

EPIDEMIOLOGY OF FILARIASIS IN INDIA

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SYNOPSIS

The author reviews the history of filarial infections in India and discusses factors affecting the filariae, their vectors, and the human reservoir of infection. A detailed description is given of techniques for determining the degree of infection, disease and endemicity of filariasis in a community, and aspects which require further study are indicated.

Filarial infections have been recorded in India as early as the sixth century B.C. by the famous physician Susruta in Chapter XII of the *Susruta Samhita* (quoted by Menon⁴⁸). The description of the signs and symptoms of this disease by Madhavakara (seventh century A.D.) in his treatise *Madhava Nidhana* (Chapter XXXIX), holds good even today. More recently, Clarke in 1709 called elephantiasis of the legs in Cochin, South India, "Malabar legs" (see Menon⁴⁹).

Lewis³⁸ in India discovered microfilariae in the peripheral blood. Between 1929 and 1946 small-scale surveys have been carried out, first by Korke^{35, 36} and later by Rao⁶³⁻⁶⁵ under the Indian Council of Medical Research (Indian Research Fund Association), and others, at Saidapet, by workers at the King Institute, Guindy, Madras (King et al.³⁹). The studies in the epidemiology of filariasis in Travancore by Iyengar (1938) have brought out many important points in regard to Wuchererian infections, especially *W. malayi*. The first description of *Mf. malayi* in India was made by Korke³⁵ in Balasore District, Orissa State; the credit for describing the adult worms of this infection is due to Rao & Maplestone.⁶⁸

The discovery of the garden lizard *Calotes versicolor* with a natural filarial infection — *Conispiculum guindiensis* — (Pandit, Pandit & Iyer⁵³) in Guindy led to studies in experimental transmission which threw some interesting light on comparative development (Menon & Ramamurti;⁴⁹ Menon, Ramamurti & Sundarasiva Rao⁵⁰). Hawking²² suggested the use of microfilaria *C. guindiensis* in the in vitro screening of drugs. The antigen from adult filarial worms, as prepared by the King Institute, is being studied for its reliability in the early diagnosis of filariasis and for possible use in epidemiological studies (Indian Council of Medical Research²⁷).

In spite of its ancient history, even today no countrywide survey of the disease has been carried out, to note either the extent of prevalence or the distribution of the two species or their vectors. It is hoped to achieve

this by 1958 under a National Filaria Control Programme recently launched in India. Megaw & Gupta⁴⁷ were the first to publish a "filaria map" of India based on examination of blood samples received from different places in the various states. Recently Jaswant Singh & Raghavan³² have brought out the salient points of filariasis as a public health problem in India and its control, with a filaria map based on replies received to a questionnaire and published reports. It has been estimated from the above sources that there are at least 25 million persons living in the filaria-endemic areas in 13 states in India (Assam, Bengal, Bihar, Orissa, Andhra, Madras, Travancore-Cochin, Hyderabad, Madhya Pradesh, Vindhya Pradesh, Uttar Pradesh, Saurashtra and parts of Bombay). Recently foci in the Nicobar and Laccadive islands, as also in the French settlement recently ceded to India, have also come to light.

The Filariae of India

Types of infection

The types of filarial infection occurring in India are *W. bancrofti* and *W. malayi*. The former is the more anciently reported and more extensively prevalent infection, while *W. malayi* has been reported since 1929 and from a few foci only. The largest single tract of *W. malayi* infection so far reported in India is in Travancore-Cochin State (Iyengar²⁸) covering over a million persons. Annex 1 sets out details of places where *W. bancrofti* and *W. malayi* infections were noted (see page 573). A case of *Loa* infestation (probably imported) (Maplestone⁴⁶) and one of thelaziasis (caused by *Thelazia callipaeda*) have also been mentioned (Friedman,²⁰). Cases of "ocular filariae" have been circumstantially diagnosed as caused by *W. bancrofti* (Charles;¹¹ Wright, Iyer & Pandit;⁸⁰ Srivastava;⁷⁵ Chatterjee¹²).

Periodicity of microfilariae

The microfilariae of *W. bancrofti* and *W. malayi* infections occurring in India are truly nocturnally periodic (Korke;³⁶ Iyengar;²⁸ Raghavan & Krishnan^{60, 61}). This interesting phenomenon makes it imperative to carry out filaria surveys at night-time.

The periodicity of microfilariae is usually worked out by taking hourly or two-hourly samples of a unit volume of blood over a 24- or 48-hour period and then smearing and staining them, and enumerating and plotting the microfilariae found in each of the slides. To work out the periodicity index, two formulae have been suggested, one by Brug⁸ and the other by Iyengar.²⁸ Brug⁸ suggested the formula "number of microfilariae at noon; number of microfilariae at midnight". Since the midnight count is not always the peak, Iyengar²⁸ suggested "Average of counts between 9 a.m. and 4 p.m./Average of counts between 9 p.m. and 4 a.m. \times 100. In his Travancore studies, Iyengar reported the index to be 0×100 , 2.3×100 , and 2.5×100 .

The two peaks in the microfilarial "tide", one at 8 p.m. and the other at 4 a.m., described by Brug as being characteristic of *W. malayi*, were not noted by Iyengar or Raghavan & Krishnan.^{60, 61} With altered sleeping habits a diurnal overflow of microfilariae has been recorded in *W. bancrofti* cases (Malaria Institute of India⁴¹). Thus, probably sleep influences the periodicity of microfilariae.

Manson⁴⁵ put forward the idea that periodicity was probably related to the biting-habits of the vector. In bancroftian filariasis this association is apparently well brought out, as *C. fatigans*, its vector, is also predominantly a nocturnal biter. *Mansonioides* sp., which are the vectors for *W. malayi* infection, have nocturnal biting-habits although not so pronounced as in *C. fatigans*; it is known, however, that *M. annulifera*, the primary vector, is very active between 7 p.m. and 9 p.m. Evidence is accumulating that *M. uniformis* is at its peak activity later, that is, at about 10 p.m. The periodicity of microfilariae would thus appear to be an adjustment to obtain optimum conditions for survival of the species and also for transmission. They circulate in the blood when the appropriate insect vector bites most freely, and during the rest of the 24 hours enjoy favourable conditions of the lungs (Hawking & Thurston²³). However, more studies need to be carried out to determine the reasons for periodicity of microfilariae of Wuchererian infections in India.

Vectors of Filariae in India

Mosquitos are the only known vectors of filariae in India. It is expected that a more detailed picture of the situation will shortly be available from the results of surveys being carried out under phase I of a national filaria control programme launched in 1955. Results of the natural and experimental infection studies are set out in Annexes 1 and 2 (see pages 573 and 574-575).

The chief species of mosquito prevalent in India are *Aedes*, *Anopheles*, *Culex* and *Mansonioides*.

Aedes

Aedes sp. mosquitos are "domestic" in their habits. They breed in even small collections of clean water, for example, rainwater accumulating in discarded tins, coconut shells, etc. The chief species in India are *Aedes aegypti* Linn., *Aedes albopictus* Skuse and *Aedes vittatus* Bigot; none of them has been reported as infected in nature or experimentally positive for Wuchererian infections. They are, however, efficient vectors of dengue fever in this country.

Anopheles

In nature or experimentally many members of this species have been found to be infected with filarial or plasmodial infection only, or with both

in the same mosquito (see Annexes 2 and 3, pages 574-575 and 576). The results of experimental studies with *A. stephensi* are interesting. This species was observed to be experimentally positive to *W. bancrofti* (Rao & Iyengar⁶⁷). On an analysis of these results, it would appear to be a better vector under laboratory conditions than even *C. fatigans*. Raghavan & Krishnan^{60, 61} noted that it was highly susceptible to *Mf. bancrofti* but refractory to *Mf. malayi*. It may thus be that *A. stephensi* (type) could be used for the diagnosis of the type of Wuchererian infections. Although combined plasmodial and filarial infections have been found in the same anophelines, for example, in *A. philippinensis* (Iyengar²⁹) in nature or experimentally in *A. stephensi* (type) (Malaria Institute of India⁴²) their rarity among other anophelines in India from available records is at present inexplicable. Iyengar²⁹ reported one of 13 *A. philippinensis* collected in nature to harbour sporozoites and infective larvae, while experimentally 6 out of 54 *A. stephensi* (type) were likewise found positive for both. The filarial infection was *W. bancrofti* in both cases, whereas the plasmodial infection in the latter case was *P. falciparum*.

Culex fatigans

This ubiquitous species, the main vector for bancroftian filariae in India, is prevalent universally. It usually breeds in collections of dirty water but is also found in comparatively clean waters. Such a change in larval environment, however, does not appear to change its infection potential (Indian Council of Medical Research²⁵). In places where it is a vector the anthropophilic index is high, as in Porbander, Saurashtra (Raghavan⁵⁸). While high infection rates have been experimentally noted in *C. fatigans* with *Mf. bancrofti* (Rao & Iyengar;⁶⁷ Raghavan & Krishnan;^{60, 61} Roy & Bose⁷¹) this mosquito has been found refractory to *Mf. malayi* (Lichtenstein;³⁹ Iyengar;²⁹ Raghavan & Krishnan^{60, 61}). *Culex sitiens* Wied. and *Culex vishnui* Theo have been occasionally reported with natural infections but are unimportant as vectors.

Mansonia

These mosquitos have an unusual biology, in that their destiny is bound up with certain types of aquatic plants, especially *Pistia stratiotes*. This belongs to the family *Aroidea* and is a small plant with a rosette of floating leaves with numerous roots at its base. In India *M. annulifera* Theo, *M. uniformis* Theo and *M. indiana* Edwards are prevalent. The first two are important as vectors of *W. malayi*. Some interesting studies on their bionomics have been recorded by Iyengar.²⁹

M. annulifera Theo

The females of this species lay their eggs on the under-surfaces of leaves of certain aquatic plants only, such as *Pistia stratiotes*, *Lemna polyrrhiza*

and *Eichornia* sp.; *Pistia* is usually preferred. In the absence of such aquatic vegetation no breeding is noted; breeding is also correlated with organic contamination.²⁹ In nature, egg clusters have been noted adhering to the under-surface of the leaves of *Pistia stratiotes*; the basal part of the eggs remain stuck to the leaves, as also to each other. On hatching out, the larvae escape into the water, attaching themselves in turn to the rootlets of the plants by their modified siphon tubes. They derive their air from the vascular channels of the plant. Fraser¹⁹ in Assam has noted egg clusters on Dolgrass blades and *Eichornia australis*; larvae and pupae of *Mansonioides* sp. were noted attached to their roots. The pupae behave similarly to the larvae, detaching themselves from the rootlets to rise to the surface just before emergence of the adult.

These small brownish mosquitos are silent in flight, biting mainly between 7 p.m. and 9 p.m. They are weak-winged, and rest in dark corners of the houses and hop about like sandflies. This species is the principal vector of *W. malayi* in India.

M. uniformis Theo

This is an efficient vector of *W. malayi*. The eggs, larvae and pupae are similar to those of *M. annulifera*. These are big, black mosquitos; they are voracious feeders and bite in the open. Evidence is now available that they are strong-winged, enter the houses during the later hours of the night, and probably rest outdoors.

The bionomics of *M. indiana* is probably similar to that of *M. uniformis*. Iyengar²⁹ reported 7 positives out of 215 *M. indiana* collected from *W. malayi* areas of Travancore. More studies on the bionomics of *M. uniformis* and *M. indiana* in India need to be undertaken.

The Reservoir of Infection

The reservoir of infection is the infected person, with circulating microfilariae. The infected persons are called "possible infectors" only when they have a minimum of 12 microfilariae per 20 mm³ of blood, as only such persons are considered to be capable of infecting the vector mosquitos (Basu & Rao, 1939). The difference in individual susceptibility to infection is reflected even in the relative prevalence of species of infection, as shown below, especially in regard to mixed infections. In a village where both *W. bancrofti* and *W. malayi* were prevalent, Brug⁷ observed that the number of mixed infections was greater than would be expected from the laws of probability. In Travancore, Iyengar²⁸ reported 3909 positive among 31 012 persons examined; of these infections, 3487 were due to *W. malayi*, 412 to *W. bancrofti*, and only 64 were mixed. Results of surveys carried out by the Malaria Institute of India⁴³ in a predominantly *W. bancrofti* area (Mattancherry, Travancore-Cochin State) showed that, of 5017 persons

examined, 727 showed microfilariae in their blood. Of these, 666 were affected by *Mf. bancrofti*, 56 by *Mf. malayi*, and 15 were cases of mixed infection. In Shertallai, a predominantly *W. malayi* area in the same state, the same workers reported 1768 persons positive among 8453 examined. Of these cases, 1752 were *Mf. malayi*, 9 *Mf. bancrofti* and 7 mixed infections.

Age, race, sex and occupation are some of the other factors likely to play a role in the epidemiology of filariasis, and a brief reference is made below to available information in respect of each of these factors.

Age

An unconfirmed report of microfilariae in placental blood suggesting congenital transmission was made by Dass (personal communication to Department of Public Health, Bihar and Orissa, 1925).

In India, *W. malayi* infection appears at an earlier age than *W. bancrofti*. Bancroftian infection, however, has been reported in a child aged 27 months (Iyengar²⁸), and Sweet & Pillay⁷⁶ have recorded *W. malayi* infection in an infant below one year and in two infants aged one year.

It is generally accepted that the infection rate rises with age in the earlier years (up to 20 or 30) but not as consistently in the later age groups. This difference is probably due to the fact that some of the persons developing disease become negative for microfilariae.

Sex

Sex does not appear to influence the infection rate. In a limited survey Pattanayak & Nayak⁵⁷ in a *W. bancrofti* area in Orissa, however, reported a ratio of 1.6 : 1 for infection and 2.3 : 1 for disease between males and females.

Race

No racial or tribal characteristics would appear to have any influence on the filarial infection rate or the course or prevalence of the disease. In South India, a certain forest tribe was reported to be immune from *W. malayi*; on an investigation, however, it was found that this could be attributed to the absence of *Pistia* and *Mansonioides* sp. locally (Raghavan, unpublished data).

Occupation

Occupation in itself does not appear to be a factor in the epidemiology of filaria, but some trades or occupations may predispose to lymphostasis in certain parts of the body, for example, washermen in Saidapet, Madras (King et al.³³) or the coir weavers in Travancore-Cochin (Menon⁴⁸), and some of them have superadded filarial infection.

Relationship of infection to terrain

W. bancrofti infections are transmitted by *C. fatigans* and are mostly urban as the breeding conditions preferred by this mosquito are to be found more frequently in towns. The infection has, however, been noted in rural areas also. With reference to the degree of infection in the various parts of such urban areas, Iyengar²⁸ noted that the infection rate in the centre of a town was greatest, tailing off peripherally, whilst in *W. malayi* infection the opposite was noted. Krishnaswamy³⁷ has shown that the degree of maximum infection was closely related to the insanitary conditions prevailing, irrespective of its relation to any part of the town—that is, it was related to the lack of sanitation which was usually directly proportional to the density of population.

In Wuchererian infections, the characteristic disease process easily detectable is elephantiasis of some part of the individual, while lymphangitis and lymphadenitis may also be observed. The influence of various factors on filarial disease are given below.

Species of infection and types of disease process

In addition to lymphangitis, lymphadenitis and lymphoedema of various parts of the body, genito-urinary lesions have also been recorded; the rarity of such lesions (hydrocele, filarial scrotum (or lymph scrotum), or chyluria), however, is the characteristic difference in the disease manifestations between *W. malayi* and *W. bancrofti*. This is brought out in Tables I and II.

Age and elephantiasis

The earliest age at which disease processes have been noted are five years in a *W. bancrofti* area (Krishnaswamy³⁷) and three years in a *W. malayi* area (Iyengar²⁸).

Sex and filarial disease

The incidence of filarial disease in males as compared to females has been studied by many workers. As females are normally less exposed to infective bites, a difference could be expected. Iyengar analysed the incidence in males and females above and below 20 years of age, and noted that in both sexes the incidence was markedly higher in the age group above 20 years of age. Table III sets out such an analysis of the results of a few surveys carried out in India which display a similar difference.

Correlation between filarial infection and filarial disease

In many instances persons with filarial disease are found to be negative for infection, thus raising the question of correlation of filarial disease and

TABLE I. CLASSIFICATION OF FILARIAL DISEASE-PROCESSES IN SOME ENDEMIC COMMUNITIES IN INDIA

Area	Filarial infection prevalent	Number of persons							References	
		examined	with filarial disease	site of disease				other		
				upper extremity	lower extremity	both limbs	genitalia			lymphatic vessels (lymphangitis)
Shertallai (Travancore-Cochin State)	<i>W. malayi</i>	6 404	1 473	9	821	76	5	561	1 (scrotum and hand)	28
Ambalapuzha (Travancore-Cochin State)	<i>W. malayi</i>	3 071	442	7	208	18	1	207	1 (scrotum and leg)	28
Alleppey (Travancore-Cochin State)	Mixed (mainly <i>W. malayi</i> : 88.7% <i>W. malayi</i> : 9.3% <i>W. bancrofti</i> : 2.0% mixed)	18 364	942	12	728	58	6	137	1 (scrotum and leg)	28
Karunagapalli (Travancore-Cochin State)	<i>W. malayi</i>	2 000	68	—	25	—	—	43	—	28
Eraniel (Travancore-Cochin State)	<i>W. bancrofti</i> 86% <i>W. malayi</i> 10% mixed 4%	912	76	4	35	13	15	7	1 (leg and scrotum) 1 (leg, hand and scrotum)	28
Trivandrum (Travancore-Cochin State)	<i>W. bancrofti</i>	31 005	1 025	547			96	380	2 (mammas)	28

TABLE II. CLASSIFICATION OF FILARIAL DISEASE-PROCESSES IN SOME ENDEMIC COMMUNITIES IN INDIA

Area	Filarial infection prevalent	Popula- tion	Number of persons							References	
			examined	with filarial disease	site of disease				other		
					lymphatic vessels (lymph- angitis)	leg	hand	leg and hand			genitalia
Sri Harikotta (Andhra State)	<i>W. malayi</i>	3 900	709	102	—	76	17	7	2 *	—	60, 61
Palacole (W. Godavari District, Andhra State)	Mixed	19 829	5 957	474	—	363	14	—	88	8 **	74
Ernakulam (Travancore- Cochin State)	<i>W. bancrofti</i>	62 283	7 672	245	16	200	24	3	2	—	43
Sherattai Taluk (Travancore- Cochin State)	<i>W. malayi</i>	254 779	8 463	2 429	919	1 300	57	148	5	—	43
Mattancherri (Travancore- Cochin State)	Mixed infec- tions but pre- dominantly <i>W. bancrofti</i>	73 904	5 030	586	199	274	18	30	65	—	43
Cuttack suburbs (Orissa)	<i>W. bancrofti</i>	3 600 (roughly)	1 446	77	—	38	3	12	23	1	Raghavan (un- published data)

* Imported cases

** Chyluria

TABLE III. INCIDENCE OF FILARIAL DISEASE IN MALES AND FEMALES BELOW AND ABOVE 20 YEARS OF AGE

Percentage incidence of filarial disease in				Places surveyed and reference
males		females		
below 20 years of age	above 20 years of age	below 20 years of age	above 20 years of age	
2.8	10.4	1.7	13.8	Trivandrum, Travancore-Cochin State; ²⁸ <i>W. bancrofti</i>
6.1	9.3	5.5	12.7	Mangalore, South India; ²⁷ a gross rate of 11.6 for males and 7.8 for females was noted which was not a statistically significant difference; <i>W. bancrofti</i> area
0.9	5.9	0.9	5.4	Ernakulam, Travancore-Cochin; ⁴³ mainly <i>W. bancrofti</i> area
2.3	8.8	1.24	4.4	Mattancherri, Travancore-Cochin State; ⁴³ mixed infection; predominantly <i>W. bancrofti</i> area
13.9	39.6	18.7	32.8	Shertallai, Travancore-Cochin State; ⁴³ predominantly <i>W. malayi</i> area
2.8	4.9	0.7	8.2	Cuttack suburbs, Orissa. (Raghavan, unpublished data, 1949-50)

filarial infection. These have been reported in *W. bancrofti* cases by Cruickshank, Cunningham & Iyer ¹³ as also by Iyengar, and in *W. malayi* by Raghavan & Krishnan.^{60, 61} Galliard, Mille & Robinson ²¹ in the Pacific, however, found a correlation between filarial infection in persons with filarial disease and the duration of such disease processes. The same analysis should be worked out in India, and epidemiological surveys in clinical as well as elephantoid cases should be carried out, using intradermal or complement fixation tests.

Survey Techniques and Interpretation of Results

Study of the community

For determining the degree of infection, disease and resultant endemicity in a community, surveys carried out so far have been based on discovering the number of persons (a) found positive for microfilariae, (b) with visible elephantoid manifestations, and (c) suffering from, or with a history of, attacks of lymphangitis. Although individually variations have been noted in the numbers of microfilariae in similar quantities of blood obtained from the same person at the same time in *W. bancrofti* and *W. malayi* infections (Bahr; ³ Malaria Institute of India ⁴²), and from the same person on consecu-

tive nights at the same hour (Raghavan, unpublished data), these differences are not of serious consequence for the interpretation of the results.

In trying to determine the possibility of obviating night studies, Korke^{35, 36} studied the microfilarial rate in the same persons by day and by night. The difference was so great that he confirmed the need for night surveys.

Another interesting point is the comparatively low number of microfilariae usually met with in 20-mm³ samples in the Wuchererian infections in India, which is at variance with the observations made, for example, in *W. malayi* infections in Malaya (Wilson⁷⁹) or in *W. bancrofti* infections in East Africa.¹⁶

To avoid missing light infections, since 20 mm³ would mean only a 1/250 000 part of circulating blood, the quantity of blood to be examined could be larger, but it is impossible to obtain more than one large drop of blood from a pricked finger. Although nearly uniform-sized drops can be obtained, it is not practicable to measure out an exact quantity in large-scale surveys. Thus the routine adopted in carrying out filaria surveys is to have as far as possible the same person to prick and obtain nearly the same quantity of blood each time. Such workers are trained in the laboratory for this purpose, and usually the average of 5 drops of uniform size (20 mm³) measured with a tuberculin syringe constitute the amount of blood they could be expected to obtain from persons examined in surveys.

Staining and identification of microfilariae

Iyengar²⁸ used a weak solution of methylene blue (15 mg per litre), stained the smears for two hours and examined them wet. For routine surveys carried out by the Malaria Institute of India, polychrome methylene blue solution, as developed by Jaswant Singh & Bhattacharji³⁰ has been found suitable. The advantages with the latter stain are that it is possible to stain or restain to the required degree, and the same smear could be used for examination of plasmodia by counterstaining with eosin. The usual routine is to lay the air-dried thick smear flat on the staining rack, add a few drops of the stain, and after five minutes pour it off, dry, and examine. Studies are in progress to determine the possibility of loss of microfilariae caused by the staining of unfixed smears in this manner.

To avoid shrinkage caused by preparations of smear or drying, Iyengar²⁸ suggested narcotizing the live microfilariae by placing a crystal of menthol under the coverslip on the slide with the infected blood to which a drop of normal saline has been added. After about 30 minutes the crystal is removed, the blood smeared, dried quickly and stained. Webber & Hawking⁷⁸ used chloroform water for the same purpose. For routine examination, such a procedure does not appear to be needed.

An interesting phenomenon is the ecdysis or ex-sheathing of microfilariae in vitro and in vivo. Abe¹ described two types of ecdysis in

C. fatigans, one by the formation of a cap at the anterior end and the escape of larvae therefrom, and the other by a longitudinal splitting in the middle of the microfilaria followed by larval escape. Jaswant Singh & Raghavan³¹ described similar findings with *Mf. bancrofti* in contact with diethylcarbamazine kept under reduced atmospheric pressure for about 45 minutes and then exposed to the air. Menon & Ramamurti⁴⁹ have described the ecdysis in vitro, as regards adherence to leucocyte-fibrin and larval escape. Slides showing Giemsa-stained *Mf. malayi* obtained from a positive case were received from Wilson⁷⁹ in Malaya. In all slides, the microfilariae were seen lying separated from their sheaths. It is felt that this could be due to the high humidity in Malaya, and larval escape could have happened during the slow drying process. As this article was being prepared, a similar phenomenon has been noted in a few slides from *W. malayi* area in Travancore-Cochin, India. *Mf. bancrofti* and *Mf. malayi* were subjected to moist-chamber treatment before drying and staining, but even when Giemsa stain obtained from Malaya was used the phenomenon was not observed. More studies are needed in this direction.

Identification of the species of microfilariae in positive smears is important, but little difficulty has been experienced in recognizing the Wuchererian infections. An interesting finding is the report of post-microfilarial, pre-larval states of *W. bancrofti*, from a focus of bancroftian infection in Orissa (Pattanayak & Raghavan, 1955, unpublished data), similar to those reported by Faust et al.¹⁸

Enumeration of microfilariae

The usual technique for enumeration of microfilariae on slides is to start at one end of the smear and work down to the other end (breadth of smear), moving the slide by one field of the microscope and repeating the procedure till the smear is covered. The low dry power is used for such purposes. Although a little time-consuming, this technique avoids the use of cells, or other contrivances.

Selection of persons to be examined

Formerly, surveys were carried out in institutions such as gaols; this, however, did not give a representative sample. The present procedure is to select a random, representative sample of the area, and a house-to-house survey is carried out. While bias should be avoided in selecting the houses for the first survey, for purposes of evaluation of control measures the same persons should as far as possible be examined at subsequent surveys.

Size of sample

The size of the sample of population to be examined varies with the type of survey needed (routine survey, or for scientific evaluation), as also with the time and facilities available for carrying out such a programme,

and with the co-operation to be expected from the public. As an instance, Iyengar in his Travancore studies was able to cover in Trivandrum town 32% of the population (96 000) and in Alleppey 42% of a population of 44 000, while in rural areas he was able to cover only 1%-4% of a population. The total number of persons examined in the three years of his work in Travancore was 78 763. The Malaria Institute of India standard is to examine 5%-7% of a community in routine surveys. For purposes of more detailed evaluation, a 20% sample to start with would appear to be sufficient.

In the context of the first phase of the National Filaria Control Programme for purposes of delimiting the extent of filaria in each of the states where filariasis is known to be prevalent, the following working method has been suggested with a view to delimiting the extent of prevalence of filariasis *only* and not the exact degree of infection or disease prevalence. From the results of surveys so far available (unpublished data) and from the work of Acton & Rao,² it appears that endemic areas fall under three types, according to the filarial disease rates. This is, of course, a rough classification.

The first step is to plot on a map of a taluk (a population of about 40 000) the villages or areas where indigenous cases of elephantiasis are reported to be prevalent. A rough idea of the percentage of cases of elephantiasis in these areas is also obtained by inquiries. The first category of prevalence will be areas with elephantiasis rates above 10% (hyperendemic area), the second between 3% and 10% (moderately endemic area), and the third below 3% (low endemic area). For the first category, a 5% filaria survey (blood) should be carried out within the area encompassed and for three miles around. For the second category a 7%-10% sample, and for the third, a 10%-15% sample, should be examined. Wherever there is evidence of possibility of introduced filaria or passive transmission (as by boatmen), and if the area is "silent"—that is, if no cases of indigenous elephantiasis are noted—at least a 20% sample should be examined.

No difference is made between urban and rural areas in regard to the size of the sample for purposes of determining the filaria rate of the area. There is, however, a great need for standardization of both terminology and technique for carrying out surveys and interpretation of results.

Study of the vector

The epidemiologist is interested in the natural infection in vectors and their relation to infection in the human host. The probability of interference by animal filariasis in the interpretation is important. Since mosquitos are the invertebrate hosts of Wuchererian infections in India, procedures for studies of natural and experimental infections must be determined.

While techniques of dissection need no alteration, recording procedures should be clarified and the nomenclature standardized, for difficulties in the

accurate interpretation of developing forms of infection has been a cause of confusion. In studies undertaken at the Malaria Institute of India a stage IV larva in the thorax, head/proboscis, or abdomen, is considered as infective and the site is entered in the records; a definition in this context is needed. The forms used in routine studies by the Malaria Institute of India are reproduced in Annexes 5 and 6 (see page 578).

Many animal filarial infections have been reported in India and their proved or suspected vectors are set out in Annex 4 (see page 577). Raghavan, Misra & Radhagovinda Roy⁶² reported infective larvae in *M. annulifera* and *C. fatigans* in an area in Orissa where bancroftian filariasis was present. As *W. bancrofti* infections are stated to be negative in *M. annulifera* (Iyengar;²⁸ Raghavan & Krishnan^{60, 61}) detailed studies were undertaken. Developing forms were noted in the Malpighian tubes of *M. annulifera*, and the caudal ends of the infective larvae had a single protuberance. *D. repens* infection was recorded in the local dogs; under experimental conditions it developed normally in *M. annulifera*. This emphasizes the need for caution in interpreting the significance of infective forms found in wild-caught mosquitos from a filarioid area.

The utility of study of experimental infections needs no stressing, but there is great need for a "standard" mosquito vector. In this context *A. stephensi* (type), which can be easily colonized and which could be used to distinguish *W. bancrofti* from *W. malayi* infection, holds promise. This mosquito appears to be a better vector of *W. bancrofti* than even *C. fatigans* (Rao & Iyengar⁶⁷). Further, Webber & Hawking⁷⁸ have reported positive infection in *A. stephensi* (type) of *D. repens* and not of *D. immitis*. Russell & Mohan⁷³ have suggested its utility as a gauge to measure the significance of plasmodial infections in nature or experimentally. Plasmodia and *W. bancrofti* can develop in the same *A. stephensi* (type).⁴² Thus *A. stephensi* (type) would appear to be most suitable as a "standard" mosquito vector for plasmodial or filarial infection either individually present or combined, in nature or for experimental work.

Annex 3 (see page 576) summarizes the results of experimental infections of filariae in India. There is, however, a need for standardization to be able to compare results of similar studies by different workers.

Studies in the experimental transmission of *W. bancrofti* are reported by Basu & Rao⁴ which has aroused interest in the question of a threshold of infection. In the context of chemotherapy with potent microfilaricides, such as diethylcarbamazine, there are two views. Hewitt et al.²⁴ claimed beneficial effects in that the drug reduced the microfilariae and maintained them at zero or at levels subinfective to vector mosquitos; they considered that mass therapy of an endemic community was thus beneficial as a method of control of filaria. Rosen⁷⁰ had shown in Tahiti that it was possible to get positive infections in mosquitos even with numbers of less than 10 per 20 mm³ of blood. This led Otto & Jackowsky (personal communication,

1953) to believe that by mass therapy a community with a level of infection unfavourable to mosquitos could be brought to a favourable transmission level. This, however, has not been borne out either by Maldonado and fellow workers⁴⁴ in Puerto Rico or in the Orissa studies (Indian Council of Medical Research²⁷).

Some work on the determination of anthropophilic indices of the vectors has been carried out at the Malaria Institute of India.

Routine methods for carrying out a filaria survey are summarized in Annex 7 (see page 579).

Climate and filariae

Climate has its effect on the vector, the vertebrate host, the parasite in either of them, and consequently on the endemicity of the disease in the area. The optimum temperature for breeding conditions for the vectors and for the development of parasites are stated to be a relative humidity above 60% and a temperature between 60° F and 90° F (15.5°-32.2°C) (Basu & Rao⁴), but retarded development is possible within limits on either side of the optimum range. Further, the existence of a microclimate as opposed to the above macroclimate has to be remembered. In addition, physical factors such as rainfall have to be kept in mind. Thus factors which may be favourable to breeding conditions for the vector may not favour the development of the parasites, and vice versa. This has been brought out by Rao & Iyengar⁶⁶ in their Calcutta studies where the maximum infection was noted during the monsoon period, which naturally would be a period of minimum density because of the flushing of breeding-places. Also in Calcutta, Knowles & Basu³⁴ have brought out the lack of correlation between optimum breeding conditions for mosquitos and development of the parasites in them during the winter months, resulting in a low endemicity. As for the parasites in the vertebrate host, Rao & Sukhatme⁶⁹ have shown a positive correlation between increased incidence of filarial lymphangitic attacks and the end of the monsoon. It is well known that when persons with filarial disease proceed to a cold climate, their troubles abate and in course of time even cease. Another such example is of lymphorrhoea patients who feel better in winter.

The endemicity of a place depends on the quantum of infection. Thus a place nearer the sea coast, as also the equator, will have favourable breeding and transmission conditions for the greater part of the year. India extends northwards to 37° latitude and in the south is separated from the equator by only 10°. Thus one would naturally expect the south of India and the coastal areas to have higher endemicity, subject, perhaps, to any special local conditions. Acton & Rao² have succinctly summarized the position regarding the degree of endemicity, noting the type of lesions, age incidence, infection and disease rates prevalent in various areas. They have based their results on studies of a few important towns, for example,

Cuttack and Puri (Orissa), Calcutta (West Bengal), Purulia (Bihar), Allahabad (Uttar Pradesh) and Cochin (Travancore-Cochin). From the available meteorological data, they classified three types of area in India according to the extent of period in the year for which favourable climatic conditions prevailed, and correlated them with other factors which are summarized in Table IV.

TABLE IV. DIFFERENT CHARACTERISTICS IN RELATION TO DEGREES OF FILARIAL ENDEMICITY

Characteristic	Low endemic area	High endemic area	Hyperendemic area
Period in the year for which favourable climatic conditions prevail	Four months or below	Four to six months	Above six months
Infection rate	Below 10%	10% to 20%	Above 20%
Site of blockage of lymphatic glands	Juxta-aortic group	Deep inguinal	Epitrochlear and inguinal
Age incidence of disease processes	20-30 years	14-16 years	8-10 years

Variations in the intensity of lesions produced by *W. bancrofti* are dependent on the degree of infection and the presence or otherwise of bacterial invasion of tissues by cocci (Acton & Rao ²). From the studies in Travancore (predominantly *W. malayi* area) Iyengar ²⁸ has classified the degrees of endemicity in relation to infection rate as follows:

- (a) areas with low endemicity (1%-5%)
- (b) areas with moderate endemicity (6%-20%)
- (c) areas with high endemicity (21%-30%)
- (d) hyperendemic areas (over 30%)

Thus there is a need for standardization of the degree and types of endemicity.

Diagnosis of filariasis

There are several stages in the development of filarial infections in the vertebrate host, and the earliest stage of invasion is the most difficult to diagnose. The second stage, the "symptomless" carrier state with circulating microfilaria, can usually be relatively easily determined by obtaining blood from a pricked finger at night or from the vein by day. The acute stage presents no difficulty in diagnosis. The "adhesion phenomenon" of Pandit, Pandit & Iyer ⁵² or complement fixation tests (Lloyd & Chandra ⁴⁰) are useful in diagnosing the chronic stage.

Early filariasis can be detected by the triad of lymphadenopathy, marked eosinophilia and a positive intradermal test. Each of these, and especially the first two, have, however, many snags in their interpretation. Since 1949, Pandit, Venkatraman & Chinnaswamy⁵⁶ have been studying the utility of the antigen of *C. guindiensis* in the early diagnosis of filariasis in a *W. bancrofti* area, and detailed studies of the group-specific effect of the antigen in Ascaris-positive persons in a non-endemic area for filariasis are also in hand (Indian Council of Medical Research²⁷). At present it can only be said that the antigen holds some promise as an aid in early diagnosis of filariasis. Its utility in this direction will also be studied in areas where infection by *W. malayi* is present.

Details of preparation and mode of testing, etc., are set out in the reports of the King Institute of Preventive Medicine, Guindy, Madras.⁵⁶

Wanhill⁷⁷ stated that an attack of malaria acted as a deterrent to filaria. It has now been conclusively proved that there is no antagonism between malaria and filaria which might prevent them from co-existing in the same place, the same person or the same vector (Iyengar;²⁷ Raghavan & Krishnan;^{60, 61} Russell & Jacob⁷²). From available data there appears to be no reason why filariasis and any other infection, be it coccal, bacterial, viral or other, should not be concomitant.

RÉSUMÉ

La filariose est connue dans l'Inde depuis le VI^e siècle av. J.-C. Pourtant les recherches sur sa répartition générale n'ont été entreprises que récemment. Les premiers résultats de cette enquête sont publiés dans cet article.

On rencontre dans l'Inde deux types de filaires: *Wuchereria bancrofti* et *W. malayi*. La première est la plus répandue et la plus anciennement connue. La seconde n'a été signalée que depuis 1929 et dans quelques foyers seulement. Les microfilaires présentent la périodicité nocturne typique de ces espèces. Il est donc indispensable, pour dépister l'infection, de procéder aux enquêtes de nuit. Divers schémas horaires de prélèvement du sang sont proposés, suivant la périodicité des microfilaires, qui est probablement sous la dépendance du rythme du sommeil de l'hôte et de l'heure de piqûre du vecteur. Cette périodicité paraît orientée de façon qu'elle assure les meilleures conditions de survie de l'espèce et de transmission de l'infection. Les modalités de cette périodicité dans l'Inde offrent encore un vaste champ d'étude.

Le vecteur le plus fréquent et le plus répandu de *W. bancrofti* est *Culex fatigans*. Certains anophèles sont vecteurs de *W. bancrofti*, mais aucun *Aedes* n'a été trouvé infecté. Deux espèces de *Mansonia* sont les vecteurs principaux de *W. malayi*.

L'individu infecté est le réservoir de l'infection. Il n'est considéré comme virtuellement infectant que si 20 mm³ de son sang contiennent au moins 12 microfilaires. Outre la sensibilité individuelle, variable selon l'espèce de filaire, l'âge et le genre de vie jouent un rôle dans l'épidémiologie de la filariose. Il semble que dans l'Inde l'infection à *W. malayi* soit la plus précoce. On l'a observée chez des nourrissons et l'éléphantiasis à *malayi* a été rencontré chez des enfants de 3 ans.

Les infections à *bancrofti* sont surtout urbaines et d'autant plus intenses que les conditions d'hygiène sont moins bonnes.

L'auteur discute les techniques adoptées dans l'enquête: quantité de sang et heure du prélèvement, coloration et détermination des microfilaries, numération, choix des sujets à examiner et importance de l'échantillon de population. L'étude du vecteur introduit la question du seuil d'infection, qui est encore controversée. Certains auteurs, à l'encontre d'autres, admettent que les microfilaricides puissants, tels que la diéthylcarbamazine, réduisent le nombre des microfilaries à un niveau subinfectant pour le moustique vecteur et que la chimiothérapie de masse serait ainsi une bonne méthode de lutte.

Le climat et le microclimat influent sur l'endémicité filarienne en affectant le parasite dès la ponte par le moustique et au cours de son développement chez le vecteur et l'hôte humain.

Après avoir énuméré les moyens de diagnostiquer la filariose — et les difficultés rencontrées — aux diverses étapes de la maladie, l'auteur mentionne qu'une crise de paludisme peut révéler une filariose. Il n'y a aucun antagonisme entre les deux parasites, qui peuvent coexister dans un même lieu, chez le même vecteur et le même homme.

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Annex 1
RESULTS OF SOME FILARIA SURVEYS CARRIED OUT IN INDIA

Place surveyed	Infection rate (%)	Disease rate (%)	Endemicity rate (%)	Species of infection	Authority
Surat, Bombay	12.4	3.6	16.0	<i>W. bancrofti</i>	Director of Public Health Services, Bombay (personal communication, 1961)
Porbandar, Saurashtra	10.1	6.8	16.9	<i>W. bancrofti</i>	Director of Health Services, Saurashtra (personal communication, 1955)
Other port towns in Saurashtra	11.0-20.4	0-9.6	11-30	<i>W. bancrofti</i>	Director of Health Services, Vindhya Pradesh (personal communication, 1955)
Satna (Vindhya Pradesh)	13.8	3.4	17.1	<i>W. bancrofti</i>	35, 36
Bihar (parts)	14.0	15.0	29.0	<i>W. bancrofti</i>	71
Puri, Orissa	27.3	22.6	49.9	<i>W. bancrofti</i>	33
Saidapet, Madras	16.3	6.6	22.9	<i>W. bancrofti</i>	14
Cochin, Travancore-Cochin	20.9	12.9	32.9	<i>W. bancrofti</i>	43
Ernakulam, Travancore-Cochin	7.7	3.5	11.2	<i>W. bancrofti</i>	
Mattacherri, Travancore-Cochin	16.2	7.6	22.9	<i>W. bancrofti</i>	
Gorakhpur, Uttar Pradesh	16.4	—	16.4	<i>W. bancrofti</i>	
Hyderabad State (Districts of Medak, Karimnagar, Nizamabad and Adilabad)	15.5	0.2-4.9	—	Both infections prevalent; mainly <i>W. bancrofti</i>	Director of Health Services, Uttar Pradesh (personal communication, 1951)
Assam (Binakandy Teagarden)	4.7	4.5	—	Both; mainly <i>W. malayi</i>	64
Cachar District	14.9	7.95	22.8	Mainly <i>W. bancrofti</i>	74
Palacole, W. Godavari District, Andhra State	15.6	5.1	20.5	Both <i>W. bancrofti</i> and <i>W. malayi</i>	28
Alleppey (Travancore-Cochin State)	18.9	3.8	21.6	<i>Mf. malayi</i>	64
Assam (Robipur)	17.8	2.2	15.5	<i>Mf. malayi</i>	63
Patnagarh (Orissa)	13.3	3.4-14.4	6.0-32.0	<i>Mf. malayi</i>	65
Dhanda (Madhya Pradesh)	16.19	3.3	13.6	<i>W. bancrofti</i>	28
Travancore (parts)	10.5	—	—	<i>W. bancrofti</i>	28
Trivandrum (Travancore-Cochin State)	5-12	—	—	<i>W. bancrofti</i>	Director of Health Services, West Bengal (personal communication, 1951)
Bengal (parts)	30.2-31.5	0.5-20.0	0.5-30.0	<i>W. bancrofti</i>	Director of Public Health, Madras (personal communication, 1951)
Madras State (parts of Tanjore, Trichy, Chingleput, Rammad, South Arcot, South Canara and Malabar Districts)	0.3-29.5	0.2-12.5	0.14-35.5	<i>W. bancrofti</i>	Director of Public Health, Madras (personal communication, 1951)
Andhra State (parts of East and West Godavari, Kistna, Vizag, Sri-kakulam and Guntur Districts)	16.4	5.4	21.9	<i>W. bancrofti</i>	Raghavan (unpublished data)

Annex 2

**NATURAL INFECTION OF *W. BANCROFTI* AND *W. MALAYI*
IN MOSQUITOS RECORDED IN INDIA**

Species of mosquito reported infected	Infection rate (%)	Area where reported	Authority
<i>W. bancrofti</i>			
<i>C. fatigans</i>	20.9	Cochin	14
<i>C. fatigans</i>		Saidapet, Madras	33
<i>C. fatigans</i>	15.0-30.4	Bihar	35
<i>C. fatigans</i>	6.5-16.9	Saurashtra	Director of Health Services, Saurashtra (personal communication, 1952)
<i>C. fatigans</i>	14.6	Saurashtra	58
<i>C. fatigans</i>	16.0-31.5 (infective stages 0.6-6.4)	Madras State (Districts of Tanjore, South Arcot, Trichinopoly, Ramnad, Malabar, South Canora)	Director of Public Health Services, Madras (personal communication, 1951)
<i>C. fatigans</i>	0.7-27.2	Chittoor, Nellore, and West and East Godawari, District of Andhra State, Guntur, Krishna, North Arcot, Vizag, Srikakulam	Director of Public Health Services, Madras (personal communication, 1951)
<i>C. fatigans</i>		Orissa State, Puri, Cuttack, Balasore	Director of Public Health Services, Orissa (personal communication, 1955)
<i>C. fatigans</i>	0.7	Vindhya Pradesh	Director of Public Health Services, Vindhya Pradesh (personal communication, 1955)
<i>C. fatigans</i>	1.8 (infective) 8.0 (all forms)	Surat, Bombay	Director of Public Health Services, Bombay (personal communication, 1951)
<i>C. fatigans</i>	15.6	Madhya Pradesh	Director of Public Health Services, Madhya Pradesh (personal communication, 1955)
<i>C. fatigans</i>	1.9-6.1 (infective forms 3.1; others 6.7)	Mangalore, Madras; Ernakulum, Mattancherri (Travancore-Cochin State)	37, 43

**NATURAL INFECTION OF *W. BANCROFTI* AND *W. MALAYI*
IN MOSQUITOS RECORDED IN INDIA (continued)**

Species of mosquito reported infected	Infection rate (%)	Area where reported	Authority
<i>W. malayi</i>			
<i>M. annulifera</i>	20.9	Patnagarh, Orissa State	63
<i>M. annulifera</i>	13.3	Palacole, Andhra	74
<i>M. annulifera</i>	19.2	Travancore	28
<i>M. uniformis</i>	6.5	"	28
<i>M. indiana</i>	3.3	"	28
<i>Culicomyia pallidothorax</i>	0.9	"	28
<i>C. gelidus</i>	0.1	"	28
<i>C. vishnui</i>	0.2	"	28
<i>C. bitaeniorhynchus</i>	0.3	"	28
<i>Lutziafuscanus</i>	0.6	"	28
<i>Armegeres obturbans</i>	2.5	"	28
<i>A. varuna</i>	1.25	"	28
<i>A. subpictus</i>	0.2	"	28
<i>A. barbirostris</i>	1.7	"	28
<i>M. annulifera</i>	14.3	Tiruvaiyar, Tanjore District, Madras State	Director of Public Health Services, Madras (personal communication, 1951)
<i>Mansonioides</i> sp.	14.2	Fort Cochin, Madras State	Director of Public Health Services, Madras (personal communication, 1951)
<i>M. annulifera</i>	12.0	Sriharikotta, Nellore District, Madras State	Director of Public Health Services, Madras (personal communication, 1951)
<i>M. annulifera</i>	24.4	Sriharikotta, Nellore District, Madras State	60, 61

Annex 3

EXPERIMENTAL INFECTIONS OF *W. BANCROFTI* AND *W. MALAYI*
IN MOSQUITOS IN INDIA

Species of mosquito	Infection rate (%)	Place where recorded	Authority	
		<i>Mf. bancrofti</i>		
<i>C. fatigans</i>	48.8	Madras	Malaria Institute of India (unpublished data)	
<i>C. fatigans</i>	69.9	}		
<i>C. vishnui</i>	25.00			
<i>A. barbirostris</i>	40.0			
<i>A. hyrcanus</i> var. <i>nigerrimus</i>	55.5			
<i>A. subpictus</i> (saline)	76.2			
<i>A. subpictus</i> (freshwater)	72.4			
<i>A. ludlowi</i>	69.8			
<i>A. stephensi</i>	92.3			Calcutta, Bengal
<i>A. fuliginus</i>	70.0			}
<i>A. pseudojames</i>	40.0			
<i>A. varuna</i>	84.2			
<i>A. pallidus</i>	66.6			
<i>A. philippinensis</i>	100.0			
<i>Aedes aegypti</i>	6.0			
<i>A. stephensi</i> (type)	86.7	Madras		
<i>C. gelidus</i>	0.1	}	Malaria Institute of India (unpublished data)	
<i>C. vishnui</i>	0.1			
<i>C. sitiens</i>	0.1			
<i>C. bitaeniorhynchus</i>	0.3			Travancore
<i>A. vagus</i>	0.2			}
<i>A. subpictus</i>	0.1			
<i>A. varuna</i>	12.5			
		<i>Mf. malayi</i>		
<i>M. annulifera</i>	26.9	Travancore-Cochin	28	
<i>M. annulifera</i>	62.8	Sriharikotta, Nellore District, Madras State	60, 61	
<i>M. uniformis</i>	66.7	}		
<i>C. gelidus</i>	0.1			
<i>C. sitiens</i>	0.1			
<i>A. hyrcanus</i> var. <i>nigerrimus</i>	1.25			Travancore
<i>A. subpictus</i>	0.2			
<i>Armigeres obturbans</i>	12.5)	28	

Annex 4
SOME ANIMAL FILARIAL INFECTIONS RECORDED IN INDIA AND THEIR VECTORS

Class	Animal	Filarial infection	Locality where recorded	Authority	Vector	Authority
Reptilia	Garden lizard (<i>Crotalus versicolor</i>)	<i>Conspiciculum guindensis</i>	Guindy, Madras	53	<i>C. fatigans</i> (experimentally)	55
Aves	Crow Drongo (<i>Dissemurus paradoxus</i>) (Corvus splendens)	<i>Chandlerella bosei</i>	Calcutta Zoo Saidapet, Madras State Poona, Bombay	9, 53, 54, 26	<i>C. fatigans</i> mites	—
	Fowl	Undetermined micro-filariae	Madras State	Ramanujachari & V. S. Alwar (personal communication, 1954)	—	—
	Grey Partridge (<i>Francolinus pondicerianus</i>)	<i>Aproctoides lissum</i> Chandler, 1929	Near Meerut, Uttar Pradesh	Raghavan & David (unpublished data, 1955)	<i>C. fatigans</i> (experimentally)	59
	White-throated Munia (<i>Munia articalia</i>)	Microfilariae of <i>Diplo-triaenia dubis</i>	Calcutta	15	—	—
Mammalia	Dog	<i>D. immitis</i>	Bengal, Punjab, Madras, Uttar Pradesh, Orissa	5	mosquitos (<i>C. fatigans</i>) (also experimentally)	5
	Dog	<i>D. repens</i>	Bihar, Orissa	5	<i>Aedes aegypti</i> (experimentally) <i>Mansonioides annulifera</i> (in nature and experimentally)	5 62
	Dog	<i>Microfilariae lewisii</i>	Karnal, Punjab	5	—	—
	Dog	<i>Thelazia callipaeda</i>	Salem, Madras	20	—	—
	Cattle	<i>Setaria labiatopapillosa</i> <i>Onchocerca reticulata</i> <i>O. gibsoni</i>	Madras, Uttar Pradesh, Orissa	5	mosquitos	—
	Horse	<i>Stephanofilaria</i> <i>Setaria equina</i> <i>Onchocerca Cervicalis</i>	Assam, Orissa, Andhra Madras, Uttar Pradesh, Orissa	51 51	mosquitos	— —
	Monkey (hanuman)	Unidentified micro-filariae	Mukteswar, Uttar Pradesh	H. N. Ray (personal communication, 1952)	—	—

In addition Chandler (1929) had described a number of adult worms including *Aproctoides lissum* and microfilariae from many birds in Calcutta Zoo (for details, see Chandler^{9, 10}). He found *Microfilaria cephalocauda* and *colubroides* in the same bird with the adult worms, and thought they might be the corresponding larvae; however, this has not been corroborated by Pandit et al.¹¹ No vector has been found for these microfilariae. Microfilarial infection was noted in a quail collected near Delhi; ¹² the infection, however, was refractory in *C. fatigans*.

Annex 5

SAMPLE RECORD OF DISSECTION OF MOSQUITOS

Table No.	Place of collection	Species	Number dissected, etc.	Head		Thorax				Abdomen				Total number of infective larvae	Malpighian tube				Remarks		
				3	4	1	2	3	4	1	2	3	4		1	2	3	4			

Please show the number of microfilariae against each column.
 1 = Sheathless (thin, short) 2 = Sausage forms (thick, short) 3 = Pre-infective larvae (thick, long)
 4 = Infective larvae (thin, long)

Annex 6

PROFORMA FOR FEEDING EXPERIMENTS

Date of feed _____ Number fed _____ Species _____

Temperature (while feeding) _____ Relative humidity (while feeding) _____

Name of subject _____

Number of microfilaria per _____ mm³

(Average of three smears of 20 mm³ each)

DISSECTION

Date	Number dissected	Abdomen	Thorax	Head

Infective form only
 Percentage with head/proboscis infection
 Percentage with infection in other sites

All forms
 Percentage of infection

Annex 7

HOW TO MAKE A FILARIA SURVEY

1. The persons to be examined should be a random representative sample of the area; all persons in the houses should be examined.
 2. Since the filariae show a nocturnal periodicity, examinations should be made only at night-time between 8.30 p.m. and midnight.
 3. Technique: approximately equivalent quantities (20 mm³) of blood should be examined from each person; length of residence, history of fever, lymphangitis, and signs of disease should be noted.
 4. When persons are examined for the survey, the following points should be covered:
 - (a) *Filarial infection rate* : Percentage of those examined who show microfilariae in their peripheral circulation; one slide should be prepared for each person. In places where malaria co-exists, a search for malaria parasites should also be made.
 - (b) *Filarial disease rate* : Percentage of those examined who show signs of filarial disease; each person should be examined once.
 - (c) *Filarial endemicity rate* : Percentage of those examined who show microfilariae in their peripheral circulation, or disease manifestations, or both.
 - (d) *Average infestation rate* : Average number of microfilariae per slide among the positive slides, each slide being made up from 20 mm³ of blood. The rate is expressed as 'x' microfilariae per 20 mm³.
 - (e) *Species of microfilaria*.
 5. A survey of the vectors should include:
 - (a) Observation of types of breeding-place
 - (b) Collection of larvae
 - (c) Observation of aquatic flora
 - (d) Collection and dissection of adult vectors (Watch should be kept for malarial infections, especially in areas where both diseases co-exist.)
 - (e) Precipitin tests.
 6. Meteorological data should be noted.
 7. Vital statistics should be recorded.
 8. Periodicity of local filariae should be observed.
 9. Infection experiments should be carried out if required
 10. Filariae in locally prevalent animals and birds should be noted.
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