1976; Olatundosun, 1975)—will better enable us to grapple with or appreciate the works of scholars who have

called for an alternative approach which favours preventive services rather than curative.

This approach is quite apt and no doubt constitutes a viable alternative to the present system. Moreso as it favours epidemiological reports stated earlier. Yet attractive as this approach may seem, it is the contention of this paper that the modalities for implementing it is fraught with numerous problems capable of making us lose sight of the very essence of health care delivery services. Indeed, as Rosett (1967), rightly observed, the difficulty is that when reforms are attempted at a deeper level ". . . new problems arise to replace the old".

We contend that the physicians who undoubtedly derive great importance from the present arrangement are in all probabilities likely to unleash all the powers at their disposal including lobby by such accredited bodies as the Nigeria Medical Association (NMA) and National Association of Resident Doctors (NARD) to frustrate such moves. While this is going on, the very essence of the whole affairs will in all certainty be disrupted and evetually defeated.

The Rational Way Out

In the light of the foregoing, we strongly advocate that the only effective means of actualizing health for all by the year 2000 AD is to follow the directive of WHO (1978) to integrate the traditional healers into the mainstream of health care delivery services in Nigeria.

A traditional healer is defined by WHO (1977) as: . . . a person who is recognised by the community in which he lives as competent to provide health care by using vegetable, animal and mineral substances and certain other methods based on social cultural and religious background as well as on the knowledge, attitudes and beliefs that are present in the community, regarding physical, mental, social well being and the causation of disease and disability.

This call for integration hardly needs substantiation. It is cheap and efficacious (WHO, 1978; Elling, 1981; Maclean, 1969; Ademuwagun, 1969; Prince, 1960; Otsyula, 1973). Eighty per cent Nigerians are already used to going to them (Oyebola, 1980; WHO, 1975; Lambo, 1966; Harrison, 1974). It adequately captivates the world view of the Africans (Ndeti, 1976; Chilivumbo, 1976), and they are available in all localities (WHO, 1975).

Anticipated Problems for Integration

One big tragedy for integration, is the lack of unanimity among scholars on the issue of how best to go about it. For example, while some of the scholars favour the idea of the traditional healers working side by side with medical doctors in the hospital setting, others think otherwise. The main arguement advanced by the former is that it will be cheaper and quicker to assimilate them that way. This stance however creates more problems than it solves. It neither takes into account the social-cultural context from which it seeks to invite the traditional healers nor takes into consideration, the present attitude of Nigerian physicains to the healers. It suffice to say for now that those social scientists who have had course to work with the medical doctors in the hospital setting have said it requires a lot of

'thick skin'.

We therefore, suggest that one way to achieve full integration and hence health for all by the year 2000 AD is for the traditional healers to remain in their persent setting. This will tentatively remove the issue of standardized concepts, eliminate complexes and accommodate role conflicts like how close to get to the patients, at what stage of diagnosis should the traditional healers be called in and whether or not to make or take diagnosis jointly.

In a recent study, the researcher discovered other problems capable of frustrating this call for integration. One of such is the literacy level of the traditional healers. A total of 44% of the traditional healers fell into the stark illiterate cohort and another 44% into the semi-literate category; whose educational attainment ranked below senior secondary one. We linked this with the Universal Primary Education prevalent in the then Western region. It is therefore, doubtful if other healers in regions that did not run this programme would not rank lower

A more fundamental problem is that of concretising the spiritual world. Science does not recognise what is cannot see. So how do we take seriously the claims by healers to be able to carry out healing arts with the aid of the supernatural? Is this a mere belief, in which case having something to do with the psychological disposition of the mind or is it realitic? Exactly to what extent can it be relied upon to yield the same result from time to time?

Other areas of problems include the purification healers drugs, lack of accurate dosage and that of procuring a winowing machine to help sever 'grain-healers' from 'chaff-healers'. We strongly believe that it is the partial recognition of some of these problems and the threat of integration to the status quo that have made some scholars and especially the modern practitioners to continue to asymetrically or lopsidedly utilize their present access to the source of power, to pass derogatory and deprecating comments on the persons and works of the traditional healers (Daily Stetch, Monday Jan., 20, 1986; Ndeti, 1976; Oyebola, 1980; Bannerman, 1981; Torrey, 1972). Even then we have reasons to believe that these problems are not insurmountable. It took time to build those well organised Euro-america model the excellence of which the Nigerian Physician now strives to achieve, despite its high cost and ill suitability for the tropics.

Recommendations

It is our expressed view that the only way the traditional healers can be integrated and logically health to the door-step of all by the year 2000 AD, is for the Federal Government to recognise them. There is no other way out. Oke (1985) reported that 79% of his respondents were quite willing to heed WHO's call for integration. My field experience and the work of Erinosho (1985) further served to confirm this.

2. Official recognition should be followed up by the setting of an all exclusive traditional healers board, by the Federal Government. This body should be charged with drawing up a uniform code of conduct for all registered traditional healers in the country and be given power to deal with erring members and fake healers (unregistered healers).

3. The Federal Government should then direct the various state governments to follow suit in setting up an all exclusive healers board. This board should be composed of healers from all the local government areas. Again, representation should be on equal basis.

4. The state boards should be responsible to the one at the federal level and infact should

be seen as an organ for meeting its ends in the country.

5. The Federal Board should then direct all the state boards to register all ohe traditional healers in their state by their specialization. Such registration should be on local government basis.

6. Numbers three and five functions if properly executed should winnow quack healers. It should also enable us to have a proper categorization of the various branches of traditional medicine in Nigeria.

7. To disband the present multifarious traditional healers association in all the shapes and sizes and to set up state association to which all registered members must belong.

8. This body should be an arm of the Federal body which should have a Federal Secretariat.

9. The State bodies should have final say in the prosecution of quacks and erring members, but offenders should be allowed to appeal to the Federal level, should they feel disatisfied with the judgements. Such rights however should not be extended to quacks—or registered members. We recommend that the quacks and dismissed practitioners should only practice at the risk of being prosectuted.

10. The Federal Government should assist the Federal healers' Board to set up a phamacological research body to determine both the medicinal potency and the chemical components of herbs.

11. In this bid, the assistance of the various states should be enlisted.

12. Based on the above, the phamacological body should come up with a classification of the list of traditional drugs in Nigeria.

13. To device effective ways of diseminating the results of such efforts at standardization of herbs. The above if conscientiously executed should standardize our drugs and by so doing take care of the issue of over doze and that of the purity or refinement of healers' drugs.

14. To tackle the problem of low literacy rate by organizing symposia debates and seminars in which members will be schedule to talk; not necessarily in English, and for adequate coverage to be given to such gatherings.

15. To further encourage healers by empowering the Board to recruit and train for a maximum of two years, school certificate holders who will act as interpreters to healers in their various local governments secretariat if need be and/or translate in native dialect and broadcast the activities.

16. The Government can help to reduce to level, the blases of medical practitioner by allowing Erinosho (1981) to persuade her into inculcating in the curricula of medical education in Nigeria, knowledge of the "ubiquitous social cultural factors which underlie the contigencies of medical care in tropical Africa".

Erinosho's suggestion if adopted will help the physicians to understand and better cope with the illness behavious of Africans. It will help the doctors to know that Africa is still a face to face society where relationships with kins and neighbours matter a lot. This will play down on their present tendency towards impersonal services. This is the key to the haven patients find in the homes of the traditional practitioner (Oke, 1982; Erinosho, 1981).

Furthermore, implementing Erinosho's recommendation will as said before, correct the present attitudes of physicians to healers. This will set off a chain of reactions like reducing complexes, resolving numerous role conflicts like the issue of how close to get one's patients; foster accommodative atmosphere that will eventually culminate in the working side by side of the traditional healers and the medical doctors in the hospital setting.

When this happens we cannot but agree that a new dawn has arrived. The common man will wax stronger in health and Nigeria will thrive. We have no doubt whatsoever that such an ideal state of a nation's health can be achieved if we integrate our traditional healers into the 'midstream' of our health care delivery services. The time to start is now.

Discussion

A search for a viable alternative in the corridors of the medical systems of Euro-america and the USSR can not guarantee health for all by the year 2000. Today, Nigeria operates the Euro-america model. But it is too riddled with insufficiently syndrome to be relied upon. Today 80% of Nigerians live in the villeges and are ill provided with medical facilities.

Therefore, a model which will be accommodated by the operators of the present system will be best adopted in the interest of our continued existence. This model calls for the integration of the traditional healers into the 'midstream' of our health care delivery services. This seemly new model is not new. In China and USSR traditional healers work side by side with the medical doctors, and are doing quite well (Prince, 1964; Maclean, 1966). Perhaps it is the perfection of such wedlocks, coupled with epidemiological reports and insufficiency syndrome that made Maclean and Bannermann (1982), Ademuwagun (1969), WHO (1973) to recommend this integration "dose" to ailing third world countries. A trillion voices cannot be wrong. A healthy nation is contigent on healthy citizenry.

Here I rest my oar!

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