HIGH PREVALENCE OF BRUGIA TIMORI INFECTION IN THE HIGHLAND OF ALOR ISLAND, INDONESIA

TANIAWATI SUPALI, HERRY WIBOWO, PAUL RÜCKERT, KERSTIN FISCHER, IS S. ISMID, PURNOMO, YENNY DJUARDI, AND PETER FISCHER

Department of Parasitology, Faculty of Medicine, University of Indonesia, Jakarta, Indonesia; German Agency for Technical Co-operation (GTZ), Kupang, Indonesia; Bernhard Nocht Institute for Tropical Medicine, Hamburg, Germany; U.S. Naval Medical Research Unit No. 2, Jakarta, Indonesia

Abstract. To identify areas endemic for Brugia timori infection, a field survey was carried out in 2001 on Alor, East Nusa Tenggara Timor, Indonesia. Elephantiasis was reported on this island by villagers as a major health problem. Bancroftian filariasis was detected in four villages in the coastal area, whereas B. timori was identified in four rice-farming villages. No mixed infections with both species were found. In the highland village Mainang (elevation = 880 m), 586 individuals were examined for B. timori infection and 157 (27%) microfilaria carriers were detected. The prevalence of microfilarial infections standardized by sex and age was 25%. The geometric mean microfilarial density of microfilaricaric individuals was 138 microfilariae/ml. Among teenagers and adults, males tended to have a higher microfilarial prevalence than females. Microfilaria prevalence increased with age and a maximum was observed in the fifth decade of life. In infected individuals, the microfilarial density increased rapidly and high levels were observed in those individuals 11–20 years old. The highest microfilaria density was found in a 27-year-old woman (6,028 microfilariae/ml). Brugia timori on Alor was nocturnally periodic, but in patients with high parasite loads, a small number of microfilariae was also detected in the day blood. The disease rate was high and many persons reported a history of acute filarial attacks. Seventy-seven (13%) individuals showed lymphedema of the leg that occasionally presented severe elephantiasis. No hydrocele or genital lymphedema were observed. This study showed that B. timori infection is not restricted to the lowland and indicated that it might have a wider distribution in the lesser Sunda archipelago than previously assumed.

INTRODUCTION

Three species of filarial parasites are the causative agents of lymphatic filariasis: Wuchereria bancrofti, Brugia malayi, and Brugia timori. Although the lymphatic filarial parasites have many biologic and epidemiologic features in common, the level of knowledge about their prevalence is different. It is estimated that 115 million people are infected with W. bancrofti and 13 million with B. malayi, but no detailed estimations about the prevalence of B. timori are available. Because the distribution of B. timori is limited to some islands of the lesser Sunda archipelago, a low prevalence is suspected and B. malayi and B. timori infections are often summarized for prevalence estimations. Detailed knowledge of the distribution of B. timori is scarce. The Timor filaria was originally reported from East Timor. It was later detected on Sumba island and more detailed studies were performed on Flores, where the species B. timori was officially described.

It was reported that B. timori occurs also on Alor island. A prevalence of B. timori microfilaricaric individuals of 10% was observed in Welai village in the coastal area of Alor, but B. timori was not found in a nearby village at an elevation of 500 m. Both villages are close to the district capital Kalabahi in the northwestern part of Alor, and the distribution of B. timori in the remaining, more remote part of the island is still unknown. On Flores island B. timori occurs also in the coastal areas and not in the highlands; therefore, it was concluded that it is a typical lowland species. So far B. timori has been reported to be co-endemic only with W. bancrofti, but not with B. malayi. Lymphatic filariasis has been targeted by the World Health Organization for elimination as a public health problem by the year 2020 because recent advances in diagnosis and chemotherapy of W. bancrofti and B. malayi infections have resulted in new and promising control strategies. However, it remains to be determined whether these new concepts can be adapted to control B. timori infection. Therefore, more detailed information about distribution and epidemiology of this parasite is required to establish sustainable control programs.

In the present study, we identified for the first time villages highly endemic for B. timori in the highland at an elevation of 880 m. We provide a parasitologic and clinical description of the population in one endemic village on Alor. In this village, B. timori is responsible for various clinical signs, most prominently, lymphedema and elephantiasis of the lower leg. The results of the study present baseline data for a control program of B. timori filariasis on Alor island.

MATERIALS AND METHODS

Study area. Alor island is located north of Timor in the province Nusa Tenggara Timor (Figure 1A). The island has approximately 164,000 inhabitants and is one of the poorest areas in Indonesia. To identify areas endemic for filariasis, eight villages situated between the co-ordinates 124°20’E and 124°40’E and 8°10’S and 8°20’S were examined (Figure 1B): Wolwal and Lola villages (southwestern Alor, elevation = 120 m), Kokar village (northwestern Alor, elevation = 10 m), Alii village (northwestern Alor, elevation = 100), Mainang village (northern Alor, elevation = 880 m); Puielang village (southwestern Alor, elevation = 660 m); Fanating village (northern Alor, elevation = 20 m), and Alemba village (northern Alor, elevation = 100 m). The highland village of Mainang was selected for a more detailed survey. This village can be reached using a four-wheel-drive car by a 1.5-hour drive (30 km) from Kalabahi, the district capital of Alor. The temperature varies between 18°C at night and 28–30°C during the day and the climate is humid. The short wet season begins at the end of October continuing through March and the long dry season extends from April to October. The village is located in a swampy valley and houses are scattered over a large area. Several fields are located some
lymphangitis of the extremities, scars at the sites of previously examined by experienced physicians for clinical signs of lymphedema in Jakarta. Following registration, the individuals were participating in the survey. The study was approved by the Indonesian Ministry of Health and the University of Indonesia. Informed consent was obtained from all persons from this village who heard about the survey and asked for an opportunity, patients with lymphedema were introduced to hygienic methods to use on their affected legs and other procedures that may help stop the progression of the disease. Following the survey, treatment using a combination of a single dose of DEC (6 mg/kg of body weight) and of albendazole (400 mg) was offered to all infected individuals.

Assessment of microfilariae. For the identification of endemic areas, finger prick blood was collected between 7:00 PM and 11:00 PM and a Giemsa-stained thick blood smear was examined microscopically for the presence of microfilariae (mf). *Brugia timori* mf were identified according to the description of Purnomo and others. To confirm the periodicity of *B. timori*, finger prick blood (30 μL) was collected from two infected individuals every two hours for 24 hours and examined for mf. For more accurate estimation of mf densities, 1 mL of venous blood was filtered using 5-μm polycarbonate filters (Millipore, Eschborn, Germany). The Giemsa-stained filters were examined using a x100 magnification and mf were counted.

Data analysis. Epi-Info version 6.01 (Centers for Disease Control and Prevention, Atlanta, GA) was used for documentation and analysis of the data. The geometric mean was used as an index of the mf density within a study group. Data on distribution and density of mf were compared using the chi-square test and the Mann-Whitney U test.

RESULTS

Identification of areas endemic for *B. timori*. Eight villages in the western part of Alor island were briefly screened for the presence of mf and for clinical signs of lymphatic filariasis. A total of 1,075 individuals were examined. Twenty-seven *B. timori* mf-positive persons in four inland villages were detected by examination of finger prick night blood (Table 1). Seventy-two *W. bancrofti* mf-positive persons in four coastal villages were detected found by membrane filtration of 1 mL night blood. The prevalence of Bancroftian filariasis was low (1–3%) in the villages on the northwestern coast of Alor, and only three patients with clinical signs were observed. Patients with lymphedema of different grades or elephantiasis were observed almost exclusively in the rice-farming villages endemic for *B. timori*. Male patients with hydrocele or genital lymphedema were recorded only in the areas endemic for *W. bancrofti*. The youngest man affected was 20 years old. In these villages, people worked in dry-field agriculture or as fishermen. However, since the participants were not randomly selected, a bias due to self-selection cannot be excluded. Mixed infections of *B. timori* and *W. bancrofti* were not found.
Identification of villages endemic for lymphatic filariasis by examination of thick smears of capillary night blood for the presence of microfilariae (mf) on Alor island, Indonesia

### Microfilaria rates

The *B. timori*-endemic village of Mainang in the highlands of Alor was selected for a more detailed study. A total of 1,582 individuals lived in Mainang and 37% (586 persons) of the population was examined. No children less than five years old were included in the study. There was no difference in the sex and age distribution of the examined individuals in Mainang. More females were studied than males, and 50% of the eligible females and 27% of the eligible males were included in the study. This was because some men stayed overnight in their fields and could not participate in the survey. Confounding factors due to self-selection were possible only in Welai Selatan and Malaipae, but not in Tominuku where participants were recruited directly from their homes. However, there was no significant difference in prevalence of microfilaricmic individuals and patients with clinical signs in three sections of Mainang (Table 2). Therefore, these three sections were analyzed as a homogeneous group.

The highest rate of microfilaricmic men and women was found in those 40–50 years of age (50% for men and 38% for women) (Figure 2). However, even in those 5–10 years of age, 18% of the children were mf positive. There was no sex difference in prevalence of microfilaricmic individuals in this age group of children (*P* = 0.8266). In older children and adults, a significantly higher percentage of males was mf positive (35% vs. 23%, *P* = 0.0022). The prevalences of microfilaricmic individuals standardized by sex and age were 25% and 22% for males and females, respectively.

When the day blood of mf-positive individuals was examined, it was observed that 62% of the individuals had circulating microfilariae. There was a positive correlation between microfilarial density in night blood and day blood (*r* = 0.38, *P* = 0.002). Among 28 individuals with microfilarial densities greater than 400 mf/ml in night blood, 27 (96%) also had mf-positive day blood. The microfilarial densities in day blood were low: 57% had loads of 1–10 mf/ml, 26% had loads of 11–100 mf/ml, 37% had loads of 101–1,000 mf/ml and 23% had loads greater than 1,000 mf/ml.

### Microfilariae in day blood

When the day blood of mf-positive individuals was examined for mf, it was observed that 62% of the individuals had circulating microfilariae. There was a positive correlation between microfilarial density in night blood and day blood (*r* = 0.38, *FG* = 67, *P* < 0.002). Among 28 individuals with microfilarial densities greater than 400 mf/ml in night blood, 27 (96%) also had mf-positive day blood. The microfilarial densities in day blood were low: 57% of the microfilaricmic individuals had 1–10 mf/ml, 39% had 11–100 mf/ml, and 4% had greater than 100 mf/ml. Therefore, we briefly examined the periodicity of *B. timori* on Alor island in a patient with greater than 400 mf/ml in night blood and in another patient with 100 mf/ml in night blood. Between 8:00 AM and 4:00 PM, 4.8% and 3.3% of the total number of detected microfilariae were found in the peripheral blood of these two patients, respectively. The peak of microfilarial density was observed in one patient between 2:00 AM and 4:00 AM and in the other patient between 8:00 PM and 10:00 PM.

### Table 1

<table>
<thead>
<tr>
<th>Village</th>
<th>No. of persons examined</th>
<th>Mf (%)</th>
<th>No. of patients with leg lymphedema (%)</th>
<th>No. of male patients with scrotal hydrocele/lymphedema (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mainang</td>
<td>83</td>
<td>11 (13)</td>
<td>12 (14)</td>
<td>0</td>
</tr>
<tr>
<td>Paelenlang</td>
<td>89</td>
<td>5 (6)</td>
<td>11 (12)</td>
<td>0</td>
</tr>
<tr>
<td>Fanating</td>
<td>176</td>
<td>4 (2)</td>
<td>6 (4)</td>
<td>0</td>
</tr>
<tr>
<td>Alemba</td>
<td>99</td>
<td>7 (7)</td>
<td>2 (2)</td>
<td>0</td>
</tr>
<tr>
<td>Wolwal*</td>
<td>229</td>
<td>0</td>
<td>35 (15)</td>
<td>0</td>
</tr>
<tr>
<td>Lola*</td>
<td>167</td>
<td>0</td>
<td>32 (19)</td>
<td>0</td>
</tr>
<tr>
<td>Kokar*</td>
<td>126</td>
<td>0</td>
<td>2 (1)</td>
<td>23 (29)</td>
</tr>
<tr>
<td>Ailis*</td>
<td>96</td>
<td>0</td>
<td>3 (3)</td>
<td>1 (1)</td>
</tr>
<tr>
<td>Total</td>
<td>1,075</td>
<td>27 (3)</td>
<td>72 (7)</td>
<td>32 (3)</td>
</tr>
</tbody>
</table>

* These villages were examined by filtration of 1 mL of night blood.

### Table 2

<table>
<thead>
<tr>
<th>Village</th>
<th>No. examined</th>
<th>Mf positive (%)</th>
<th>No. with lymphedema (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male Female</td>
<td>Male Female</td>
<td>Male Female</td>
</tr>
<tr>
<td>Welai Selatan</td>
<td>88 120</td>
<td>30 (34) 23 (19)</td>
<td>14 (16) 21 (18)</td>
</tr>
<tr>
<td>Malaipae</td>
<td>87 112</td>
<td>27 (31) 24 (21)</td>
<td>11 (13) 14 (13)</td>
</tr>
<tr>
<td>Tominuku</td>
<td>65 114</td>
<td>23 (35) 30 (26)</td>
<td>4 (6) 14 (12)</td>
</tr>
<tr>
<td>Mainang (total)</td>
<td>240 346</td>
<td>80 (33) 77 (22)</td>
<td>28 (12) 49 (14)</td>
</tr>
</tbody>
</table>

* Microfilariae (mf) were detected by filtration of 1 mL of night blood.

![Figure 2](image)
B. timori ON ALOR ISLAND

These results confirmed the nocturnal periodicity of B. timori, but showed that in individuals with high parasite loads a small number of microfilariae can be also found in day blood.

**Prevalence of clinical signs in Mainang.** The most prominent clinical sign of lymphatic filariasis in Mainang was lymphedema (grades 1–3) of the leg: 35% of the affected patients had elephantiasis (grade 4 lymphedema). Twenty-nine (12%) males and 49 (16%) females had lymphedema, but this difference was not significant ($P = 0.466$). The distribution of the grades of lymphedema was similar in both genders. The prevalence of individuals with lymphedema standardized by sex and age was 12%. Lymphedema of grades 1–3 was first observed in individuals 10–15 years old and elephantiasis was first observed in patients greater than 15 years old (Figure 4). Ninety percent of the individuals with lymphedema were amicrofilaremic. The geometric mean microfilarial density of the eight microfilaremic individuals with lymphedema was 311 mf/ml (range 1–6,028 mf/ml). Both legs were affected in approximately 40% of the cases. Only one older woman had lymphedema of the right arm (grade 2). In all other cases, lymphedema was limited to the leg, mostly below the knee. Hydrocele in men and lymphedema of the genitals were not observed. About 33% of the examined individuals reported a history of acute filarial attacks. Malaria was reported to be highly endemic in the area and most participants were not able to differentiate filarial fever from malaria. Therefore, data on acute filarial fever were not analyzed in detail. During the survey, no patients with acute filarial fever and acute lymphadenitis or lymphangitis were observed. Filarial scars were observed in 12 (2%) of the individuals examined. The overall prevalence of B. timori filariasis in individuals with microfilaremia and/or obvious clinical signs of filariasis was 39%.

**DISCUSSION**

The results of the present study show that lymphatic filariasis is highly endemic on Alor island. Wuchereria bancrofti was endemic only in the coastal, rice field–free areas, whereas B. timori was found in areas with extensive rice culture. The ecotype for B. timori was characterized as foothills along a riverine valley with irrigated rice fields. $^{11,12}$ This characterization is supported by the results of the present study. Since B. timori infection was prevalent also at an elevation of 880 m, it is suggested that it is not restricted to lowland areas, but is linked to rice fields. Anopheles barbirostris is the only known vector for B. timori. $^{13}$ In a preliminary study, local A. barbirostris mosquitoes were found by polymerase chain reaction (PCR) pool screening to be infected with B. timori (Fischer and Supali T, unpublished data), published data), but because it is not a homogeneous species, the vector strain remains to be further characterized by molecular methods. However, A. barbirostris is a typical rice field–breeding species and is responsible for transmission of brugian filariasis in many areas in East Asia. $^{7}$ In contrast, A. subpictus was reported to be the most important vector of W. bancrofti on Flores, an island near Alor. $^{14,15}$ The distribution of members of the A. subpictus complex is more variable than that of A. barbirostris, and certain cytospecies are known to occur only in coastal areas and breed in brackish water. Highland areas with an elevation more than 500 m predominate on most of the islands of the lesser Sunda archipelago, and it is likely that B. timori occurs in all rice-farming areas in this region where A. barbirostris is abundant. No reports of a co-endemicity of B. timori and B. malayi have been published, and it can be hypothesized that B. timori replaces B. malayi in this region.

We found a standardized prevalence of mf carriers of 25% in Mainang. Previous studies on Alor also reported high prevalences of B. timori infection, but the exact sampling strategy, disease rates, and standardized prevalences were not provided. $^{4,6}$ Dennis and others detected on Flores a prevalence of mf carriers of 25% in a defined community of 201 persons. $^{11}$ No significant differences in sex and age distribution of microfilaremic individuals were found in this village. In our study, men tended to be mf positive more often and a positive correlation was observed between the prevalence of microfilaremic individuals and the age. A similar observation was made by Partono and others on Flores, but due to the small sample size these differences were not statistically significant. $^{12}$ Many studies on W. bancrofti and B. malayi infection provide evidence that a positive correlation between mf prevalence and age exists and it is not unusual for filariasis that men are infected more often than women. $^{16}$ It can be assumed that B. timori has an epidemiologic pattern similar to the other lymphatic filarial parasites.

Although B. timori was confirmed to be nocturnally periodic, the presented results indicate that persons with mf den-
sites greater than 400 mf/ml of night blood can be identified by the filtration of day blood with a sensitivity of 96%. For the identification of endemic areas with high parasite loads, this might be an alternative to the night blood collection. However, for areas with low endemicity and low infection density, as well as for the monitoring of intervention programs, the parasitologic examination of day blood for microfilariae is not suitable. More sensitive methods, such as the PCR, need to be used for this purpose.7

In the opinion of villagers in Mainang, the development of leg edema was due to extensive work in rice fields. In accordance with this belief, in rice field-free areas, where Bancroftian filariasis was endemic, almost no edema of the leg was observed. It is now well known that external bacterial superinfections contribute to the pathology of lymphatic filariasis.18 Therefore, working in irrigated rice fields may be a risk factor in the development of elephantiasis of the leg in individuals infected with B. timori. Although individuals with open lesions and advanced lymphedema try to avoid contact with water because of the pain, they are forced to continue to work in their rice paddies for economic reasons. Further studies are needed to elucidate the risk factors for the development of lymphedema in individuals living in areas endemic for B. timori. However, the prevalence of lymphedema in Mainang was in agreement with results of two studies on Flores, which reported B. timori microfilaria rates of approximately 25% and lymphedema rates ranging between 11% and 15%.11,12

Dennis and others found that elephantiasis due to B. timori most often developed during the third decade of life.11 This observation is supported by our results, but we found that lymphedema up to grade 3 was common even in teenagers and may lead to serious social and economic consequences at an early age. In a sample of 42 previously uninfected immigrants to an area endemic for B. timori, nine individuals were reported to have developed lymphedema or elephantiasis within two years following settlement.19 Although the development of lymphedema may be faster in individuals who have not been previously exposed to B. timori, this observation is in agreement with our data that show that lymphedema due to infection with B. timori often affects teenagers and younger adults. The high prevalence of microfilaremic individuals and persons with clinical signs such as lymphedema in Mainang shows that B. timori filariasis is still an important local public health problem.

As of the year 2000, approximately 3.9 million people lived in Nusa Tenggara Timor and approximately 0.75 million people lived in East Timor.20 These people are at risk of lymphatic filariasis due to W. bancrofti and B. timori. The prevalence of B. timori on Timor appeared to be low,3 but it likely that on the other islands its prevalence may reach values similar to those reported for Mainang in the present study. Although it may be premature to estimate the global number of B. timori infections based on the available data, it probably does not exceed 800,000 persons. In comparison with W. bancrofti and B. malayi, this number is relatively small and may be controlled more easily.

A few decades ago B. timori filariasis was successfully controlled in a small community of 202 individuals on Flores island by mass administration of DEC (5 mg/kg of body weight) for 10 consecutive days.21 However, a dramatic advance in the control of W. bancrofti and B. malayi infections was achieved by the observation that a single dose of DEC (6 mg/kg) has similar effects as the same dose of DEC given daily for two weeks.8,22,23 Community-based treatment using a single, annual, or semi-annual dose of DEC is considered to be more feasible compared with a 10-day or two-week treatment course. It is still not known if this single-dose treatment strategy is also safe and effective in the control of B. timori infection. So far, no animal reservoir for B. timori has been identified. The distribution of B. timori is restricted to a few islands and anopheline mosquitoes are the only known vectors. It has already been discussed that filarial parasites transmitted by anopheline mosquitoes can be more easily controlled than culicine-transmitted infections.24 For these reasons, the prospects for the elimination of B. timori filariasis may be more promising compared with the elimination of filariasis due to B. malayi and W. bancrofti in many other regions.

The present study showed that B. timori filariasis is abundant in the highlands of the lesser Sunda archipelago and that this infection is still a public health issue of local importance. Further studies are needed to show if B. timori infection can be controlled by the new treatment strategies for the control and elimination of lymphatic filariasis proposed by the World Health Organization.

Acknowledgments: We thank Dr. Paul Manoepnil, Sudiman, Dr. Lani Harjianti, Simone Klüber, and the district health workers for their help during the field survey. We are grateful to all inhabitants of Mainang and the other villages examined who participated in the study. We also thank Professor Bernhard Flescher for his encouragement and Professor W. Büttner for critically reading the manuscript.

Financial support: This investigation was supported by the UNDP/World Bank/WHO Special Program for Research and Training in Tropical Diseases (TDR). Peter Fischer was supported by the Scholarship Program ‘Infectiology’ of the German ‘Bundesministerium für Forschung und Technologie (BMFB)’.

Authors’ addresses: Taniawati Supali, Herry Wibowo, Is S. Ismid, and Yenny Djuardi, Department of Parasitology, Faculty of Medicine, Salewba 6, University of Indonesia, Jakarta 10430, Indonesia; Paul Rücker, German Agency for Technical Co-operation (GTZ), Jalan Wolter Robert Monginsidi H/2, PO Box 1217, Wallikota Baru, Kupang 85000, Indonesia; Kerstin Fischer and Peter Fischer, Bernhard Nocht Institute for Tropical Medicine, Bernhard-Nocht-Strasse 74, D-20359 Hamburg, Germany; Purnomo, U.S. Naval Medical Research Unit No. 2, Jakarta, Indonesia.

Reprint requests: Peter Fischer, Bernhard Nocht Institute for Tropical Medicine, Bernhard-Nocht-Strasse 74, D-20359 Hamburg, Germany, E-mail: Pfischer@bni.uni-hamburg.de.

REFERENCES

6. Joesoef A, Dennis DT, 1980. Intestinal and blood parasites of...