20 YEARS OF PROGRESS IN LYMPHATIC FILARIASIS RESEARCH

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The accomplishments and progress that have occurred over the past 20 years in collaborative filariasis research between NAMRU-2 detachment staff, National Institute of Health, Research and Development (NIHRD), and Directorate General of Communicable Disease Control and Environmental Health (CDC&EH), University of Indonesia, and other health institutions have produced an impressive array of important contributions to the study of human lymphatic filarial disease. Over this time, no less than 62 publications specifically addressing filariasis studies have come about as a direct result of close cooperation between interorganizational investigators (Figs. 1, 2). Beginning in 1972 with observations on diethylcarbamazine (DEC) provocation for diurnal diagnosis of *W. bancrofti*[1], publications have covered a wide range of different disciplines all with the common goal of understanding and ultimately controlling this disease.

Numerous biomedical and filariasis surveys have been recorded over this period, adding greatly to our knowledge of the diverse epidemiology and disease distribution across the archipelago. The following is an attempt to highlight the major milestones among selected categories that have made significant contributions and have provided insights into filariasis research.

TAXONOMY/BIOLOGY

The knowledge and understanding we acquire from the classification and basic biology of the filarial parasites and their vectors allows for more sound approachs to research and control; without such studies we would be proceeding down an unsteady path.

Three events, in particular, standout - the discovery and morphologic descriptions of both *Brugia timori* and *Wuchereria kalimantani*, and the reclassification of *Brugia malayi* into defined epidemiologic terms. In the mid-1960's *Brugia* microfilariae of man in Timor were being referred to as a new species or morphologic variant of *B. malayi* based on physical characteristics. However, conclusive evidence only began to emerge with a series of papers describing *B. timori* ten years later. In 1976, Purnomo *et al.*[2] were able to show that the first-stage larva in the mosquito could be morphologically distinguished from *B. malayi* and that the microfilariae of the Timor *Brugia* were, in fact, morphologically different from *B. malayi*[3]. This eventually lead to the description of adult worms from experimentally infected

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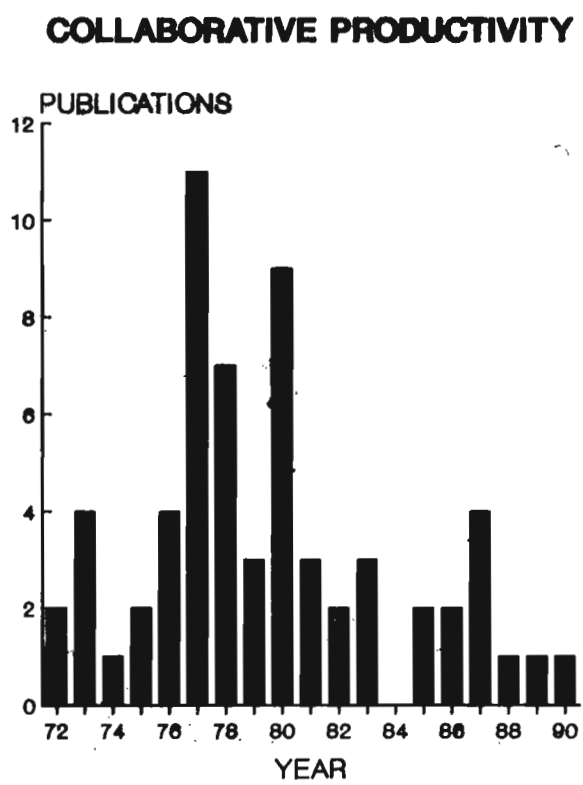

Fig. 1 Research productivity as measured by numbers of publications on lymphatic filariasis by year.

Fig. 2 Breakdown of publications by category from 1972 to 1990.

mongolian jirds and a new species[4]. Since that time more research has been amassed on the epidemiology and control of this disease.

In 1980, Palmieri and others[5] described for the first time a new species of *Wuchereria* from *Presbytis cristatus* (= cristata) in South Kalimantan. Previously this genus of filariid had not been known outside of humans. Such a finding was very significant because laboratory work with *W. bancrofti* has long been hampered due to lack of a susceptible and adequate laboratory model. Because of the close taxonomic relationship of this simian parasite with *W. bancrofti*, antigens from *W. kalimantani* produced in *P. cristata* may have potential value as reagents in immunodiagnostic tests for the human disease. The model itself has already proven useful in various immunologic and chemotherapeutic trials.

In 1987, Partono and Purnomo[6] re-evaluated the classification of *B. malayi* strains based on periodicity studies and the biological behavior of the parasite in animals to arrive at two useful epidemiologic types of *B. malayi*, zoophilic and anthropophilic. This has helped to sort through the confusion dealing with different geographic strains and periodicity patterns.

IN VITRO CULTURE

Because animal models can often create problems from financial constraints to ethical issues, *in vitro* culture systems are desirable and often sought. In 1987, Franke *et al.*[7] provided results that were a significant step in that direction. She and others were successful in developing third-stage larvae of *W. bancrofti* to the fourth-stage using commercially available reagents. Because *W. bancrofti* exhibits a narrow host specificity, a reliable *in vitro* culture system will provide an important tool for immunologic and biochemical studies and for screening antifilarial compounds.

Recently, Riberu *et al.*[8], using the same media as Franke, was able for the first time to successfully culture third-stage larvae of *B. malayi* to mature adult worms that produced living microfilariae. Besides the advantages previously mentioned, this development should allow investigators the ability to study worm development under controlled conditions and place less reliance on existing animal models.

EXPERIMENTAL HOSTS

Host models are often sought because they make possible important studies on host susceptibility, response to infection, immunology, and the effect of filaricidal drugs; work not yet practical with *in vitro* systems. Unlike *B. malayi*, a number of attempts in the past have met with little success in establishing an animal model for *W. bancrofti*[9]. However, results of studies begun at NAMRU using various species of nonhuman primates for establishing this parasite in the laboratory have been encouraging. In 1979, Cross *et al.*[10] were the first to show that this parasite is capable of reaching sexual maturity and producing microfilariae in monkeys. Subsequently, Palmieri *et al.*[11] and Campbell *et al.*[12] were able to achieve a similar breakthrough using *Presbytis cristata*. These results, towards producing working models, will provide the opportunity for more detailed parasite and vector research, which may lead to more effective treatment and disease prevention.

The study of *Brugia timori* also lacks a nonhuman natural reservoir host and patent infections in most experimental animals have been difficult to produce[13]. However, Sartono *et al.*[14] have been successful in producing a patent infection in *P. cristata* and thus further research on this parasite could be performed.

Experimental models are not restricted to vertebrate animals but can extend to insect vectors as well. Purnomo *et al.*[2] have successfully infected *Aedes togoi*, a valuable laboratory vector for many filarial species, with *B. timori* to describe the life cycle and larval stages of this parasite.

VECTORS

Although, in the past, vectors of filariasis have been studied in many areas, more intensive studies were needed. The incrimination of mosquito vectors for filarial infections is epidemiologically essential for designing effective control and protective measures against transmission. Significant accomplishments along these lines for *W. bancrofti, B. malayi* and *B. timori* have been made across the archipelago through experimental studies and discovery of natural infections. The use of various laboratory vectors has proven very useful indescribing different biologic strains of *B. malayi* and *W. bancrofti* based on susceptiblity patterns. (unpublished reports)

Among the important findings was the first time incrimination of *Anopheles aconitus* and *Anopheles subpictus* as vectors of *W. bancrofti* in Flores[15] and *Anopheles barbirostris* as the natural vector of *B. timori* in Flores[16]. The incrimination of *An. barbirostris* lead to an exhaustive review on the systematics of this species to determine if the vector and non-vector forms could be distinguished morphologically. Although the forms are very different behaviorally (i.e. host preferences), they are still considered nonspecific until a more definitive study can be made[17].

In 1977, Partono *et al.*[18] demonstrated that *Mansonia uniformis*, a vector of both periodic and subperiodic *B. malayi*, could not develop *B. timori* beyond the first-stage. This was further evidence that *B. timori* is a distinct species from *B. malayi.*

In 1986, Campbell *et al.*[19] reported *Anopheles balabacensis* was able to experimentally develop infective stage *W. kalimantani;* however, only recently has it been shown to be the natural vector in Kalimantan as well (Atmosoedjono *et al.*, in press). Because *An. balabacensis* is the principal vector of malaria in the region, the potential of *W. kalimantani* as a zoonotic disease is an intriguing question.

DIAGNOSIS

Currently, the definitive diagnosis of filariasis is the demonstration of microfilariae in the peripheral blood. However, the sensitivity of various tests are affected by the degree (i.e. density) of microfilaremia. In 1973, Partono *et al.*[20] provided a valuable field evaluation comparing various conventional and more sophisticated methods of detecting *W. bancrofti* microfilariae from Jakarta patients. The results proved conclusively that the membrane filtration technique of whole blood was superior to either the standard 20 mm^3 thick smear or Knott's concentration technique for detecting low microfilarial densities. This more reliable

method is important with regards to assessing where accurate diagnoses are required or when monitoring post-treatment microfilaremias[21].

Entomologically, Sim *et al.*[22] provided a simple method to accurately identify *B. malayi* larva in vectors using a species-specific DNA probe. This development is important in having the ability to separate out *B. malayi* from other sympatric filariids that can also develop in mosquitoes. This tool has great promise for use in both monitoring and studying local vectors in different geographical areas. Additionally, this and other probes represent an important breakthrough in the quest for species-specific reagents as a promising approach for the diagnosis of infection in humans. In 1987, Carlow *et al.*[23] developed an enzyme-linked immunoassay suitable for third-stage species specific detection of *B. malayi* in mosquitoes using a stage and species specific monoclonal antibody. This has a significant advantage over DNA probes which cannot discriminate between the different developmental stages within the vector, thereby reducing the errors in overestimation of disease transmission potential. Both advances are significant contributions that enable field workers to differentiate *B. malayi* from other filarial parasites, including the morphologically indistinguishable parasite of animals *Brugia pahangi*.

IMMUNOLOGY

Serological tests have been slow in development, especially those that can offer conclusive, definitive results in the diagnosis of human infection[24]. The research activities over the last decade have been attempting to elucidate the complex cellular and humoral immune responses to the lymphatic dwelling filariids.

In 1980, Piessens *et al.*[25] revealed a striking correlation between the presence of immune responses to microfilarial antigens and the absence of patent microfilaremia, suggesting that more than one type of immune reaction might be involved in the resistance to and the elimination of filarial infections. The increase in numbers of antigen-specific suppressor T cells was found to correlate with reduced immune responses in patients with microfilaremia rather than in persons with other pathologic manifestations of Brugian filariasis[26]. In essence, filarial parasites seem to impair the function of their host's immune system. The immunosuppression induced by filarial parasites is a possible mechanism of survival of these organisms in an immunocompetent host. Furthermore, it has been suggested that the immune unresponsiveness is due to active suppression of immune responses directed against the parasite and not to an intrinsic inability of infected patients to react to parasite antigens[27].

In 1982, Kurniawan *et al.*[28] studied cellular and humoral immune responses in bancroftian filariasis and found no difference between microfilaremic and amicrofilaremic individuals to specific anti-sheath antibody as contradictory to reports by others, thus indicating further the complex nature of the immunologic response to lymphatic filariasis. In 1985, Campbell[29] gave possible explanations for the minimal immune response to *W. bancrofti* antigens in *P. cristata* (i.e. molecular mimicry or use of host proteins to conceal parasite) and made a cautionary statement on future vaccine development based

on parasite antigens alone without a thorough understanding of the host response to these antigens. In 1980, Piessens *et al.*[30] reaffirmed the hypothesis that the pathogenesis of various clinical syndromes may result from different types of immune reactions to distinct antigens associated with different developmental stages in the life cycle of the parasite.

A number of studies documented that patent infections are associated with a state of immune unresponsiveness to parasite antigens. Observations reported by Piessens *et al.*[31] indicated that elimination of detectable circulating antibodies by treatment with DEC partially reverses the state of cellular unresponsiveness to filarial antigens and may play a role in the observed extended amicrofilaremic period after successful treatment.

A study by Piessens *et al.*, in 1983[32], proved most interesting in describing that lymphatic filariasis and chronic malaria (Tropical Splenomegaly Syndrome) have opposing effects on immunoregulatory lymphocytes. It was shown that patients with dual infections tended to yield results similar to uninfected control donors. This immediately calls into question the value of various serologic tests for diagnosis of filariasis, especially in areas with high malaria endemicity.

EPIDEMIOLOGY/CONTROL

A tremendous amount of time and effort has gone into describing the epidemiologic factors responsible for disease transmission and clinical manifestations. Many of the control projects have been pioneer attempts at disease intervention based on past epidemiologic knowledge. The three species of lymphatic filariids found in Indonesia present diverse and complex situations with regards to distribution, pathology, transmission dynamics and reservoir hosts. The introduction of "non-immunes" into endemic areas through transmigration or military movement has been of particular interest and concern[33-34]. One of the first published attempts at large scale control of *B. malayi* in Indonesia involved mass treatment with DEC in an area of Central Sulawesi[35]. Results were considered satisfactory after a 6-day treatment in which the microfilaremia rate dropped from 24% to 5% after 2-3 months. Oemijati et al., in 1978[36], described the unintentional effects of environmental change in a community over a ten year period (i.e. agricultural development) on the endemicity of *B. malayi* as a result of dramatically decreased breeding sites for *Mansonia* mosquitoes. The microfilarial rate and density showed a marked decrease (22.2% vs 2.5%) between surveys in the absence of any organized control efforts. This paper was an important example of the potential use of environmental management towards the control of various vector-borne diseases.

Even before the taxonomic question of *B. timori* was clarified, Dennis *et al.*[37] were the first to describe this disease in detail concerning the epidemiology and clinical manifestations in a defined community on Flores and identified the only known vector, *An. barbirostris,* as responsible for endemic transmission. A dramatic and simple intervention method using DEC to control *B. timori* was described by Partono *et al.* in 1979[38]. One year after a single mass treatment, selective re-treatment was done which resulted in reduction in the microfilaria rate from 30% to 2.5%. The shorter treatment

schedule using a higher dosage of DEC was accompanied by minimal adverse reactions providing a more simple and practical control measure for rural areas. Partono *et al.*[21] were able to ascertain the long-term effects of repeated DEC use to control *B. timori*. Among their many conclusions was that a single mass DEC treatment is not sufficient to adequately control Timorian filariasis, but that re-treatment of active cases is necessary. It was suggested that for active foci, a second mass treatment should be given 3 years after the initial regimen.

Spectacular results were obtained in a study involving community participation to administer low doses of DEC weekly over a 18 month period that eradicated *B. timori* from six villages in Flores[39]. Rusch *et al.*, in 1980[40], evaluated the effectiveness of long and short-term mass treatment of *B. malayi* with DEC in South Kalimantan. It was concluded that because of the greater side effects with the short-term treatment (22% vs 3%), the long-term treatment was considered to be the more acceptable regimen.

In all, many of these field studies were successful in reducing the number of persons acquiring active infections over time and decreasing morbidity (the total number of lost working days) due to filariasis.

CONCLUSION

The past 20 years of lymphatic filariasis research has been varied and productive from the standpoint of advances in the multifaceted fields. However, it seems clear that the results so far obtained are only the beginning to further and greater accomplishments and dividends in the future. Many areas require increased research efforts, such as improved *in vitro* techniques, advanced vector studies, taxonomy, more sensitive and field applicable diagnostic methods, and advanced levels of immunology and epidemiology.

This is particularly true for control or elimination of this disease from endemic areas. Trials involving new antifilarial compounds (e.g. Ivermectin), greater use of environmental management techniques to reduce transmission, and increased reliance and promotion on community participation are only a few of the ways past activities and experience can be transformed into future positive results.

Lymphatic filariasis will continue to be a problem in many parts of Indonesia in the years ahead. The close collaboration between organizations and disciplines joining forces will increase the speed at which knowledge and effort are able to overcome this debilitating disease.

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