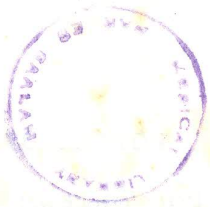
STUDIES ON THE PREVALENCE OF HUMAN ONCHOCERCIASIS AND EXPERIENCE WITH SERODIAGNOSIS OF THE DISEASE IN TANZANIA

By



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DEDICATION

To my father mzee Stefano Mwaiko and my mother staff nurse Rhoda Ma-msenga of Magila, Muheza; and family.

D E C L A R A T I O N

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....10/9/92.....

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A C K N O W L E D G E M E N T S

First the author wishes to say that these studies were carried out when he was a research scientist for the National Institute for Medical Research, Amani Centre, Tanzania. He is grateful to Mr. P. Wegesa, the former Director of the Amani Centre when it was formerly known as East African Institute of Malaria and Vector-Borne Diseases Amani, for initiating the research study.

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A B S T R A C T

Prevalence of human onchocerciasis in Tanzania is described. The prevalence is high in Bwakira (63.6%), Mahenge (58.6%) and Ruvuma (31.9%); and low in Amani (22.4%) when compared to that reported in 1970. The increase and decrease on the prevalence indicate that the disease transmission has been increasing or decreasing in the respective foci. It is speculated that bush-clearing and deforestation in general, had positive effects on the transmission of the disease where Simulium damnosum complex is a vector and the negative effects with Simulium neavei complex.

In this study, Indirect Haemagglutination Antibody (IHA) test was also established for serodiagnosis of onchocerciasis, using crude heterologous antigen of adult Onchocerca gutturosa. With this antigen, IHA titres (serum dilutions) of 1/320 and above were used as positive for onchocerciasis because they discriminated cross-reaction effects due to other helminths and under these circumstances, the IHA test was considered to be sufficiently valid for the diagnosis of the disease in Tanzania.

The established IHA test for diagnosing onchocerciasis was found to be complementary to the skin snip and clinical diagnostic methods. High sensitivity of the IHA test was found in Amani (93.7%), Bwakira (89.7%), Kilosa (81.8%) and

and Ruvuma (75.0%) foci. However, the IHA test was complementary to the parasitological diagnosis in assessing age disribution of the disease. There was significant association between IHA response and parasitological diagnosis, and skin changes due to onchocerciasis in certain foci; indicating a well established balance between the O.volvulus parasite and the host, and also emphasizing the importance of IHA test in ascertaining the disease in these people with skin changes due to disease.

A report on the development of O.gutturosa to infective stage in Simulium vorax Pomeroy is presented (Appendix D4). A more descriptive report on O.gutturosa in Tanzania cattle is presented (Appendix D3). This second report highlights the importance of continuing checking for O.gutturosa in cattle in all areas where the presence of O.volvulus has been established, because there is evidence that S.vorax which transmits O.gutturosa in cattle is also a potential vector of O.volvulus. Raising infective stage larvae of O.gutturosa in S.vorax for antigen was attempted. But the average number (2.3) of the microfilariae per fly, taken during the infective blood-meal from a cow infected with the parasite, was considered to be quite small for sufficient recovery to provide sufficient amount of the infective stage larvae for antigen.

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D. List of Publications

1. Mwaiko, G.L., Mtoi, R.S, and Mkufya, A.R, (1990). Onchocerciasis Prevalence in Tanzania Centr.Afr.J.Med. 36, 94-96.
2. Mwaiko, G.L. (1980). Indirect Fluorescent Antibody Test for diagnosis of Onchocerciasis in Tanzania. Dar.med. J. 8: 13-15.
3. Mwaiko, G.L. (1979). ONCHOCERCA GUTTUROSA IN TANZANIA CATTLE: Its prevalence and distribution in North-Eastern Tanzania. Tanz.vet.Bull., 1: 8-12.
4. Mwaiko, G.L. (1981). The development of Onchocerca gutturosa Neuman to infective stage in Simulium vorax pomeroy. Tropenmed.Parasit., 32: 276-277.
5. Mwaiko, G.L. and Mkufya, A. (1977) Demonstration of Antibodies in people infected with Onchocerca volvulus using Adult Ohchocerca gutturosa Antigen. E.Afr.med.J. 54: 681-683.

1. INTRODUCTION

Onchocerciasis is a disease which is caused by a filarial nematode Onchocerca volvulus Leuckart (Blacklock, 1926a). The O.volvulus is transmitted by black flies of Simulium species (Blacklock, 1926a, 1926b, 1927). The disease then became widely known as river blindness because vectors which are responsible for transmission develop in rivers at some stage of their life cycle.

Male and female adult worms of O.volvulus live in human subcutaneous tissue and the females after fertilization by the male worms, produce millions of microfilariae which then invade the skin and eyes. When a female blackfly bites an infected person, microfilariae are ingested and develop without multiplication into infective stage larvae which invade a new host during a later blood-meal and therefore, completing the life cycle as shown in Plate 1 (Blacklock, 1926a, 1926b).

In the skin of man, O.volvulus then causes pathological changes which are manifested by presence of pruritis, skin depigmentation and atrophy, nodules, hanging groins and eye lesions which may eventually culminate in blindness (Ouzilleau, 1913, 1923; Rodhain, 1916, 1920; Nelson, 1955, 1970; Duke, 1972).

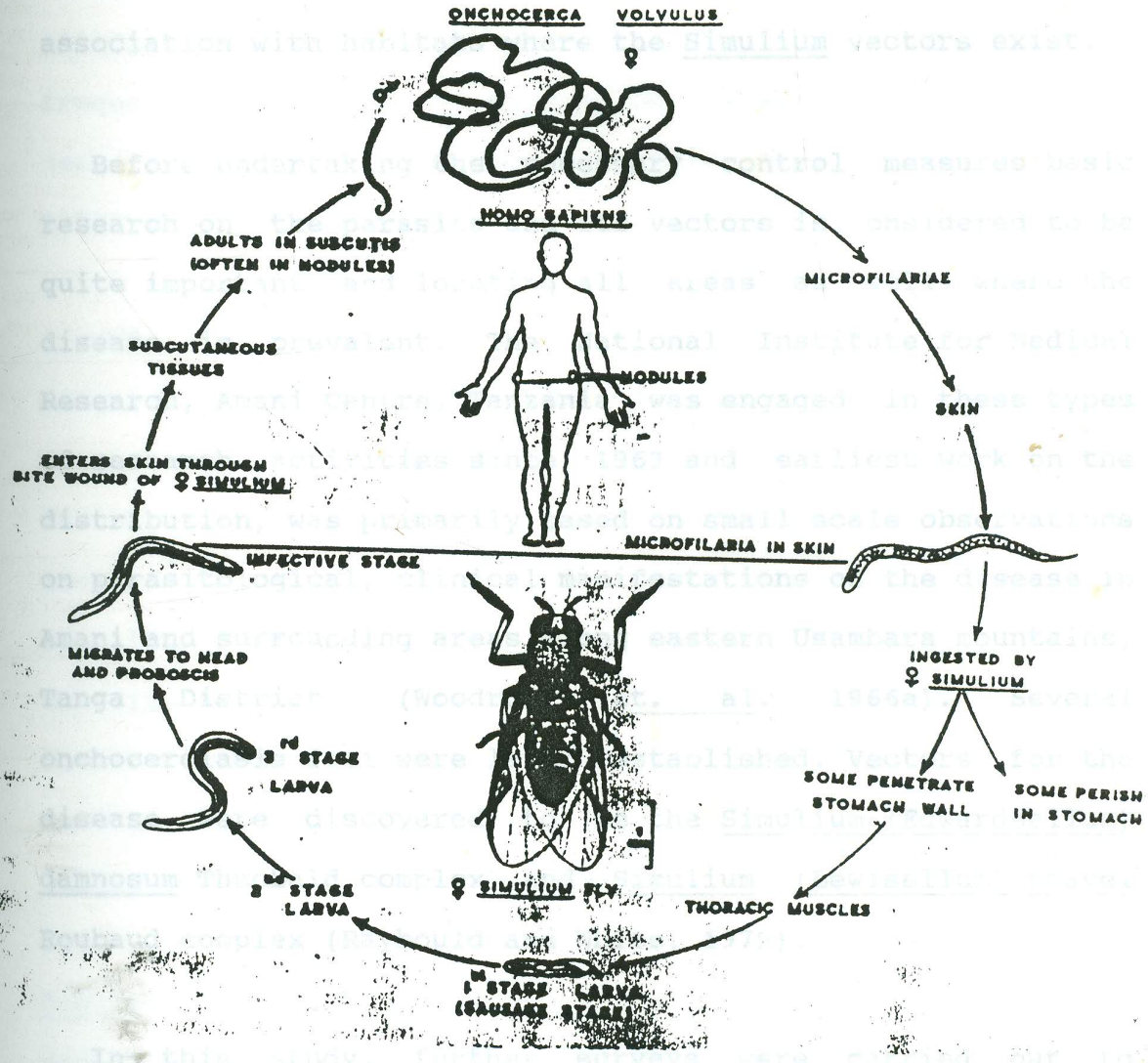


Plate 1. Life cycle of O.volvulus.

Onchocerciasis is endemic mainly in two regions: the tropical zone of Africa and the Central and South America; and a small focus in Yemen (Wegesa, 1969; Nelson, 1970; Sasa, 1976). The disease affects about 20 million people in these zones (Raybould, 1976). Endemicity tends to be focal, in association with habitats where the Simulium vectors exist.

Before undertaking the necessary control measures basic research on the parasite and its vectors is considered to be quite important and locating all areas as well where the disease is prevalent. The National Institute for Medical Research, Amani Centre, Tanzania was engaged in these types of research activities since 1963 and earliest work on the distribution, was primarily based on small scale observations on parasitological, clinical manifestations of the disease in Amani and surrounding areas along eastern Usambara mountains, Tanga District (Woodruff et. al. 1966a). Several onchocerciasis foci were later established. Vectors for the disease were discovered to be the Simulium (Edwardsellum) damnosum Theobald complex and Simulium (Lewisellum) neavei Roubaud complex (Raybould and White, 1979).

In this study, further surveys were carried out to determine the prevalence of onchocerciasis in several parts of Tanzania as part of research needs recommended by WHO (1976) in order to improve knowledge of natural history of the disease generally. Parasitological, clinical and

serological methods were used in studying the prevalence of the disease. Indirect Haemagglutination Antibody (IHA) test was used as a serological method. Antigenic extracts of O.volvulus and O.gutturosa were used in the IHA test and compared. O.gutturosa is a filarial parasite which infects cattle and is closely related to O.volvulus and has been frequently used as a source of heterologous antigen for serodiagnosis of human onchocerciasis (Kagan, 1963; Wegesa, 1968; Ambroise-Thomas, 1974; Bartlett et. al. 1975).

WHO (1983) also realised that there is no suitable diagnostic test which is currently available for accurately diagnosing early prepatent O.volvulus infection. One of the reasons for the absence of the suitable diagnostic test is lack of necessary reagents. Therefore, it was suggested that specific parasite antigens from developmental stages of the parasite as well should be collected and made available for such a purpose. From these suggestions, attempts were made also as part of this study, to obtain the infective stage larvae of O.gutturosa from Simulium vorax Pomeroy black flies which were artificially fed on a cow with a natural O.gutturosa infection. S.vorax was shown to be a potential vector of this parasite species (Appendix 2). The prevalence surveys including all other related experiments were conducted between 1976 and 1983.

II. LITERATURE REVIEW

1. Historical Note

Onchocerca volvulus was first described by the name of Filaria volvulus by Leuckart in 1895 (Prout, 1901; Brumpt, 1904; Railliet and Henry, 1910; Fulleborn , 1911) from specimens which were obtained from nodules under the skin of a native in the Gold Coast (Ghana). This filarial species was placed by Railliet and Henry in (1910) into the genus Onchocerca Diesing, 1841, together with other species parasitic in animals; the genus Onchocerca was created by Diesing, 1941, with O.reticulata as the type species, which is the parasite of the horse, donkey and a mule in Europe. Yamaguti (1961) used an amended name Onchocerca Creplin, 1884, for Onchocerca Diesing.

In the Americas, the occurrence of human onchocerciasis was noted first by Robles (1919), in Guatemala; both Pacheco-luna (1918) and Robles (1919) described the eye lesions due to onchocercal infection; Brumpt (1919) studied the material sent by Robles (1919) which consisted of one male and fragments of the two female worms, and named the parasite as Onchocerca caecutiens because he considered that the specimens differed from the African form in the distribution of male papillae and in the larger size of spicules. Subsquent studies by Fulleborn (1929) and Sandground (1934)

showed that there existed no consistent differences in the American and African forms, thus O.caecutiens was probably a synonym of O.volvulus.

A black fly species in Africa, Simulium damnosum was found to be an efficient vector of O.volvulus by Blacklock (1926a, 1926b), in experimental infection studies with wild-caught flies. In Guatemala (Nelson, 1970; Sasa, 1976) larval development of O.volvulus was discovered to take place in the following species of the black flies: Simulium ochraceum, Simulium metallicum and Simulium callidum.

Ruiz Reyes (1952) presented the idea that onchocerciasis was brought to tropical America and became established when Negros were imported from Africa to cultivate sugar cane and coffee. On the other hand, studies by De Leon and Duke (1966), suggested that the Central American and African parasites are at least distinct physiological races in view of their compatibility with the Simulium intermediate hosts.

Onchocerciasis in Tanzania was first reported in the south, by Fischer in 1932 (Hawking, 1940) in indigenous people of Itete and Matema in Ulanga District. Hawking (1940) continued examining as many people as possible in the country, and the places he visited were Chunya, Mbeya, Tukuyu, Ilembula Njombe, Uwemba, Malangali, Iringa, Bukoba and Kigoma. 3 out of 39 people he examined in Tukuyu, were

found infected. Other hospital cases were reported from Mahenge in Morogoro region, and the Ruondo Island in the lake Victoria by Young in 1946 (Gabathuler and Gabathuler, 1947), and subsequently followed by an epidemiological survey in the same area by Gabathuler and Gabathuler (1947). 26 years later, a new onchocerciasis focus was discovered in Amani area of the eastern Usambara mountains in Tanga District by Giaquinto in 1957 (Woodman, 1958).

2. Distribution of Onchocerciasis

a) General Distribution in the World

According to Nelson (1955, 1970), Gratama, S. (1966) Wegesa and Raybould (1969), Abaru (1976a), Raybould and White (1979), onchocerciasis occurs in rainforest regions and savannah belt of tropical Africa which stretches from coast of Senegal to the Indian Ocean in Tanzania and Malawi, South and Central America, and Yemen in Arabia. In South and Central America the disease is found in Mexico, Guatemala, Venezuela and Colombia. In Africa, onchocerciasis is associated with the main river systems south of Sahara and north of the equator, from Cape Verde right across the continent to Ethiopia. The disease also occurs south of the Equator as far as southerly limit about 10 degrees south in Tanzania, Congo (Kinshasa) and Malawi areas (Wegesa and Raybould, 1969). Areas having a high incidence of blindness

are confined to the savannah belt which runs across Africa generally from 8 degrees east to 12 degrees north and in this category, the following countries are included: eastern Senegal, eastern Guinea, Mali, all countries related to the Volta River Basin (Upper Volta, Ivory Coast, Ghana, Togo and Dahomey), Nigeria, Chad, Congo (Kinshasa), Central African Republic, northern Uganda, Sudan and Ethiopia.

b) General Distribution in Tanzania

A review of early surveys which were concluded in Tanzania between 1940 and 1969 was given by Wegesa (1970a). The surveys led to the establishment of major foci which are Mahenge in Ulanga District; Amani in eastern Usambara mountains, Tanga district; western Usambara mountains Tanga district with highest infection rate of 37.0% in Tewe village; the largest focus south of Tanzania covering two districts of Songea and Mbinga; Bwakira areas centred around in the Uluguru mountains immediately south of Morogoro; and finally, the Nguru mountains about 96 kilometres north of Morogoro. Just before the review had come out, a new focus was discovered in Kilosa district (Wegesa, 1969). 2450 people were examined in all parts of this district and 588 (24.0%) out of these were found infected giving a range of infection rate of 5.0% in Nongwe to 46% in Msowero divisions

By 1971, much of the country had already been covered.

Extreme west of Tanzania however, had not yet been visited. Enroute Wegesa et.al. (1971a), had the opportunity of looking at places such as Masailand, Kondoa and Tabora respectively . Absence of running water near those places for most of the year, made it extremely unlikely that O.volvulus could be transmitted there. A total of 4181 people were examined and 18 (0.43%) were infected. These infected people were not indigenous; they all originated from onchocerciasis area of Morogoro. A preliminary reconnaisance of Shinyanga and Kahama districts gave no evidence for man biting simuliids. The survey of western Tanzania, covered areas extending from Kibondo near Burundi border in the north to the southern tip of lake Tanganyika at the Zambian border in the south. S.damnosum complex was found biting man in the Kigoma, Kasulu and Kibondo districts, but they were not found in Mpanda and Sumbawanga districts further south. A total of 1800 people were skin snipped and none of them was found infected. In the following years more emphasis was made in Kigoma where the man biting S.damnosum complex was found. The concept here was that if the fly was found to support the development of the parasite then it would indicate that there was a possibility of transmission if infected individuals settled in the area. However as much of the western edge of Tanzania is rather isolated from the rest of the main foci in the country, it was concluded that possibly the absence of the disease was due to simply not having been introduced. Earlier investigations (Wegesa, 1966a) showed that O.volvulus

microfilariae can develop to infective stage in Simulium woodi at 23 degrees centigrade - 26 degrees centigrade and therefore the fly species was incriminated as a vector. Since the temperatures in Kigoma were found to fluctuate between 19.1 degrees centigrade and 28.3 degrees centigrade, it was then felt desirable to test the vectorial capacity of the S.damnosum complex in the region. Thus a male volunteer infected with O.volvulus from Amani accompanied a research team to this Kigoma region (Owaga, 1974-75). There he was exposed to Simulium bites. The mean temperatures during that time of study was 23.5 degrees centigrade, within the ranges known to be favourable for the development of O.volvulus in S.woodi. Microfilariae of this parasite species were picked up by this S.damnosum complex species, but unfortunately, the developmental stages of these parasites could not be followed up because the flies survived for not more than 24 hours under the experimental conditions. Further surveys by Bogh in 1978 (Pedersen and Kolstrup, 1982) revealed the presence of onchocerciasis in indigenous people in this region. 580 people were examined in Bitale, Kalinzi, Mahehe, Mukongoro and Manyovu villages, and 17 (2.9%) were found infected. The range of infection was betwen 0.6% at Kalinzi and 8.7% in Mukongoro. These villages were situated at an average altitude between 502-1003 m. above sea level.

Tunduru, the third district in the southern region of Tanzania was not included in the surveys (Wegesa, 1968a, 1969

and 1970a) because transmission could hardly extend interior as most of the district lies below 600 m above sea level. 74 people however, were examined at Masasi dispensary far east in Masasi district and 2 (2,7%) non-indigenous people were found infected (Wegesa, 1972). Observations were made in the north of Songea and Mbinga districts to find out how far this focus of the south extended and therefore, 197 people were examined at Kyela and Isoko in Rungwe districts and 2 (1.01%) were found infected. 223 people were examined at Wino, a little north of Ruvuma and 23 (10.3%) out of these had onchocerciasis. Raybould (1976) did not find an infected person in 1979 people he examined in Mtera area of Iringa district.

Surveys which were carried out near Njombe township revealed no natural infection; temperatures were found to be low for transmission to take place (Wegesa, 1968c, 1970a). However, onchocerciasis was found present in remote places of Njombe township at Lugalawe, Matema, Igongwe, Itete and Mawengi where 190 people were examined and 8 (4.2%) of them, had the infection (Wegesa, 1972).

Discovery of Tukuyu focus is also historical in nature. Like other foci, Hawking (1940) was the first person to report a few positive cases. Wegesa (1972) later obtaibned an infection rate of 1.9% in 661 people he examined. Granbacka (1973) got an infection rate of 4.4% in 606 people he skin

snipped. Bogh in 1978 (Pedersen and Kolstrup, 1982) reported a much higher infection rate of 28.7% after examining 973 people and the range of infection was between 2.9% at Ipinda division and 56.4% in Lufilyo. Pedersen and Kolstrup (1982) reported highest prevalence of onchocerciasis in a zone 3-6 km. wide along the Lufilyo river and more specifically at Kilungu village where they found an infection rate of 62.8% together with 27 positive cases at Ruhuhu on the shores of Lake Nyasa.

Surveys in the vicinity of Pangani river south of eastern Usambara, did not provide evidence of onchocerciasis transmission (Wegesa et.al. 1971a). A total of 1066 people were examined and 10 (0.94%) only were positive. These positive cases originated from onchocerciasis areas.

Abaru (1976b) obtained an infection rate of 18.4% in 873 people of Maramba, north east of eastern Usambara mountains.

Repeat surveys in certain major foci by Abaru and Van Dam (1972-73) and Bogh in 1978 (Pedersen and Kolstrup, 1982) gave further details on the pattern of the distribution of onchocerciasis. For example, they found that onchocerciasis is more wide spread in Bwakira areas.

Table 1 gives the literature which describes onchocerciasis in Tanzania between 1932 and 1982. This

information on the general distribution of the disease in the country is also given with other details in Appendix **D1**.

3. Vectors of Onchocerciasis

In South and Central America S.callidum, S.metallicum, S.ochraceum, S.gonzales, S.verscruzanum and S.haematobium are vectors of O.volvulus of which the third species is most important (Nelson, 1970; Sasa, 1976).

In Africa O.volvulus is transmitted by man-biting forms S.(Edwardsellum) damnosum Theobald complex and S.(Lewisellum) neavei Roubaud complex (Nelson, 1970; Raybould et.al. 1972; Raybould and White, 1979). S.damnosum which is by far the most important vector, occurs in a wide variety of permanent and temporary streams and rivers in areas ranging from the rain forest to savannah. The larvae and pupae of the most S.neavei complex species are found attached to fresh-water crabs in permanent streams and small rivers, often in highland areas covered with forest and woodland.

In Tanzania, S.damnosum is the main vector with exception of the Usambara and Nguru mountains, where the vector is S.neavei complex; S.woodi de Meillon which is a species of S.neavei complex particularly, is responsible for transmission in both eastern and western Usambara (Hauserman, 1969; Raybould et.al. 1972). With most of the main

Table 1. Literature describing onchocerciasis in Tanzania

Year	Author	Place Place	District	Region	Total examined	Infected in the place		Infected elsewhere	
						No. mf positive	% mf positive	No. mf positive	% mf positive
1932	Fischer	Itete and Matema	Ulanga	Eastern	Observed nodules containing O.volvulus from infected people				
1940	Hawking	Chunya	Mbeya	Mbeya	28	0	0	0	0
		Mbeya, Sumbawanga	Mbeya	Mbeya	26	0	0	0	0
		Tukuyu	Tukuyu	Mbeya	39	3	7.1	0	0
		Ilemba, Njombe, Uwemba	Njombe	Iringa	69	4	5.7	0	0
		Malangali, Mufindi	Mufindi	Iringa	29	1	3.7	0	0
		Iringa	Iringa	Iringa	43	0	0	0	0
		Dodoma	Dodoma	Dodoma	2	0	0	0	0
		Bukoba	Bukoba	West Lake	9	0	0	0	0
		Ufipa (Examined at Kigoma)	Sumbawanga	Mbeya	0	0	0	0	0
		Songea	Songea	Ruvuma	4	0	0	0	0
1947	Gabathuler and Gabathuler	Mahenge Plateau: Mahenge, Vegai, Isonga Upomera, Epanko and Ntololo	Ulanga	Eastern	375	0	0	0	0
		Ulanga Valley: South of the river Kichangani, Iragua, Itete, Mtimbira, Sofi, Ngombo and Malinyi	Ulanga	Eastern	150	0	0	0	0
		Ulanga Valley: North side of the river: Ifakara,							

Table 1 (cont).

		Mageta, Utengule, Taveta and Kilosa kwa Mpepo	Ulanga	Eastern	12	0	0	0	0
		Ruaha and Luhombero: Ruaha, Mbiji, Ilonga and Sali	Ulanga	Eastern	1763	685	38.9	0	0
1957	Giaquinto-Mira	Eastern Usambara: Amani							
1958	Woodman	Eastern Usambara: Amani	Tanga	Tanga	Not fully described		50.0	0	0
1963-1964	Laing and Wegesa	Eastern Usambara:							
		i) Amani Plateau: Ngambo Tea Estate and Kwamkoro Tea Estate (Kwakoro Division, Ngua and Ndole) Amani, Msasa and Mlesa			675	179	26.5	0	0
		ii) Zigi River Valley: Chemka, Kisiwani, Lewa and Bukoba			99	32	32.3	0	0
		iii) Magorotto Palm Oil Estate	Tanga	Tanga	77	24	31.1	0	0
		iv) Foothills: Lunguza, Bombani, Zimbiri, Magila and Kiwanda			276	37	13.4	26	9.5
		v) Sisal Estates: Zigi Mambeni, Mruazi and Lwengera			323	18	5.5	10	3.1
		Western Usambara: Mlalo, Soni, Bumbuli, Herkulu Tea Estate and Korogwe	Tanga	Tanga	366	21	5.7	7	1.9
1965	Geigy et al.	Ulanga Area (Sati Ruaha areas)	Ulanga	Morogoro	281	55	19.6	0	0
1966a	Woodruff et al	Eastern Usambara: Amani and Zigi Valley	Muheza	Tanga	390	109	28.0	0	0

Table 1 (cont).

1967	Wegesa	Songea Hospital, Peramiho Hosp., Namtumbo Health Centre, Gumbiro Health Centre	Songea	Ruvuma	584	30	5.0	0	0
1967	Sprengel	Western Usambara: Tewe and Lunguza	Lushoto	Tanga	271	111	40.9	0	0
1968	Sprengel	Kigonsera Dispensary, Litembo Hosp., Lundo/Ngindo Refugee settlement, Liuli Pr. School, and Nang'ombe Dispensary	Mbinga	Ruvuma	481	90	18.7	0	0
			Songea	Ruvuma	233	23	10.3	0	0
1968	Wegesa	Wino	Mahenge	Morogoro	449	247	54.7	0	0
1969	Hauserman	Mahenge	Kilosa	Morogoro	2450	588	24.0	0	0
1969	Wegesa	Kilosa: Nongwe, Mkobwe, Mandege, Msilibula, Uvidunda Kidodi and Msowero	Njombe	Iringa	331	0	0	4	1.2
		Kilena Hospital	Mahenge	Morogoro	1763	686	29.0	0	0
1970a	Wegesa	Mahenge	Lushoto	Tanga	523	104	19.8	0	0
		Western Usambara: Tewe	Tanga	Tanga	2070	788	38.1	0	0
		Eastern Usambara: Amani	Morogoro	Morogoro	167	14	8.4	0	0
		Nguru Mountains: Gonja, Bombo, Pemba and Ubiri	Morogoro	Morogoro	947	272	29.0	0	0
		Bwakira							
1971	Wegesa	Mbulu Hosp., Oldeani Hosp., Mangola Disp., Oldeani School, Magara, Babati Hosp., and Magugu Dispensary	Mbulu	Arusha	929	0	0	0	0

Table 1 (cont).

		Nyamhongo Pr. School, Kongwa Pr. School, Manungu School, Hogoro School, Sagara Village and Sch., Chamhawi Pr. Sch., Mlaya Sch., Wotta Disp., Lumuma Pr. School, Mlanga School, Kibakwe, Iduo Disp., Masa Disp., Rudi Disp., Mlali Pr. School, Panda mbili Disp., Mgeta Disp., Pembemoto Pr. School, Mlali Community Centre	Mpwapwa	Dodoma	2482	0	0	18	0.7
		Masange Disp., Kikila Disp., Puhi	Kondoa	Dodoma	527	0	0	0	0
1971	Wegesa (cont.)	Katuma Ussuya Dispensary	Mpanda	Tabora	243	0	0	0	0
		Mpanda Hosp., Uriula Disp.	Mpanda	Tabora	532	0	0	0	0
		Sumbawanga Hosp., Kirandu, Milepa and Pombwe Disp.	Mbeya	Mbeya	652	0	0	0	0
		Kigoma Hospital	Kigoma	Kigoma	138	0	0	0	0
		Mayange Ujamaa Village, Simbo Disp., Mwindiga Dispensary	Kasulu	Kigoma	438	0	0	0	0
		Kokonko Health Centre, (Besunza) Ujamaa Village, Mabambe Rural Health Centre	Kibondo	Kigoma	409	0	0	0	0
		Hale Mwelingwambi sisi kwa sisi, Kalekwa, Magungu Hosp., Mgombezi Disp., Mkanyeni, Kwakombo, Hale Disp., Kwa Msisi Disp., Maumi, Magoma, Mswaha, Kiloza, Lwengera Mafulora, Mkanyeni Matungu	Korogwe	Tanga	1066	3	0.3	7	0.7

Table 1 (cont).

1972	Wegesa	Tukuyu Hosp., Suma Disp., Masoko Disp., Mwakaleli, Ipinda Disp., Ifumba Hosp., Bulongwea Hospital	Tukuyu	Mbeya	661	13	1.9	0	0
		Matoma Disp., Lugalawe, Igongwe Hosp., Itete Hospital, Msuda Disp., Mawengi Disp., Liuli Hosp., Lituhi Hospital, Kyela Hosp., Isoko Hospital Kamba Segera	Njombe	Iringa	190	8	4.2	0	0
			Songea	Ruvuma	117	19	16.0	0	0
			Rungwe	Mbeya	197	2	1.0	0	0
			Mbinga	Ruvuma	51	5	9.8	0	0
		Masasi Dispensary	Masasi	Lindi	73	2	2.7	0	0
1872-1973	Abaru and van Dam	Dutumi, Bwakira Chini, Bwakira Juu, Mngazi	Morogoro	Morogoro	479	320	66.7	0	0
	Abaru	Dutumi Sch., Bwakira Chini Sch., Bwakira Juu School and Mngazi School	Morogoro	Morogoro	267	116	43.4	0	0
		Amani School, Zigi School, Marvera and Kwamkoro Schools	Muheza	Tanga	398	86	21.6	0	0
1976b	Abaru	Mhinduro, Mavengero, Bamba Sch., Churwa Sch., Dukani Kisiwani, Bosha Kwamtindi, Bosha Kichangani, Lanzoni Darajani	Muheza	Tanga	875	161	18.4	0	0
1976	Raybould	Mtera, Kinyari, Chapuya, Makonge, Izazi, Migole, Kifusi, Ihani, Chipogoro, Kisima, Kisima School, Chipogoro and Legi Schools	Iringa	Iringa	1079	0	0	1	0.09

Table 1 (cont).

1982	Pedersen and Kolstrup	Makuyuni School, Doma, Mtombozi, Singiza School, Lukunguni Sch., Maharaka, Msongozi School	Morogoro	Morogoro	193	58	30.1	0	0
		Malinyi, Ngombo, Sofi, Majiji, Sofi Mission, Kilosa kwa Mpepo, Mtimbira	Ulanga	Morogoro	441	22	4.9	0	0
		Mbinu, Mngeta, Kinegere, Kisawasawa, Mangula, Mlimba, Kidatu	Kilombero	Morogoro	291	25	8.5	0	0
		Mbinga, Mbambabay,. Liuli, Mkali, Mango, Tambi, Kihangara, Lituhi, Ruanda	Mbinga	Ruvuma	381	60	15.7	0	0
		Lufilyo, Lufilyo School, Mwakaleli, Masoko, Ipinda, Matemba, Isoko and Ikuti	Tukuyu	Mbeya	973	279	28.68	0	0
		Bitale, Kalinzi, Mahehe, Mukongoro and Manyovu	Kigoma	Kigoma	580	17	2.9	0	0
		Sali Ruaha and Isongo	Ulanga	Morogoro	419	309	73.8	0	0
		Kilimanjaro	Moshi	K'manjar0	448	0	0	0	0
		Nguru Mountain	Morogoro	Morogoro	152	0	0	0	0
		Eastern Usambara Kisiwani	Muheza	Tanga	87	44	50.6	0	0
		Dutumi	Morogoro	Morogoro	44	40	90.0	0	0
		Kilugu, Kisege, Kibole, Kifunda, Ngubati, Kipapa, Kambasegela, Mababu, Samu, Masiko, Mbambo, Masululi, Ngeleka, Kasyabona, Ntaba, Matama, Ndobo, Busilya, Kipoke, Isange and Ibungu	Tukuyu	Mbeya	2045	449	22.0	0	0
		Ruhuhu	Tukuyu	Mbeya	27	27	100.0	0	0

transmission areas in which S.damnosum predominates, however, there are one or more localities in which S.neavei complex species also bites man and probably contributes to the transmission to a small extent (Raybould et.al. 1972). The S.damnosum also consists of a complex of sibling species and include both anthropophilic and non-anthropophilic forms (Dunbar, 1966). Other species S.vorax Pomeroy and S.nyasalandicum (Amani form) bite cattle (Raybould et.al. 1972), while S.neavei mainly bites chicken (Raybould, 1967); these species were also found to be potential vectors of O.volvulus in the eastern Usambara (Wegesa, 1967b, 1970b).

4. Biology of Onchocerca volvulus

The life cycle of O.volvulus is already described (pg.1). O.volvulus microfilariae have no outstanding morphological characteristics. However, Denham (1978) was able to characterize them, that they are large 300-350 u long and 5-8 u diameter; tail is sharply pointed with no nuclei at its tip; the cephalic space is approximately 1.5 - 2 times the width of the body and this identity, allows its distinction from Wuchereria bancrofti, Loa loa, Dipetalonema perstans, Brugia malayi, Meningonema peruzzii which can also be found in man. Characteristic morphological features of the infective larvae of O.volvulus (Nelson 1962; WHO 1966) are:-

	Mean measurements (u)
Head	9.0
Nerve ring	80.0
Anterior oesophagus	108.0
Posterior oesophagus	254.0
Intestine	163.0
Oesophageal/intestinal ratio	2.2
Maximum breadth	18.7
Tail	600.0

The extremity of the caudal region is rounded with a very small papilla and two inconspicuous ventral subterminal papillae. Those characteristic morphological features, distinguish the infective larvae of O.volvulus from those of other filarial species.

It is known that the microfilariae of O.volvulus are not constantly present in the skin and that their densities are affected by the time of the day within the local climatic conditions (Nelson, 1970). In East Africa, Tanzania, Wegesa (1966b) found that microfilaria densities are highest between 8, 10 and 18 hours, coinciding with the peak biting activity of the local vector S.woodi. The periodicity appears to be an adoption to the biting cycle of the local vector in much the same way as there is an adoption by various strains of W.bancrofti, Brugia and Loa loa to their local vectors

(Nelson, 1970; Mosha, 1981).

Experiments in West Africa (Duke, 1966a, 1967a, 1967b) demonstrated that microfilariae from persons infected in the forest zone, which develop well when ingested by forest S.damnosum show almost no development to infective larvae when ingested by S.damnosum from Sudan-savannah zone. The reverse is also true; microfilariae from Sudan-savannah zone carriers fail to develop to infective stage in the forest S.damnosum. The observations led to the conclusions that the strains of O.volvulus and the Simulium transmitting forms differ in the zones, and the parasite-vector complexes therefore, also different. Similar experiments which were carried out in Central and South America, established that parasites in Guatemala and Venezuela exhibit many similarities but were found to be widely different from the strains in Africa (Woodruff et.al. 1966a, 1966b, 1977; Duke, 1972).

5. Clinical Symptoms and Signs of O.volvulus in man

All serious complications of onchocerciasis are due to heavy infections persisting for long periods and there is evidence (Nelson, 1970) suggesting that the disease is progressive even in the absence of continued transmission; life span of the parasite is thought to be 16 years.

Clinical features of onchocerciasis comprise pruritic skin rashes, depigmentation, atrophy, nodules, hanging groins and eye lesions which may eventually culminate in blindness. Comprehensive aspects on these signs are described (Nelson, 1955, 1970; Abaru, 1976a), that pruritic lesions are often associated with relatively light and recent infections. Severe itching may lead to intensive scratching, resulting in gross thickening and roughening of the skin.

There is also skin atrophy with loss of elasticity resulting in premature aged appearance. The loss of elasticity of skin results in hanging groins; these are pendulous sacks most commonly found in adult males and they often contain inguinal or femoral lymph glands which may go down as far as the knee. In Africa the loss of elasticity of the skin of the groin results in high prevalence of hernias (Nelson, 1970; Abaru, 1976a).

Patchy depigmentation is another common feature of long-standing onchocerciasis and the most characteristic eye lesions are formed in the anterior part of the eye and their severity is related to the number of parasites reaching the cornea from the skin of the face and conjuctiva (Nelson, 1970; Abaru, 1976a).

In long-standing infections proportion of adult worms are found in the characteristic nodules which can be felt and

sometimes easily seen (Nelson, 1970; Abaru, 1976a).

The strain differences in the parasite coincide with marked differences in pattern of clinical manifestations; in the southerly forest areas of West Africa for example, gross skin lesions and hanging groins are encountered but eye lesions are of relatively limited significance; further north of the hot Sudan-savannah areas, on the hand, the dermatological complications are less evident but the incidence of eye lesions and blindness is between 10% and 15% (Duke, 1972).

Pruritus, skin depigmentation, atrophy of skin, nodules, hanging groins and hydrocoeles are common manifestations in onchocerciasis areas of Tanzania (Wegesa, 1970a). Ocular complications have not been studied extensively except in Amani and Bwakira (Wegesa, 1970a; Abaru and Van Dam, 1972-73) where cataract and corneal opacity were detected in onchocercal patients.

6. Treatment and Control of O.volvulus

a) Chemotherapy

Two drugs are used for treating onchocerciasis, namely diethylcarbamazine (DEC) and suramin. A third drug merlarsonyl potassium (Mel W) which also acts effectively

turned out to be excessively toxic and therefore became virtually out of use (Mazzotti, 1948; Wegesa, 1969; Nelson, 1955, 1970; Hawking, 1978a and 1978b); Taylor, 1983). DEC kills the O.volvulus microfilariae rapidly while adult worms are not affected. The allergic reactions accompanying the death of microfilariae are particularly noticed during treatment and thus becoming unsuitable for mass treatment. Suramin is effective against onchocerciasis, killing adult worms while not having such an immediate lethal effect on the microfilariae as DEC does; pruritic reactions are milder or do not occur; infection may be cured by a single course of treatment lasting 5 to 6 weeks (Wegesa, 1969). It is however not a suitable drug for mass treatment because of its severe toxic effects (Hawking, 1978b).

Mebendazole and flubendazole which fall into the group of benzimidazoles, have been found to be effective on the microfilariae and they seem to have few side effects than DEC (Taylor, 1983). But there is no clear indication yet that they can be a substitute for the existing drugs. However there are renewed interests in the organic arsenical compounds; it is considered that the toxicity of Mel-W for example may result from its readiness in breaking down releasing melarsenoxide in the body; but stability and activity have been increasing by substituting cationic substituents for the anionic groups on the compound (WHO, 1982).

Ivermectin offers a new dimension to the treatment of onchocerciasis. This drug is a semi-synthetic macrocyclic lactone and active in low doses against many animal parasites, including nematodes (roundworms) and arthropods (insects, ticks and mites). The human experience with ivermectin was reported by Aziz et.al. (1982a, 1982b). They found that the drug reduced drastically microfilariae of O.volvulus in the skin, one month after doses of treatment of 100 mg/kg body weight and even at 50 mg/kg body weight. The drug was reported to have mild adverse reactions (Awadzi, et.al. 1985).

b) Nodulectomy

Another method of treatment is nodulectomy. This was practised systematically in Guatemala and Mexico for many years (Wegesa, 1970a; WHO, 1976; Taylor, 1983). The results of this practice in these two countries indicated that nodulectomy only alleviates the seriousness of the disease and the possibility of eventual blindness in the infected person but cannot remove the reservoir of infection. Also it is hardly applicable in some cases whereby nodules can neither be palpated nor recognized for surgical removal.

c) Vectors

While more search in chemotherapy is necessary and should

be encouraged, the main line of attack on onchocerciasis at present is directed against the vectors. The most widely applicable method is the application of insecticide to the breeding sites. 1,1,1-trichloro-2-bis (4-chlorophenyl) ethane (D.D.T.) has been successifully used in the past but abate which is biodegradable and Bacillus thuringiensis H-14 (B.T.H.-14) which is much more selective are used (Wegesa, 1969; Raybould, 1976; WHO, 1976). For successful use of insecticides against the Simulium vectors of onchocerciasis, precise knowledge of their breeding sites is essential. The interval between insecticide applications is determined according to the minimum length of the local life of the Simulium species in the area and the treatment needs to be continued over a period longer than the life of those simuliids already emerged before the insecticide application (Raybould et.al. 1972).

Experiments on bush-clearing and DDT larviciding in running water of streams, resulted in elimination of S.neavei complex in onchocerciasis areas of Kenya (Garnham and McMahon, 1947, 1954; McMahon et.al. 1958). It was also considered that S.woodi had almost disappeared from the type locality at Magombe in Malawi, probably as a result of deforestation (Raybould, 1968). Furthermore, Raybould (1975-76) found that deforestation might be responsible for the fall in population density of the S.neavei complex females in Eastern-Usambara and he suggested that the control of

onchocerciasis in this area is feasible and could be attempted.

7. Diagnosis of O.volvulus

a) Clinical Diagnosis

People who complain of itching and having hyperpigmented thickened skin and scratches all over the body should be suspected for onchocerciasis (Abaru, 1976a), In newly or lightly infected people this type of diagnosis may be problematic because at this stage, the respective symptoms and signs may not yet have appeared. Under these circumstances a reaction in the skin that follows within 1-24 hours of injection of 50 mg of diethylcarbamazine (Mazzotti test) may be useful (Duke, 1972). Over 90% of cases usually show positive Mazzotti reaction (Abaru, 1976a), but not frequently used because severe itching usually occurs.

In cases with established disease, palpation of nodules is diagnostic. But the nodules should be differentiated from lipomas and other tumours. Depigmentation is also suggestive but should be differentiated from burns, leprosy and other types of diseases (Abaru, 1976a).

Examination of the eyes may reveal conjuctivitis, corneal opacities, iritis, choriodorenititis, etc. (Nelson, 1970). The eye lesions usually cause blindness. Careful examination is

quite essential because those manifestations may be caused by factors other than onchocerciasis (Abaru, 1976a).

b) Parasitological Diagnosis

Diagnosis usually depends on the demonstration of microfilariae in the skin (Duke, 1972). The generally accepted method is the skin snip. The skin snips are usually taken by the insertion of the needle into the skin to elevate a cone of the skin, which is then cut off with a razor blade or sharp bladed scapel (WHO, 1966). The area of the skin snip is usually approximately 3 mm in diameter and the weight of the skin snip varies; Schultz-key (1977) reported a range between 0.6-4.8mm. After cutting the skin, weighing it and teasing in saline, the emerging microfilariae may be counted to give a quantitative estimate of their density usually expressed as microfilariae per mg skin. Another development involves the use of a modified Walser punch by Schaffel (Duke, 1972), which takes more or less standardized piece of skin snip of approximately 1 mg for similar examination. Aseptic conditions are quite necessary in taking the snip. The optimum site for the biopsy will vary depending on the geographical strain of the parasite. In Africa, the preferred site is below the iliac crest, whereas in Mexico the preferred site is behind the shoulder (WHO, 1976). However, neither of these sites would be of value in Yemen, where the highest concentration of microfilariae occurs around the

ankles. It is recommended that anatomical distribution of microfilariae in the skin should be determined in an area, so as to select the best site for biopsies.

c) Immunological Diagnosis

Parasitological examination may be inadequate in a situation whereby the microfilariae are too scanty to be reasonably found in a number of skin snips. The microfilariae may be scanty in newly or lightly infected people or because of the periodic nature and quantitative variations of the parasitaemia, and in a situation of gross thickening and roughening of the skin resulting from severe itching after a person has become infected. From the practical viewpoint, this explains importance attached to immunological diagnosis. From the theoretical viewpoint, research in this field is more justified because O.volvulus and other filariases develop over very long periods parasitic to man for several years and these parasitic diseases are often accompanied by allergic manifestations that reflect the importance of immunological phenomena they cause (Ambroise-Thomas, 1974; Walls and Schantz, 1986).

Several important publications which describe the general veiw on the sero-immunology of filariases, in which O.volvulus is included, and various immunological methods established, have been presented by Kagan (1963) and

Ambroise-Thomas (1974). Several immunological methods or antigenic reagents have been used with varying degrees of success. Parameters which do frequently impose limitation in immunodiagnosis are lack of sensitivity and specificity. The review of Kagan (1963) and Ambroise-Thomas (1974) gave details on the application of various immunological techniques.

(i) Skin Test

The test involves intradermal injection of antigen in the arm of the individual. Positivity is judged by the size of the ring formed soon after the antigen has been injected. The skin test based on this phenomenon is relatively easy to perform. The skin response is of the immediate type and is usually within 15 to 30 minutes. Delayed skin-test reactions however, have sometimes been noted and causes for them are not clearly known. The criteria established in differentiating between negative and positive tests usually are so varied that the comparison of the results of many experiments is difficult. Some workers (Kagan, 1963) considered a specified increase in the size of the antigen wheel over that of the control antigen as a positive test. Standards for a positive reaction include an increase of 3 mm in diameter of the antigen wheel over that of the control wheel, or a diameter of 2 to 3 mm when small amounts of antigen are used (WHO, 1966). In Tanzania, Wegesa (1968d)

took a wheel of 6 mm diameter or more as positive, using antigen extracts of adult O.gutturosa which were prepared with physiological saline; nine of the ten onchocercal patients gave positive reactions as did two of eight uninfected persons. Specificity of this test is dependent on the antigen and the conditions under which the test is read. False positive may also be observed in cases when the solvent alone is injected. Incidence of cross-reactions is high and reaches as far as 54.9% in tropical regions (Ambroise-Thomas, 1974). Because of the frequent and non specific false positive reactions observed by many investigators, the test fails to give satisfaction. Unless specific and well characterized antigens are utilized, this test is known to have little value for epidemiological purposes (WHO, 1976).

(ii) Complement-fixation (CF) Test

The complement system of fresh guinea-pig serum has the capacity to combine irreversibly with antigen-antibody complexes and if the antigen is associated with the sheep erythrocyte cell surface, such a combination may result in lysis of indicator system of the complement activity (Weir, 1967).

This is a serological test with the longest history of application to onchocerciasis (Rodhain and Van den Branden,

1916). Other workers employed homologous antigens in this assay (Montpellier and Beraud, 1921; Van Hoof, 1934; Lartigue, 1966). Gutierrez (1931) found CF giving cross-reactions with sera from syphilitic patients. Van Hoof (1934) reported high sensitivity using an O.volvulus antigen achieving 86% positivity in microfilariae positive patients and non - positive in the group of microfilariae-negative patients.

Woodruff et.al. (1958) reported a positive CF in 63% of patients; however, a similar percentage of individuals with stronglyoidiasis also reacted. They did not find any correlation between skin changes and CF, but showed that 84% of seropositive persons also had eye lesions.

Lartigue (1966) reported that 65% of microfilariae-negative individuals tested had detectable antibodies. McRae et.al. (1977) found an inverse relationship between microfilarial load and CF response i.e., high microfilariae associated with low CF. They also noted that females had greater antibody response than males. Reliable antigen and a standardized test procedure is required for the test to be effective. Areas which require careful study are the preparation of the antigen, standardization and evaluation of a test procedure. With Sawada antigen (Ambroise-Thomas, 1974) the CF test produced 60% positive reactions for onchocerciasis. For epidemiological viewpoint, workers seem

to have attached less importance to it. The wide range of sensitivity and specificity of this assay makes it unsuitable. Large quantities of antigen are also required.

(iii) Precipitation Test

The earliest report of gel diffusion being used as a diagnostic test for onchocerciasis was by Culbertson et.al. (1944) using Litomosoides carinii antigen. In his review, Kagan (1963) found that precipitation tests were applied to several types of filarial infections including O.volvulus. But the sensitivity and reactivity of these precipitation tests were found to be so much lower than other conventional serological tests that their use for routine diagnosis could hardly be recommended. Ulrich et.al. (1970) carried out a study on the sera from 50 patients with soluble extracts of both adult O.volvulus and microfilariae. A difference in response to these antigens was found: 64% had precipitating antibodies to the adult antigen, but 60% responded to the microfilarial antigen. Intradermal skin tests using the latter antigen proved positive in 87%.

Using L.loa as antigen, Petithory et.al. (1972) found 58% sensitivity with onchocerciasis sera, but in a later study (Petithory et.al. 1973) reported greater sensitivities (80-90%) using antigen preparations from Ascaris suum, Neoascaris vitulorum and Parascaris equorum by the Ouchterlony test.

A modified Ouchterlony assay, the closed hexagonal immunodiffusion technique was utilised by Neppert (1974a, 1974b, 1975, 1978, 1979), who found 64% positivity in patients with dermal microfilariae and 25.3% positivity in those free of microfilariae. However, the antibody titre did not correlate with microfilarial load, the number of nodules present, or the presence of pruritus. The sera from onchocerciasis patients reacted with antigens of other filariae as well as Ascaris and hookworm. In another study Niel et.al. (1972) compared the antigens of O.volvulus, D.viteae and A.suum using O.volvulus sera and reported sensitivity of 96% with O.volvulus antigen as compared with 85% with the heterologous antigens.

Immuno-electrophoresis then became attractive in the study of onchocerciasis (Ambroise-Thomas, 1974). The Ouchterlony test is generally used for screening, as a kind of preliminary test to immuno-electrophoresis. It may take several days to carry out immuno-electrophoresis, but the process can be speeded up much faster by allowing the antigen/antibody system to diffuse under the influence of electric current and a technique called countercurrent immuno-electrophoresis (CIEP) was subsquently developed. The CIEP is capable of detecting antibodies in human and animal filariasis (Desowitz and Una, 1976). It is also simple like the gel diffusion, rapid to perform and permitting a relatively large number of serum samples to be tested at the

same time.

These double gel diffusion and immuno-electrophoretic tests in onchocerciasis can demonstrate characteristic arcs which are obtained not only with the homologous antigens, but also with extracts from other types of filarial species. The techniques can reveal antigenic fractions common to Loa loa, Wuchereria bancrofti, O.volvulus, D.witei and A.suum These common fractions vary so that one heterologous antigen ultimately leads to specific results (Ambroise-Thomas, 1974). O.volvulus antibodies were detected using antigens of adult O.gutturosa in agar gel double diffusion (AGD) and CIEP tests; higher percentage 67.3% of positive was observed with CIEP than with AGD test 54.5% (Appendix D5). In both tests, the precipitation bands disappeared at serum dilution of 1/16 and therefore showed to be of little use in quantitative antibody levels assessment. However precipitation bands were not observed with sera of people infected with W.bancrofti.

(iv) Flocculation Test

The principle of technique is described by Kagan et.al. (1963), that standard size of bentonite particles (1-2 u in size) are mixed with antigen. After the antigen is absorbed onto the bentonite particles, the excess is removed by centrifugation. The particles are then stained with thionin so that they become visible and the preparation is usually

adjusted with Tween 80 to eliminate spontaneous saline flocculation. This adjusted antigen is then used by adding a known small amount of anti-serum on a wax-ringed slide and rotated for 15 minutes before being read. Sera diluted with saline also are screened and those positive are titrated to end point usually taken as the last dilution which gives detectable flocculation.

This technique does not seem to have wider application in diagnosing filariasis because of low sensitivity. It is believed to be most sensitive with sera from patients with active infection. Only a few positives could be diagnosed in those people when microfilariae of O.volvulus could not be detected in their skin (Kagan, 1963).

(v) Indirect Fluorescent Antibody (IFA) Test

In this test, the test serum is reacted with slide-antigen. After that slides are washed so that unfixed serum is removed. The slides are then treated with a fluorescein-labelled antiglobulin which is attached to the onchocercal antibody-parasite complex on the slide. Illumination with the U.V-blue light permits visual observation of the fluorescent dyes attached to the complex. The amount of fluorescence is proportional to the quantity of antibody absorbed on the slide-antigen from the test sera. This factor can then be utilised as increasing dilution of the test sera yields

weaker fluorescence and an end-point can be established, usually taken as the last serum dilution which gives a detectable fluorescence. Very small quantitites of antigen and sera are required for this technique. Flourescein-labelled antiglobulin with defined specification are commercially available.

The first report of the IFA test being used in onchocerciasis was made by Lucasse (1962) using the whole microfilariae treated with a range of fixatives, together with bovine serum albumin (BSA) and rhodamine. He obtained positive results only when the microfilariae were treated with formalin or alcohol; the strongest reaction being found with microfilariae which had been rhodemine-BSA treated and then fixed in 10% formalin. In a later study, this group reported 100% sensitivity with onchocerciasis sera (Lucasse and Hoeppli, 1963). They found that the microfilarial level did not correlate with antibody titre. However, there was a relationship between nodules with skin changes and antibody levels. Woodruff and Wiseman (1968), following the method of Lucasse, had no success in using the whole microfilariae.

Duxbury and Sadum (1967) used soluble extracts of adult D.uniformis, D.immitis and D.witei, absorbed onto cellulose acetate filter paper disks and then dried. Onchocerciasis sera were added to the disks and specific antibody detected by fluorescein – labelled reagents. Cross-reactions were

observed with sera from patients with S.haematobium.

Hedge and Ridley (1977) using a soluble antigen from sonicated B.pahangi microfilariae found 96% of onchocerciasis sera positive by this method. However, cross-reactions were seen in sera from individuals with malaria, Strongyloides and other nematodes.

Collins and Skinner (1979) and Collins et.al. (1980) prepared sections of Guatemalan onchocercal nodules which were formalin fixed. This antigen was employed in fluorescence assays on sera from Africans and Guatemalans. They found that IFA and microfilariae positivity in patients increased with age, but that the levels of positive IFA response were low and at first attributed this to antigenic variations (i.e., African sera on Guatemalan parasites). However the Guatemalan sera gave a similar response with high rate of false-negatives.

A number of workers carried out the IFA test on frozen sections using either O.volvulus or other filarial worms like D.immitis, D.witei , W.bancrofti, and L. loa. Coudert, et.al. (1968), Ambroise-Thomas and Kien Truong (1974), Ambroise-Thomas et.al. (1980) and Gentilini et.al. (1972) carried out studies using cryostat sections of these adult worms. Higher titres were obtained with the homologous antigen. With D.witei , the test gave positive results in

nearly 90% of cases; there was an indication of good correspondence between the IFA test and the endemic level of onchocerciasis which was clearly shown by the percentage of positive tests; 89.6% for hypoendemic levels respectively. The sections could be frozen for storage for up to 2 years. Sensitivity of their methods ranged from 85-100% with cross-reactions in about 12% of non filarial sera. Greatest sensitivity was achieved by using O.volvulus material. The authors found that the main advantage of the technique is the ease with which such antigens could be used. Since the test was carried out on frozen sections 5 um thick, very few adult parasites were capable of providing antigen for several thousand tests.

Ten Eyck (1973a, 1973b) using frozen unfixed sections of adult O.volvulus and infective larvae of W.bancrofti found 97% sensitivity with onchocerciasis sera and 86% with W.bancrofti sera. Cross-reactions occurred to a titre of 1/64 with non-filaria sera and to 1/16 with control population. He found no difference between the antibody titres of males and females, but found that the titres increased with age, reaching a maximum at 20-29 years, then dropping slightly to remain constant thereafter.

Ngu and Blackett (1975) used microfilariae of O.volvulus which were fixed with acetone on microscope slides for the detection of specific antibodies. But screening was first

performed on the patients selected, to avoid interference by other filarial species. Bruijining (1975) found that in areas where helminthic infections which cause cross-reactions have high prevalence, the test is much less valuable than in areas where cross-reacting infections are absent or rare.

Diesfield et.al., (1981), in their study to differentiate filarial diseases used a range of antigens, which included B.malayi, B.pahangi, D.witei, L.carinii, O.volvulus, Ancylostoma caninum and Trichinella spiralis. Onchocerciasis sera gave stronger reactions in cytosomal areas of the worm than in the cuticle with adult B.malayi, O.volvulus and A.caninum. The results of cytosomal and cuticular staining could not differentiate sera of W.bancrofti patients from those of onchocerciasis, although the lack of cuticular staining with sera from other helminths infections narrowed the selection to a filarial infection. It is interesting to note that the authors found 100% positivity rate with O.volvulus sera, whereas other workers using different assay systems found that only around 10% of onchocerciasis patients produce antibodies to surface antigens of live microfilariae (Mackenzie, 1980).

Diesfeld et.al., (1981) found that there was no difference in the antigenicity of male and female adult O.volvulus worms. This was later confirmed by Daveau and Ambroise-Thomas (1982) who also found homologous O.volvulus and the

heterologous D.witei antigen preparations to be equally sensitive, although slightly higher titres were found in onchocerciasis patients when the homologous antigen was used. Immunofluorescence has often been compared with ELISA (Speiser, 1980; Lujan et.al. 1983; Lujan et.al. 1984); in both these studies, ELISA was found to be more sensitive, but less specific. The degree of specificity is mostly affected by the nature of the antigen.

Several reports have attempted to correlate antibodies detectable by immunofluorescence with the disease status in onchocerciasis. Wery et.al. (1970, 1981) reported that antibodies are produced from the early age, but found no relationship between antibody titre and ocular lesions, except that the prevalence of punctate keratitis increased with antibody titre. Antibody titres were low when patients had very high microfilarial loads, i.e., 1000 mf/mg or greater than 20 nodules. Prod'Hon et.al. (1982) reported that, in a zone with continuous disease transmission, the mean antibody level and microfilarial load were both higher, but blindness rate much lower in comparison with a zone of discontinuous transmission, Nozais et.al. (1986) found that the immunofluorescence, in conjunction with the Mazzotti test (DEC provocation), greatly improved the detection rate achieved by skin snip alone, with 80 instead of 11.8%, respectively.

In Tanzania, IFA test was performed using microfilariae of O.volvulus harvested from an infected person (Appendix D2). Cross-reaction was observed with sera of people infected with W.bancrofti and therefore would be of limited use in the country where both types of disease prevail. The test for example, would not give valid results in onchocerciasis areas like the Eastern and Western Usambara which are adjacent to coastal areas where bancroftian filariasis is prevalent. Finally it has been noted that this technique requires elaborate equipment and experienced personnel as well as standardized fluorescein-labelled anti-immunoglobulins.

(vi) Enzyme-linked Immunosorbent Assay (ELISA)

ELISA is a relatively new test and was developed by Engvall and Perlmann (1972) for detection of human serum albumin antibodies using enzyme alkaline phosphatase or peroxidase - labelled anti-immunoglobulin in antigen-coated tubes or microtitre plates. The principle of the ELISA is that soluble antigen is coated onto a solid phase (e.g. polystrene plate) to which it adheres physically. Adherence is achieved by incubating the plate with antigen. At the end of incubation, excess antigen is removed by washing the plate. The test serum which is suspected to contain specific antibodies to the antigen is then incubated in the antigen-coated plate. The test serum in the plate is then washed to remove any unbound antibody. A conjugate consisting of an

anti-species immunoglobulin (e.g. Rabbit anti-human IgG) linked with an enzyme, is then incubated in the plate. The anti-species component of the enzyme-conjugate reacts with the antibody fixed to the antigen. Excess conjugate is then removed by washing. A specific enzyme substrate is then added. The substrate reacts with enzyme part of the conjugate fixed to the antibody and its change of colour indicates the amount of enzyme which is attached to the antibody. This amount of enzyme is proportional to the concentration of antibody in the test serum.

The colour change can be assessed visually and also be measured in the spectrophotometer. This makes ELISA both a qualitative and quantitative serological test (Kihamia, 1976; WHO, 1976).

Bartlett et.al., (1975) carried out preliminary studies on application of ELISA to onchocerciasis. The heterologous antigens derived from N.americanus, A.suum, D.witei, O.gutturosa, L.carinii and B.pahangi were found to react with a positive O.volvulus reference serum. In these preliminary studies, however, the major drawback found was extensive antigenic cross-reactions between filarial and other parasitic nematodes, making the interpretation of a positive serological test difficult, especially when the parasitological status of the individual being tested was not known. Surprisingly, a homologous crude extract of O.volvulus

used as antigen, gave high positive ELISA values with sera of infected and uninfected groups of people. This discrepancy was attributed to contamination of the antigen with human material which reacted readily with the anti-human globulin-enzyme conjugate. The crude homologous extract was obtained from adult O.volvulus which were obtained from nodules surgically removed from infected people. O.gutturosa antigen was the more easily available and appeared to give a better differentiation between the positive and negative serum, it gave 100% specificity and 87% sensitivity and therefore, was employed for the test sera. The authors found ELISA inferior to the IFA for onchocerciasis.

Ngu (1978), using a soluble O.volvulus antigen, studied onchocerciasis in the Cameroons. He reported differences in mean ELISA values between a group of patients with generalised onchocerciasis and those with the localised form; the latter giving the lowest values.

Marcoullis and Grasbeck (1976), and Marcoullis et.al. (1978) used physicochemical and immunochemical techniques to purify O.volvulus worm antigens for use in ELISA. "Frequent" antigens (i.e. those corresponding to antibodies that predominate in pooled onchocerciasis sera) were purified by sequential affinity chromatography using human IgG from onchocerciasis sera absorbed onto Sepharose. A good distinction between onchocerciasis sera and normal human

controls was achieved using isolated antigens. Values for specificity and sensitivity were not given. Affinity techniques were applied to the antigen in attempt to remove human immunoglobulins, thus reducing the "background" reactivity which Bartlett et.al. (1975) had experienced.

Taking all subjects with O.volvulus, Ambroise-Thomas et.al. (1980) found that the overall percentage of positive reactions to ELISA test was not higher than that obtained using IFA test (85%) with which it was compared. It was also found that this test appears to be more sensitive for detecting strongly positive serological reactions. Daveau and Ambroise-Thomas (1982) used the antigens which were produced only by the female O.volvulus in the ELISA. They found that these antigens gave higher specificity than did somatic antigens. Buttner et.al. (1982) applied the ELISA to study onchocerciasis in Yemen. They used a saline extract of D.immitis antigen, which clearly distinguished between sera from proven onchocerciasis cases and those from European and non-endemic Yemen controls. Mean antibody levels were higher in patients with generalised or mild localised form. No correlation was found between ELISA activity and microfilarial levels in the skin, but ELISA was able to detect antibody levels in persons from the endemic area who had skin lesions but no detectable microfilariae in the skin.

Korenaga et.al. (1983) compared blood collected onto

filter paper with corresponding venipuncture sera for use in detecting IgG antibodies in onchocerciasis and found a close correlation between the two techniques. O.volvulus and O.gutturosa were similar in ability to detect IgG antibodies in sera of inhabitants of endemic areas, and ELISA values closely correlated with the microfilarial loads. Cross-reactivity with other parasitic infections occurred.

Campbell et.al. (1983) found ELISA to be preferable to the Mazzotti test as an auxiliary test to skin biopsy and nodule examination in Guatemala. The ELISA and Mazzotti correlated well regardless of presence or absence of palpable nodules, but ELISA appeared to have enhanced sensitivity and lacked the inconvenience risk involved with DEC provocation. Further evaluation of ELISA was conducted using samples from Guatemala (Lujan et.al. 1983) and using finger-pricked blood the investigators found a degree of false-positivity from other helminth infections.

Immunofluorescence has often been compared with ELISA (Speiser, 1980; Lujan et.al. 1983); in both these studies, ELISA was found to be more sensitive but less specific. The degree of specificity is mostly affected by the nature of the antigen.

ELISA is easy to perform, requires small quantities of antigen, economical, simple to perform and reproducible. The test however, appears to have lower specificity than IFA but

there is a general view that it will become more of practical interest as better defined specific antigens become available; as pointed out by WHO (1976).

(vii) Indirect Haemagglutination Antibody Test

Indirect haemagglutination antibody (IHA) test relies on the agglutination of antigen-sensitised particle in the presence of antibody. The filarial antigen is usually attached to red blood cells and the agglutination is assessed by the settling of the sensitised cells, in the presence of serum or serum dilution, in wells made in small plastic plates. Apparatus is available for making multiple rapid dilution in these plates with a minute quantity of serum.

The simplicity for large field testing and suitability for large-scale surveys, have made it recommendable as an epidemiological tool and this has led to its being applied in several studies on onchocerciasis. WHO (1976) accepts the IHA test as the most probably sensitive tool, but its value in the serology of onchocerciasis needs to be further evaluated as purified and isolated specific antigens become available.

However, while the need for the purified antigen is still recognised, some research workers managed to define different threshold positive titres which discriminate the cross-reaction effects. Rose' et.al. (1966) made antigen extracts

from both O.volvulus and A.suum and carried out IHA on sera from onchocerciasis patients and those infected with D.perstans, Dracunculus medinensis, L.loa, W.bancrofti, and syphilis. They achieved 100% positivity with filarial sera using their O.volvulus antigens with a maximum titre of 1/56,536, but also detected weak positive reactions with sera from patients with pyrexic infections or syphilis. When A.suum antigen was used in testing onchocerciasis sera, 25-50% of these were found positive, although the highest titres were detected with the homologous antigen. Ikeda et.al. (1978, 1979) used a threshold titre of 1/520 with homologous crude O.volvulus antigen in a seroepidemiological study in Guatemala, to examine the relationship of antibody level to nodules and microfilariae. No differences were detected in the number of females or males with positive IHA in highly endemic zones, but in less endemic areas more males had positive titres. He also found high correlation in antibody titre between standard venous blood samples and those collected on filter paper. Buck et.al. (1969) working with D.immitis antigen, found that mean IHA titres and the intensity of infection both increase with age.

Bryceson et.al. (1976) used IHA to define antigenic diversity in savannah and forest forms of onchocerciasis. They cross-absorbed pooled sera from savannah and forest zones with insoluble antigens from these two zones and carried out IHA using soluble antigens from both areas. Their results

indicated forest zone sera contained antibodies which could not be removed in insoluble extracts of O.volvulus and therefore confirmed the existence of differences in immunological reactivity between savannah and forest patients. On reflection, it is possible that the differences in haemagglutinating antibodies in these two groups might have been due to class differences in the antibody- or antigen-binding sites. It is thought that microfilarial loads can affect the class of antibody present.

Formalinised ("fixed") erythrocytes can be lyophilized for long-term storage, but this may give inferior settling patterns (Herbert, 1973). Importantly, this test can be used with blood collected onto filter paper (Neal and Miles, 1970), which makes it an attractive procedure for large field studies. False positive results may occur due to nonspecific agglutinins with an incidence of up to 1 in 40 in some tropical areas (Meuwissen, 1974); however, these factors can be absorbed out. This test requires relatively large quantity of antigen but is considered to be most suitable because of its high sensitivity and technical ease; no specialised equipment is necessary as well and a rather simple test for which no highly qualified personnel is required (Rose' et. al. 1966; Buck et. at. 1969; Ikeda et. al. 1978, 1979).

8. Study objectives

The objectives of the present study were:

(a) To carry out surveys on onchocerciasis in Tanzania in order to assess its prevalence.

(b) To use serodiagnosis in addition to clinical and parasitological diagnosis for the prevalence studies of onchocerciasis in the country.

III. MATERIALS AND METHODS

1. Parasitological and clinical examination

People were examined for onchocerciasis as follows: total number of the village(s) Balozi* was first determined at each particular locality. Households of each Balozi were then identified. 20-30% of the total households belonging to each Balozi were selected randomly. In any one day, a research team would then examine people of those selected house-holds which were gathered at a local dispensary, court house or under a tree. Examination involved taking a skin snips from the upper and outer quadrant of each buttock using a needle and sharp scapel (WHO, 1966; Abaru, and Van Dam, 1972-73). The skin snips were then weighed by means of a torsion balance (White Electronic Co. Ltd. England) and examined in normal saline under a microscope. Microfilariae counts were made, the snips being teased on and off. Males and females

*Leaders of a cluster of about ten households in a village.

adults were examined together with children. Additional adjustments were sometimes necessary when it was found that insufficient numbers were examined for some of the age groups. At times all people in a village had to be examined when it was found that the population was small to make significant 20-30% random selection in the household. Presence of nodules and observations on skin changes which include pruritus, atrophy and depigmentation, were also specifically looked for as other manifestations of the disease.

2. Development of the IHA test

a) Establishment of antigen bank

(i) Adult O.gutturosa and O.volvulus

Adult O.gutturosa were found attached onto ligamentum nuchae of cattle which were slaughtered at local abbatoir which is situated at Korogwe (Appendix D3). The ligamentum nuchae were wrapped in polythene material and kept in the thermos flask containing ice at 4 degrees centigrade. On the same day, the ligamentum nuchae were then brought to the Amani Centre laboratory and preserved at -70 degrees centigrade for subsquent uses.

Adult O.volvulus were obtained from nodules which were

removed surgically from infected people of Bwakira areas in Morogoro district. The nodules were frozen in liquid nitrogen immediately after they were removed from the infected people and finally brought to the Amani Centre laboratory where they were stored at -70 degrees centigrade for subsquent uses.

(ii) Attempts to obtain infective stage larvae of O.gutturosa

Attempts were made in three stages. A cow infected with O.gutturosa was needed first to act as a source of the microfilariae. Second stage was collection of wild S.vorax. Third stage involved experiments on feeding S.vorax on the infected cow and assessment of quantity of the microfilariae of the O.gutturosa which were ingested by the S.vorax after the infective blood-meal before continuing further with rearing the flies for subsquent development of these microfilariae to infective stage larvae.

A search for a cow infected with O.gutturosa was performed by conducting a survey for the infection in cattle in Lushoto district where the disease was reported as being prevalent (Appendix D3). During the survey, cattle were examined for the presence of the parasite as described in Appendix D3. A cow with highest microfilariae count of 34 per skin snip, obtained in Yogoi village (Table 2), was then selected as a parasite source.

Table 2. O.gutturosa infection in cattle of Lushoto district

Village	No. cattle examined	No. cattle positive	% positive
Yogoi	17	3	17.6
Miegeo	14	8	57.1
Kwembago	15	2	13.3
Total	46	13	28.3

Wild S.vorax were caught and collected as they came to feed on a non-infected cow using a fine netting. In the laboratory however, some of the flies were found to contain traces of blood-meal when their abdominal regions were squashed on white papers and therefore they had to be maintained in the laboratory for 24 hours, at room temperature of 25 degrees centigrade, to allow possible further digestion of these traces of blood-meal before they were later fed on the cow infected with the O.gutturosa. For the maintenance, the flies were kept in groups of 50-100, in compartments made in polystrene material which was 5 cm thick. The size of each compartment was 7 x 8 x 5 cm. 44.0% of the flies survived. This survival rates however, was enhanced to 66.5% when the flies were fed with saturated sucrose solution for 5-10 minutes on the same day they were collected. 2496 of these wild caught flies were dissected for checking possible local transmission of O.gutturosa in Amani area as was previously done (Appendix D4) and none of these flies was found with filarial parasite.

The wild caught flies were experimentally fed on the cow infected with O.gutturosa using a method described by Raybould (1969), with the same fly-species feeding on ears of a rabbit. However, a cow being a larger animal than rabbit, a slight modification on the methodology of handling the cow was adopted; the infected cow was confined to a wooden paddock which was built inside the experimental hut, but the

head of this cow was left completely outside this paddock-hut system. A sleeve of nylon netting was then enclosed over each ear of the cow and then closed at each end with a rubber band as shown in Plate 2. 50 flies were then released onto each sleeve using a sucking-tube and then allowed to feed for 15 minutes. Five sets of feeding experiments were performed.

b) Sera

Positive control sera were collected from onchocerciasis patients confirmed by the skin snip method. The negative control sera were obtained from Europeans who had not been exposed to tropical filarial and other helminthic diseases. To check the degree of cross-reaction with other helminths, Wuchereria bancrofti, Schistosoma haematobium, Ancylostoma doudenale, Ascaris lumbriccoides, Dipetalonema perstans, Trichuris trichiura, Strongyloides stercoralis and Necator americanus, control sera from people infected with these conditions, diagnosed specifically using the methods described by Jacobson (1971), Denham (1978) and Mosha (1981) were used.

c) Procedure of the IHA test

Procedure used for IHA test was that described in Appendix D5.



Plate 2. A feeding trial of S.vorax on a cow infected with O.gutturosa

VI. RESULTS

1. Parasitological and Clinical Observations

a) Parasitological Observations

Microfilaria rates in all surveyed areas are shown in Tables 3 and 4. In some of the known onchocerciasis foci, the rates were found to be different from those reported by Wegesa (1969, 1970a). Higher microfilaria rates, for example, were observed in Bwakira (63.6%), Mahenge (58.6%) and Ruvuma (31.9%); whereas in Amani, a lower rate of 22.4% was recorded. The rate of 20.6% in Kilosa with a range between 9.2% in Chanzuru and 40.2% in Msowero was almost the same as reported before (Wegesa, 1970a).

Onchocerciasis was not encountered in Ngara, Kibondo, Kasulu, Sumbawanga except in Kigoma where microfilaria rates of 4.0% and 2.9% were recorded in indigenous people of Mukongoro and Mkingo villages respectively. The rate of 4.0% in Mukongoro village was lower than that of 4.7% reported by Pedersen and Kolstrup (1982). A survey in Karagwe and Ngara districts, revealed five positive cases out of 2743 people examined. Those infected, however, had lived in Burundi previously. In Ifakara, microfilaria rate of 10.6% was recorded with a range between 3.4% in Kikwawila and 19.2% in Kilama villages. In Ubembe, on the foothills of Eastern

Table 3. Prevalence and clinical manifestations of onchocerciasis in

Area	District	Village	People examined	Microfilaria Positive	Microfilaria % positive	Skin changes* Positive	Skin changes* % positive	Nodules Positive	Nodules % positive
Amani	Muheza	Mbomole	224	13	5.8	49	21.8	1	0.4
		Shebomeza	165	3	18.8	48	29.1	1	0.6
		Msasa	279	65	23.3	55	19.7	9	3.2
		IBC	152	26	17.1	43	28.3	0	0
		T o t a l	820	135	16.5	195	23.8	11	1.3
Ubembe	Muheza		47	8	17.0	0	0	0	0
Korogwe	Korogwe	Mgobe	310	0	0	0	0	0	0
		Rutuba	170	0	0	0	0	0	0
		Maurui	114	0	0	0	0	0	0
		Goodshed	73	1	1.4	0	0	0	0
		T o t a l	667	1	0.15	0	0	0	0
Nguru	Morogoro	Pemba	114	8	7.0	0	0	0	0
Kilosa	Kilosa	Chanzuru	196	18	9.2	39	19.0	0	0
		Msowero	122	49	40.2	119	97.5	0	0
		T o t a l	318	67	21.1	158	49.7	0	0

Table 3 (cont).

Bwakira	Morogoro	Dutumi	275	203	73.8	110	40.0	41	14.9
		Milengwelengwe	71	40	56.3	15	21.1	5	7.0
		Mngazi	101	71	70.2	30	29.7	10	9.9
		Sesenga	160	123	76.9	61	37.9	1	0.6
		Kolero	72	53	72.2	27	37.5	15	20.8
		Kisaki	67	24	35.8	18	26.9	0	0
		Lukange	31	20	64.5	9	29.0	2	6.5
		T o t a l	777	553	68.6	270	34.7	74	9.5
Ifakara	Kilombero	Kilama	172	33	19.2	8	4.7	0	0
		Kining'ina	201	21	10.4	13	6.5	0	0
		Kikwawila	89	3	3.4	33	37.1	9	10.1
		Mang'ula	103	11	10.7	5	4.6	0	0
		Mkula	103	5	4.9	6	5.8	0	0
		Mkamba	60	4	6.7	22	36.7	1	1.6
		T o t a l	728	77	10.6	87	12.0	10	1.4

Table 3 (cont).

Mahenge	Ulanga	Ruaha	134	109	81.3	40	29.9	2	1.5
		Itete	143	36	25.2	60	0	13	9.1
		Mahenge Hosp.	93	55	59.1	64	68.8	7	7.5
		Chikuti	112	100	89.3	44	39.6	6	5.4
		Total	482	300	62.2	208	43.2	28	5.8
Bukoba	Karagwe	Nyamiaga	441	0	0	0	0	0	0
		Lwabukata	398	0	0	0	0	0	0
		Kihinda	235	0	0	0	0	0	0
		Murongo	501	2	0.4	0	0	0	0
		Rutungulu	233	0	0	0	0	0	0
		Kibingo	356	0	0	0	0	0	0
		Kaisho	27	0	0	0	0	0	0
		T o t a l	2191	2	0.09	0	0	0	0
Ngara	Ngara	Ruganzo	190	1	0.5	0	0	0	0
		Chivu	100	0	0	0	0	0	0
		Ntobeye	262	2	0.7	0	0	0	0
		T o t a l	552	3	0.5	0	0	0	0

Table 3 (cont).

Kibondo	Kibondo	Magugu	181	0	0	0	0	0	0
		Kibadaduye) Kasaga)	199	0	0	0	0	0	0
		Bayungu) Kanyanza)	153	0	0	0	0	0	0
		Nyamibunge) Nyaronga)	104	0	0	0	0	0	0
		T o t a l	639	0	0	0	0	0	0
Kasulu	Kasulu	Kususenga Sch.	245	0	0	0	0	0	0
		Mulole School	104	0	0	0	0	0	0
		Kitumbuka Disp.	104	0	0	0	0	0	0
		Makere School	117						
		T o t a l	570	0	0	0	0	0	0

Table 3 (cont).

Kigoma	Kigoma	Mukongoro	100	4	4.0	21	21.0	0	0
		Mkingo	207	6	2.9	9	4.3	0	0
		Nyarubanda	208	2	0.9	13	6.3	0	0
		Matiazo Hosp.	152	2	1.3	9	5.9	0	0
		Mukongoro School	312	7	2.2	13	4.2	0	0
		Mwandiga School	78	0	0	0	0	0	0
		T o t a l	1057	21	1.9	65	6.2	0	0
Sumbawanga	Rukwa	Mtowiza	190	0	0	0	0	0	0
		Zimba	130	0	0	0	0	0	0
		Muze	150	0	0	0	0	0	0
		T o t a l	470	0	0	0	0	0	0
Tukuyu	Tukuyu	Kilugu	100	56	56.0	32	32.0	4	4.0
		Isange	56	0	0	8	14.3	0	0
		Mbambo	87	8	9.2	26	29.9	0	0
		Kambasegesa	111	17	15.3	29	26.2	3	2.7
		T o t a l	354	81	22.9	95	26.8	7	2.0

Table 3 (cont).

Ruvuma	Mbinga	Liuli	43	5	11.6	7	16.3	1	2.3
		Mango	69	32	46.4	46	66.7	11	15.9
		Puulu	76	34	44.7	20	26.3	2	2.6
		Lundo	40	19	47.5	26	65.0	4	10.0
		T o t a l	228	90	39.5	99	43.0	18	7.9
Tunduru	Tunduru	Njenga	212	0	0	0	0	0	0
		Likwezo	234	0	0	0	0	0	0
		Wenje	215	0	0	0	0	0	0
		Kitalo	122	0	0	0	0	0	0
		Naikula	230	0	0	0	0	0	0
		Ligoma	297	0	0	0	0	0	0
		Kidodoma	214	0	0	0	0	0	0
		T o t a l	1524	0	0	0	0	0	0

*Atrophy, depigmentation and pruritus

Usambara mountains, 17.0% of the people examined were found infected. A single person was found infected in Korogwe; but this person had previously lived in Western Usambara mountains where onchocerciasis in prevalent.

Table 4 also shows crude and standardised microfilaria rates in the onchocerciasis foci; the age distribution of the people examined differed and therefore, there was a need to make them comparable. The standardised microfilaria rates were determined using the total population examined in all foci.

In this group, onchocerciasis endemicity criteria of WHO in 1973 (Pedersen and Kolstrup, 1982) was used: i.e. areas with high endemicity (Prevalence 70%), medium endemicity (prevalence 40-60%), low endemicity (prevalence 10-40%), sporadic cases (prevalence 0-10%), and villages with no infection. Using the standardized microfilaria rates in Table 4, Bwakira, Mahenge were therefore classified as the foci of medium endemicity. Whereas Ruvuma, Kilosa and Amani became the foci of low endemicity. In Nguru, the endemicity was found to be sporadic.

Males were found to be more infected in Amani, Nguru and Ruvuma with respective microfidlaria rates of 21.6%, 12.9% and 44.7% (Table 5). In Bwakira, females were found to be more infected (82.1%) than males (56.4%). In other remaining

foci, there were no marked differences in the microfilaria rates between sexes.

Tables 6-11 and Figs. 1-6 show age distribution of the microfilaria rates in the different foci. The rates for Bwakira, Mahenge and Ruvuma were generally high and were found to increase with age. Whereas in Tukuyu, Amani and Kilosa, the rates were relatively lower but were also found to increase with age. The rates for Bwakira, Amani, Kilosa, Tukuyu and Ruvuma were generally high in females than males and were found to increase with age in both sexes. In Mahenge, the trend looked slightly different in that the microfilaria rates were higher in males than females at the age of 0-29 years; whereas at the age of 30-50+, the rates were higher in females. In both sexes, however, the rates were found to increase with age.

Mean microfilaria counts per mg skin were found to be highest in Bwakira (12.3) and lowest in Nguru (3.1). In Mahenge, Tukuyu and Ruvuma, the counts were similar being 10.6, 10.5 and 10.4 respectively. The counts of 6.3 were recorded in Amani and Kilosa. Figs. 7-12 show the age distribution of the microfilaria counts. In Bwakira, the counts were generally higher in males than females but in both sexes, they were found to increase with age. In Mahenge, the counts were much higher at the age of 20-30, and 50+ years in males than females. Ruvuma focus had a different

Table 4. Crude and standardized microfilaria (mf) rates

Focus	% mf crude	% mf standardized	Endemicity levels (WHO, 1973)	% mf (Wegesa, 1969, 1970a)
Bwakira	68.6	63.6	Medium	29.0
Mahenge	62.2	58.6	"	39.0
Ruvuma	39.5	31.9	Low	12.0
Tukuyu	22.9	22.8	"	0
Kilosa	21.1	20.6	"	24.0
Amani	16.5	22.4	"	38.0
Nguru	7.0	5.8	Sporadic	8.0

Table 5. Microfilaria (mf) rates in males and females

Focus	Males			Females		
	No. examined	No.mf positive	% mf positive	No. examined	No. mf positive	% mf positive
Bwakira	379	214	56.4	398	319	80.1
Mahenge	220	142	64.5	262	151	57.6
Ruvuma	114	51	44.7	114	45	39.3
Tukuyu	183	49	26.7	171	32	18.7
Kilosa	148	35	23.6	166	32	19.3
Amani	391	85	21.6	429	50	11.6
Nguru	54	7	12.9	60	1	1.7

Table 6. Age distribution of microfilaria (mf) rates in Bwakira focus

Age group (years)	No. examined	No. mf positive	% mf positive
0 - 9	36	8	22.2
10 - 19	224	125	55.8
20 - 29	178	115	64.6
30 - 39	141	112	79.4
40 - 49	88	77	87.5
50+	110	96	87.3
T o t a l	777	533	68.6

Linear regression estimates:-

Microfilaria rates = 28.60 + 10.24t

r = 0.89

Table 7. Age distribution of microfilaria (mf) rates in Mahenge focus

Age group (years)	No.examined	No.mf positive	% mf positive
0 - 9	47	19	40.4
10 - 19	144	62	43.1
20 - 29	89	55	61.8
30 - 39	62	39	62.9
40 - 49	88	59	67.0
50+	78	66	84.6
T o t a l	508	300	59.1

Linear regression estimates:-

Microfilaria rate = 35.62 + 8.32t

r = 0.86

Table 8. Age distribution of microfilaria (mf) rates in Ruvuma focus

Age group (years)	No. examined	No. mf positive	% mf positive
0 - 9	12	1	8.3
10 - 19	38	9	23.7
20 - 29	43	9	20.9
30 - 39	41	17	41.5
40 - 49	32	16	50.0
50+	62	36	58.1
T o t a l	228	90	39.5

Linear regression estimates:-

Microfilaria rates: = 16.20 + 8.8t

r = 0.94

Table 9. Age distribution of microfilaria (mf) rates in Tukuyu focus

Age group (years)	No. examined	No. mf positive	% mf positive
0 - 9	53	1	1.9
10 - 19	140	19	13.6
20 - 29	36	5	13.9
30 - 39	31	11	35.5
40 - 49	34	8	23.5
50+	60	37	61.7
T o t a l	354	81	22.9

Linear regression estimates:-

Microfilaria rates: = 8.27 + 9.08t

r = 0.93

Table 10. Age distribution of microfilaria (mf) rates in Kilosa focus

Age group (years)	No. examined	No. mf positive	% mf positive
0 - 9	54	2	3.7
10 - 19	88	14	15.9
20 - 29	40	5	12.5
30 - 39	53	20	37.7
40 - 49	30	12	40.0
50+	53	14	26.4
T o t a l	318	67	21.1

Linear regression estimates:-

Microfilaria rates: = 5.51 + 4.7t

r = 0.70

Table 11. Age distribution of microfilaria (mf) rates in Amani focus

Age group (years)	No. examined	No. mf positive	% mf positive
0 - 9	217	3	1.3
10 - 19	277	25	9.0
20 - 29	151	43	28.5
30 - 39	92	29	31.5
40 - 49	42	18	42.9
50+	41	17	41.5
T o t a l	820	135	16.5

Linear regression estimates:-

Microfilaria rates = -0.41 + 7.16t

r = 0.90

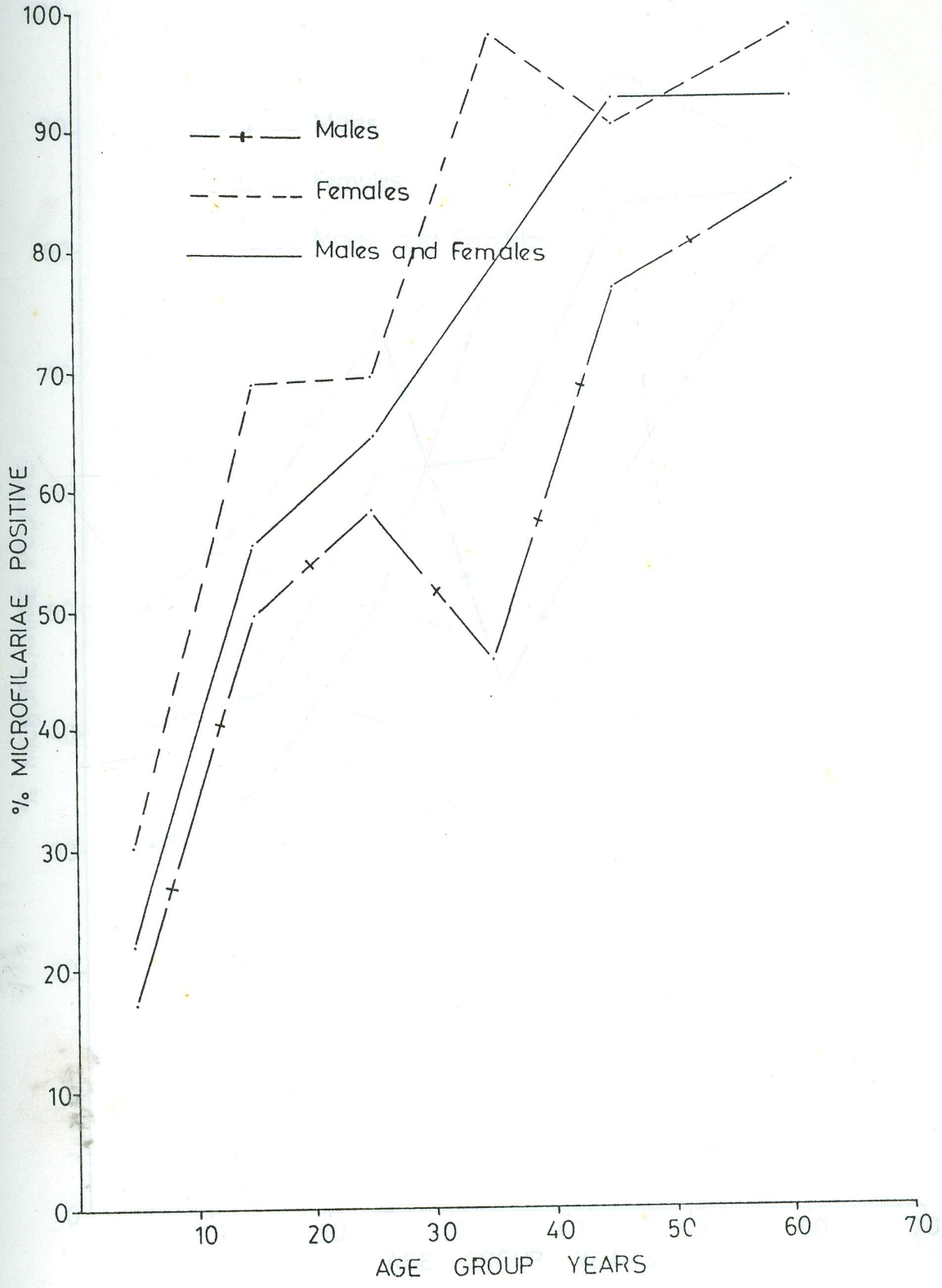


Fig.1. Age distribution of microfilaria rates in Bwakira focus

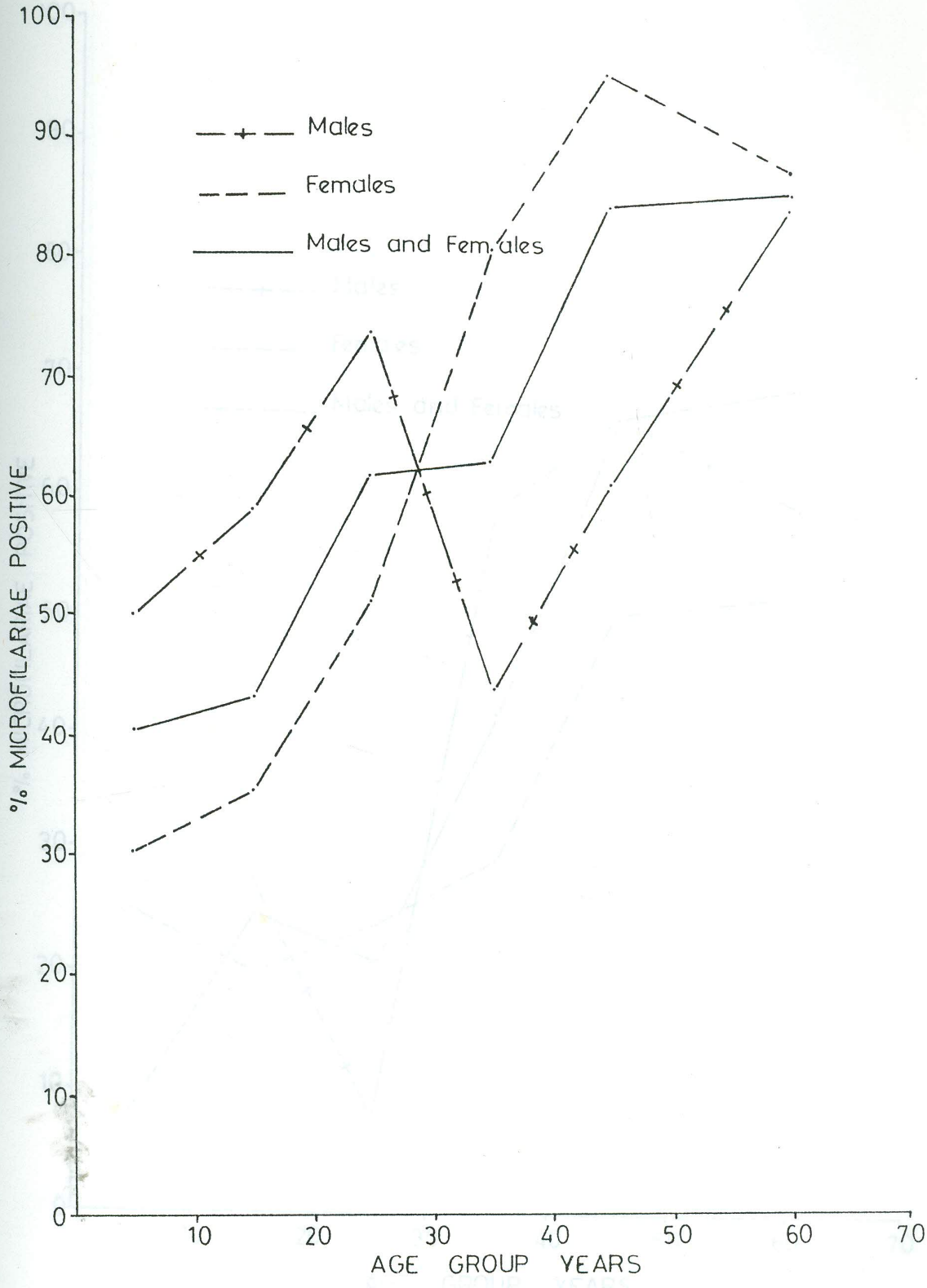


Fig.2. Age distribution of microfilaria rates in Mahenge focus

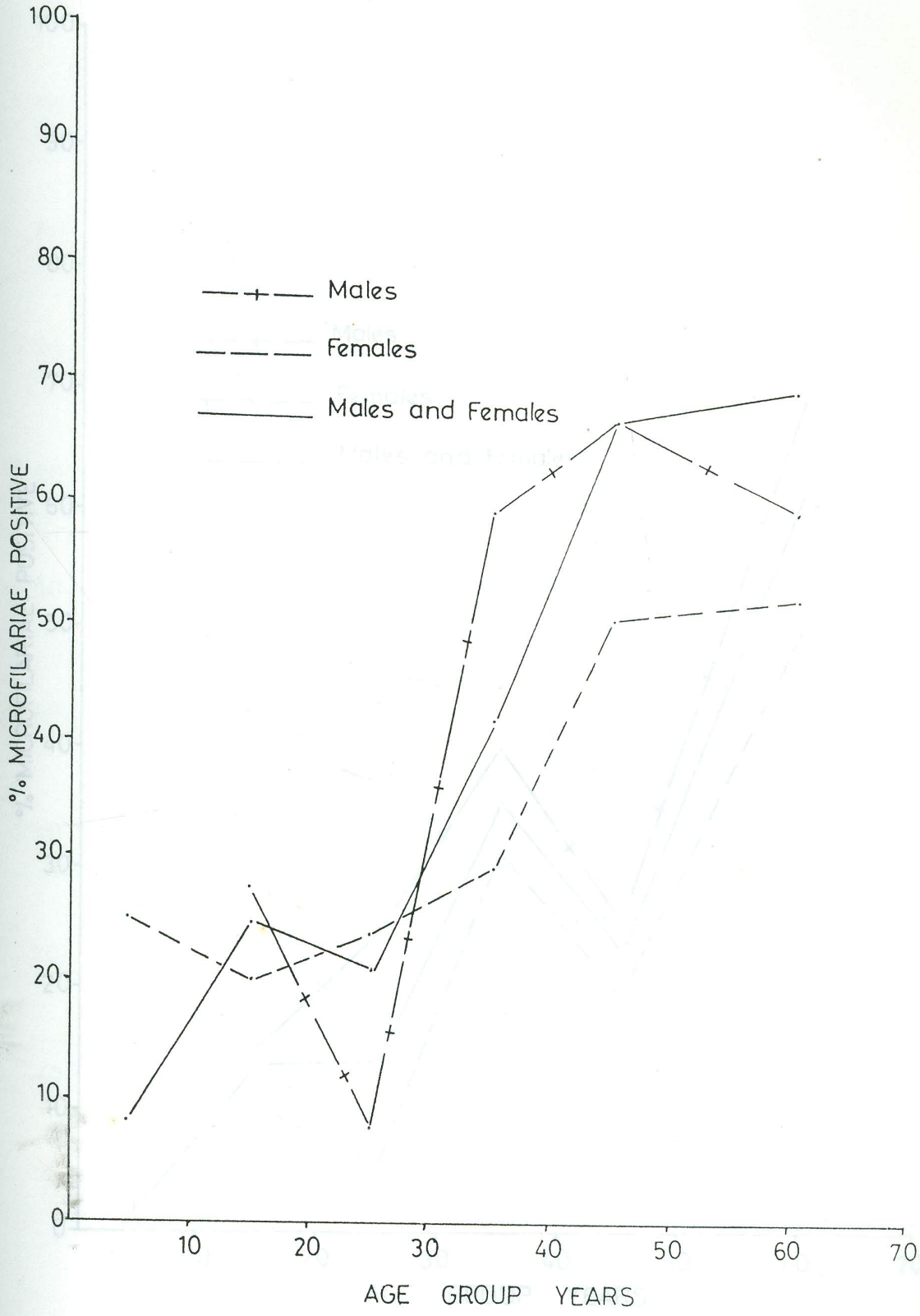


Fig.3. Age distribution of microfilaria rates in Ruvuma focus

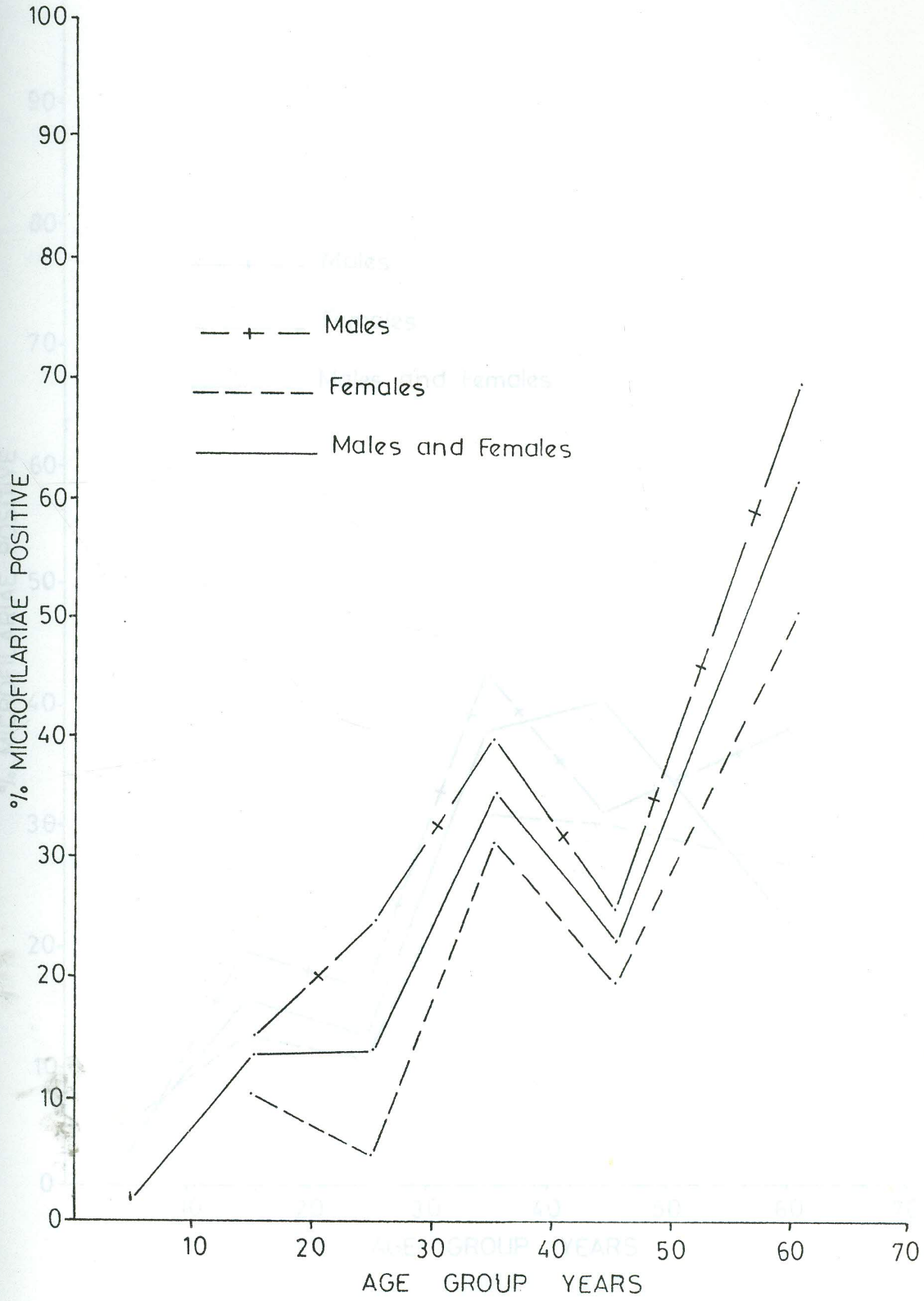


Fig.4. Age distribution of microfilaria rates in Tukuyu focus

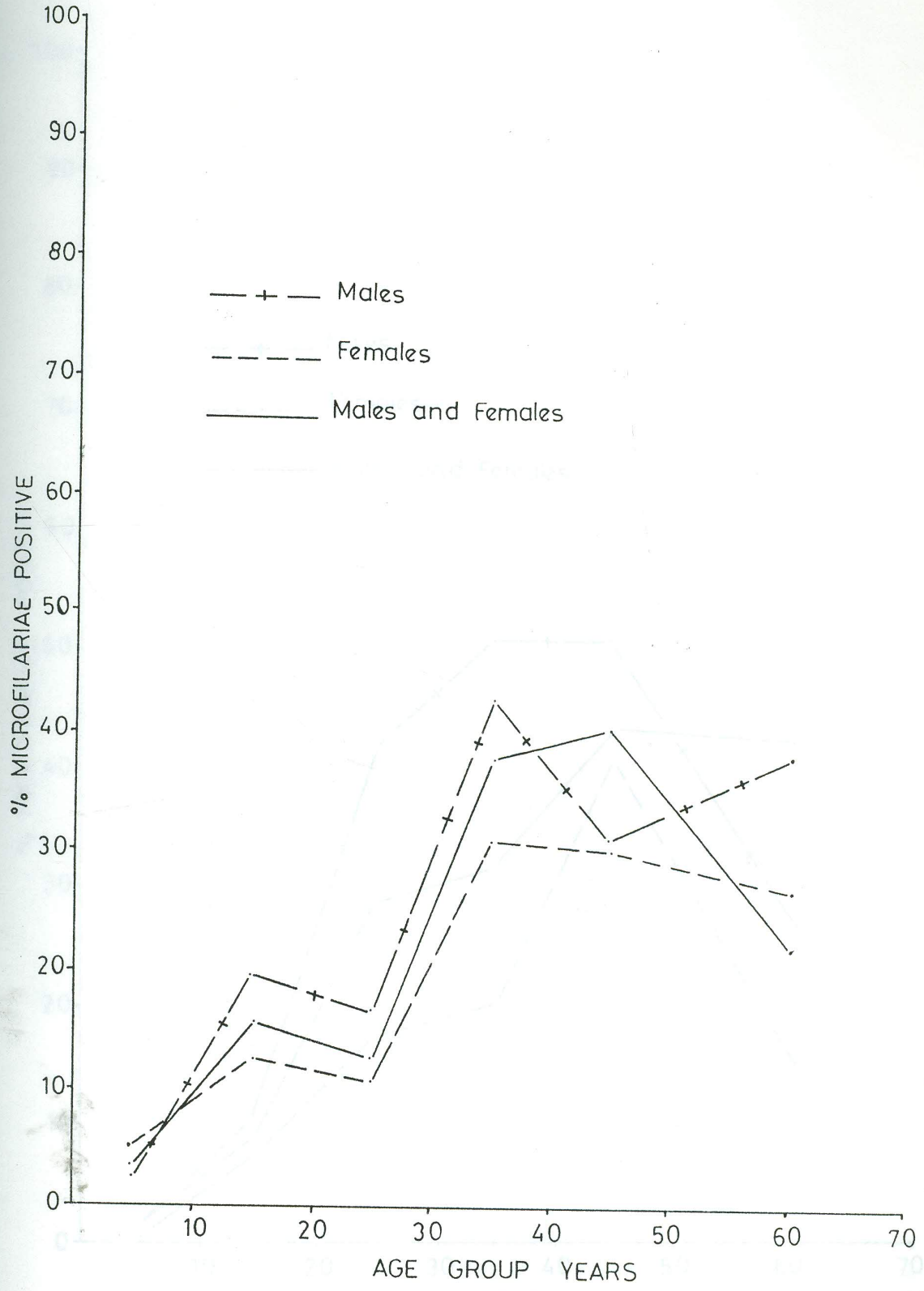


Fig.5. Age distribution of microfilaria rates in Kilosa focus

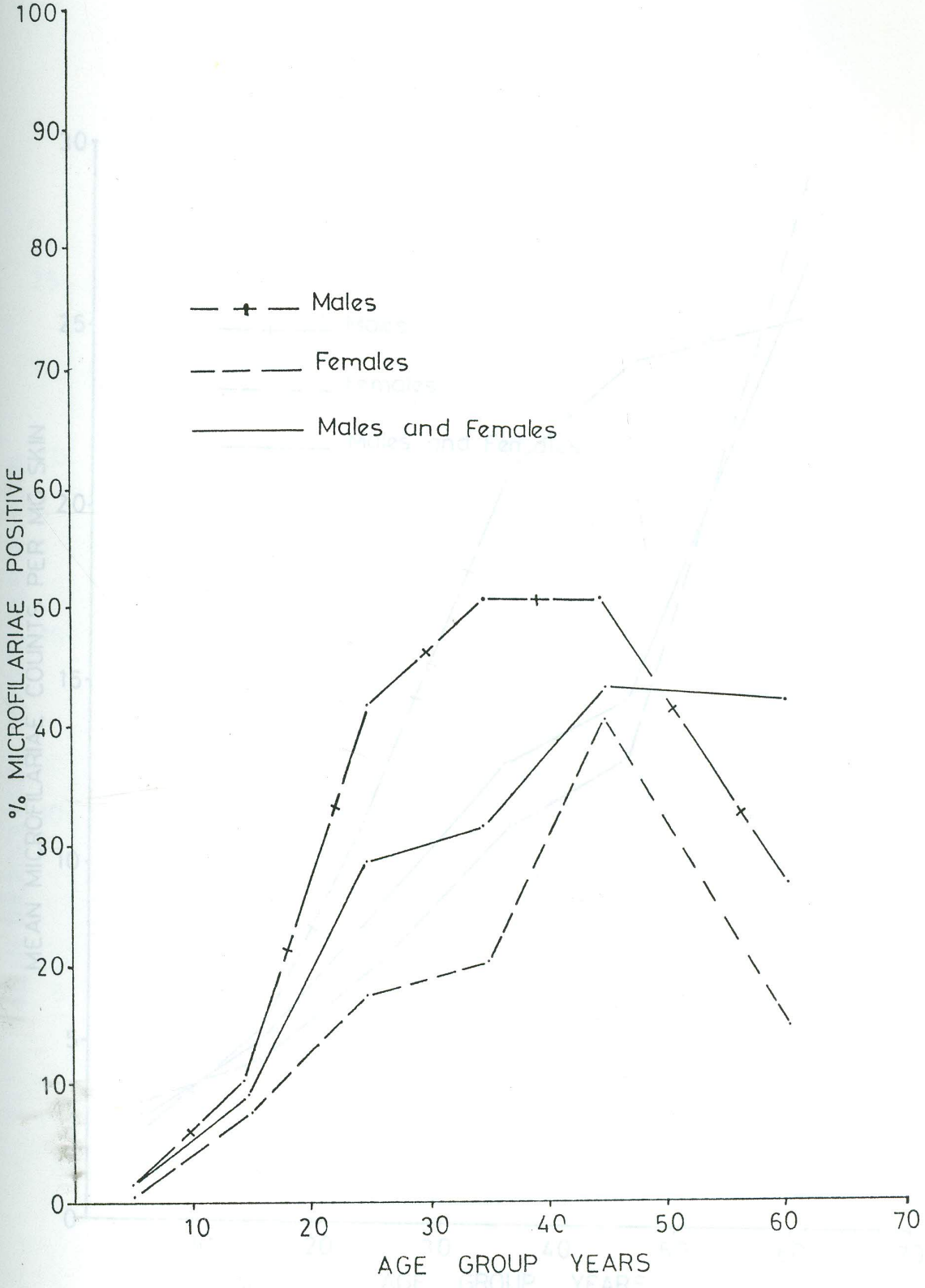


Fig.6. Age distribution of microfilaria rates in Amani focus

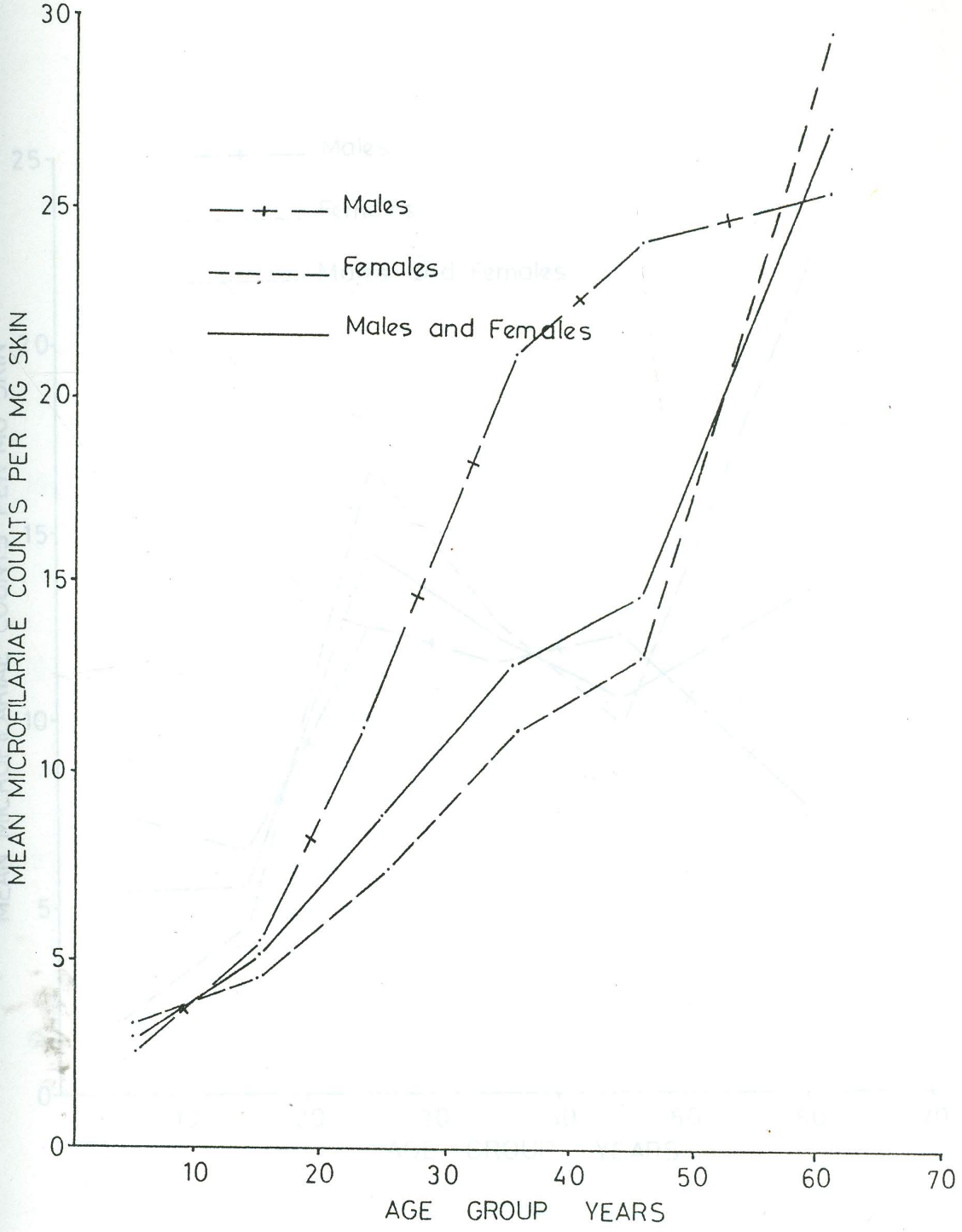


Fig.7. Age distribution of mean microfilaria counts in Bwakira focus

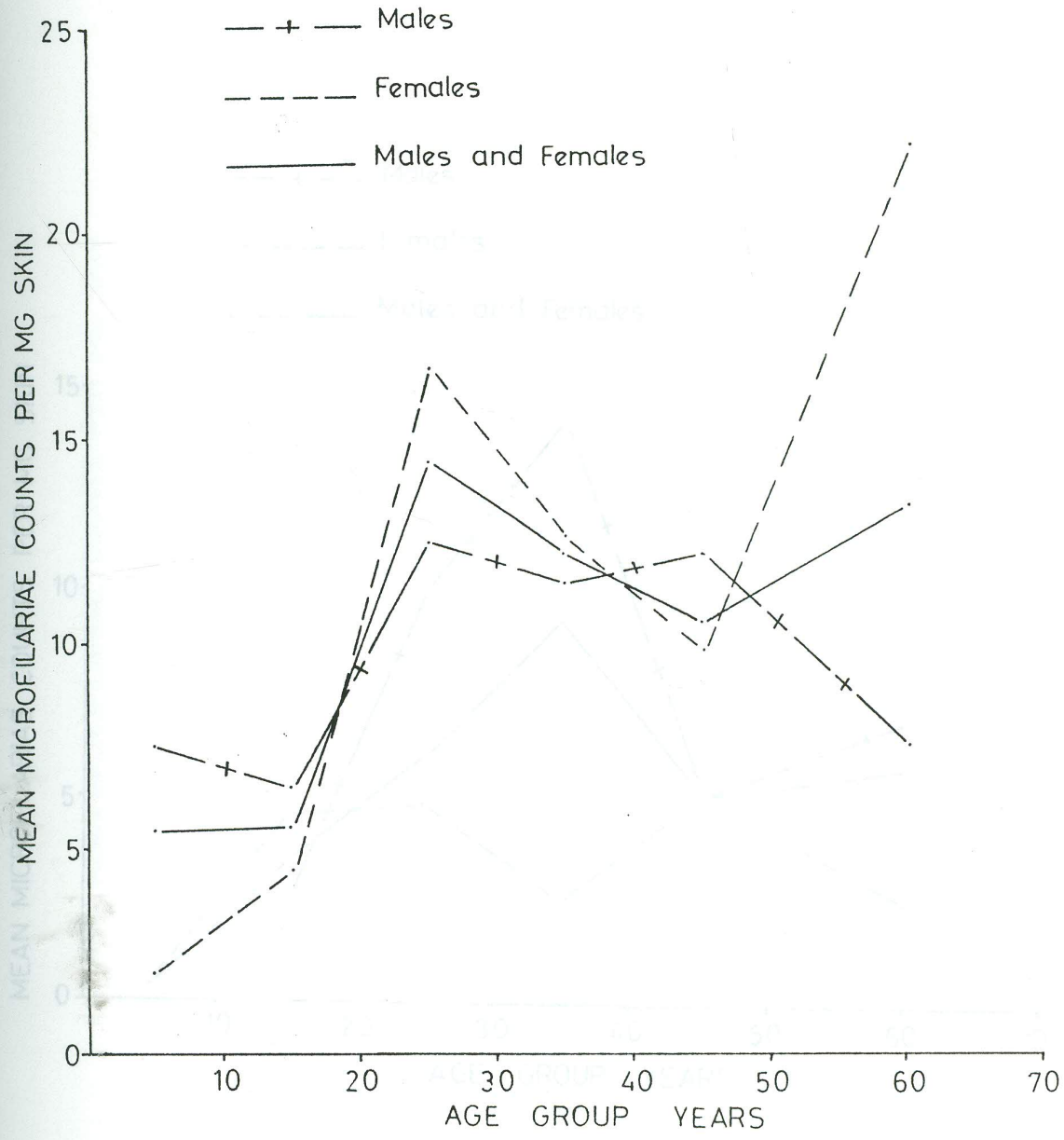


Fig.8. Age distribution of mean microfilaria counts in Mahenge focus

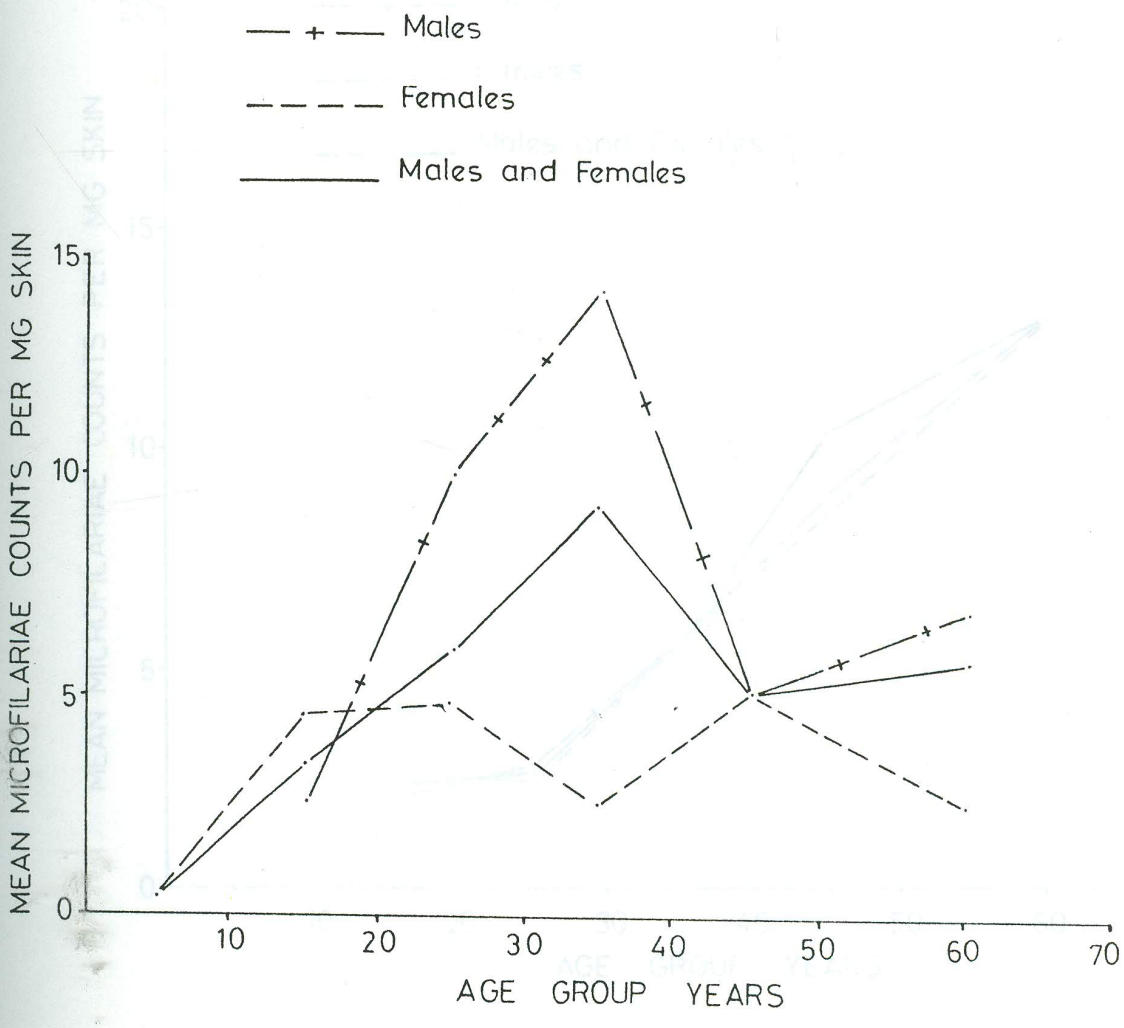


Fig.9. Age distribution of mean microfilaria counts in Ruvuma focus

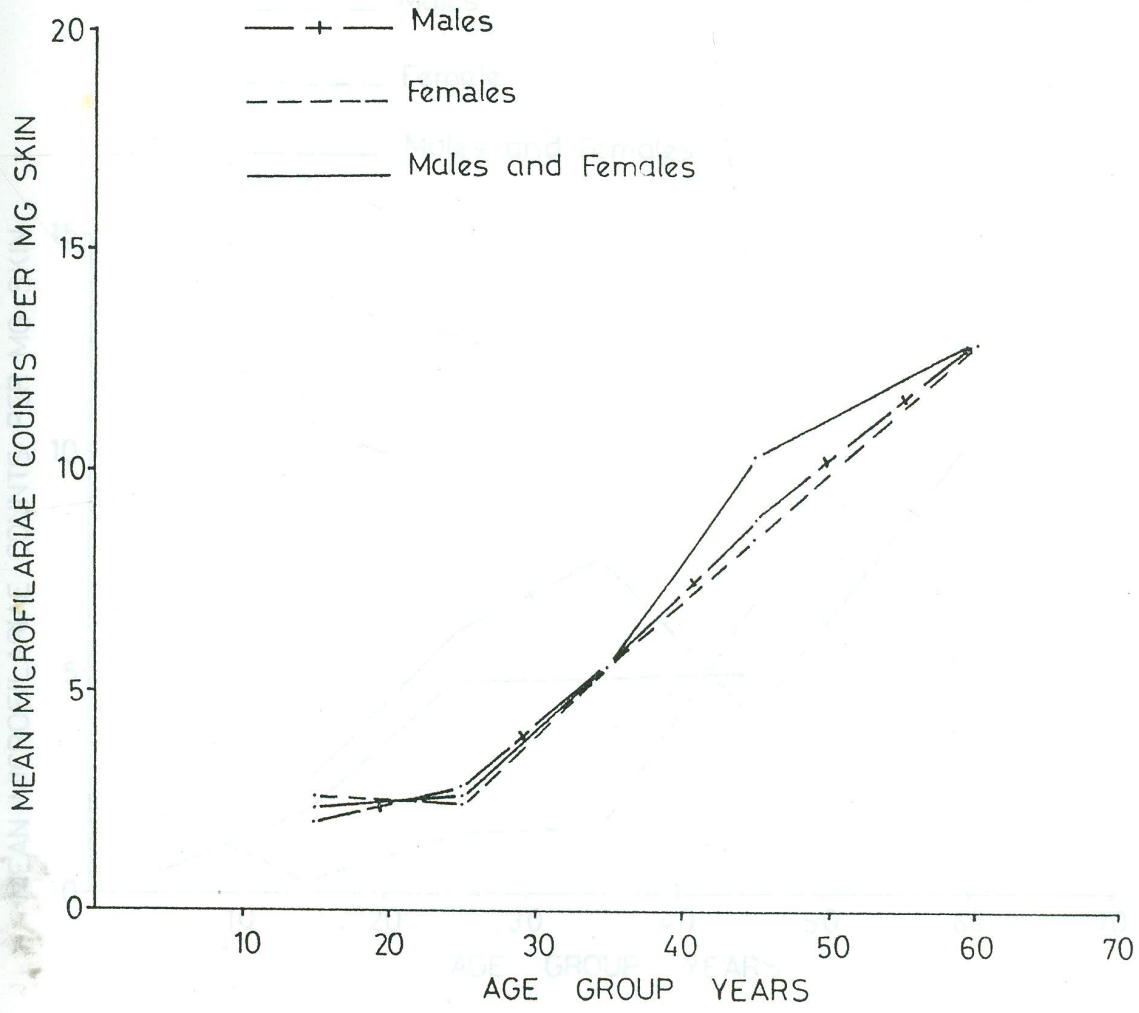


Fig.10. Age distribution of mean microfilaria counts in Kilosa focus

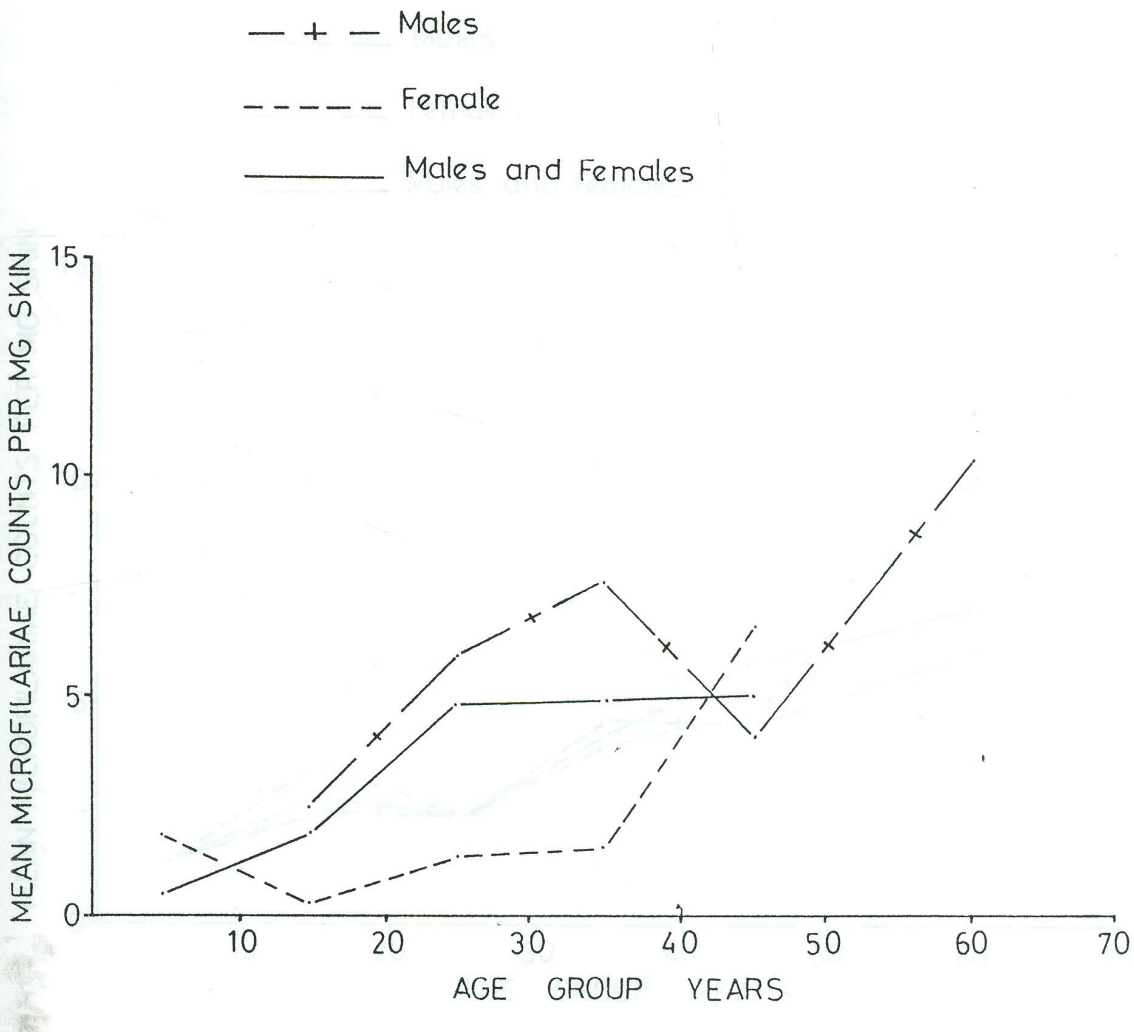


Fig.11. Age distribution of mean microfilaria counts in Tukuyu focus

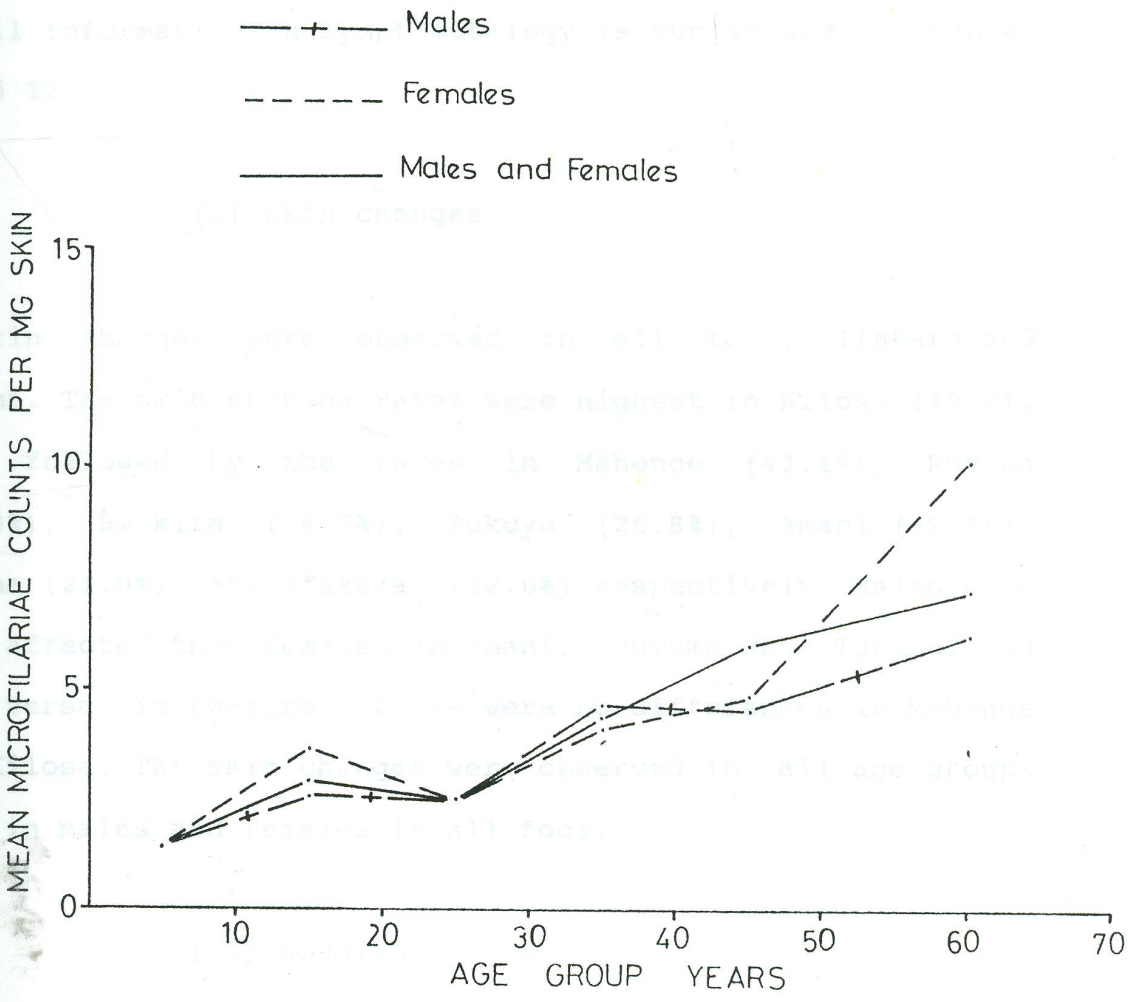


Fig.12. Age distribution of mean microfilaria counts in Amani focus

pattern of the counts as well, which were found to be high in males at the age of 20-40 years. In Amani and Kilosa, there were no marked differences of the counts in males and females and they were found to increase with age in both sexes.

b) Clinical observations

All information on symptomatology is summarised in Tables 3 and 12.

(i) Skin changes

Skin changes were observed in all foci, Ifakara and Kigoma. The skin changes rates were highest in Kilosa (49.7). Then followed by the rates in Mahenge (43.1%), Ruvuma (43.0%), Bwakira (34.7%), Tukuyu (26.8%), Amani (32.8%), Kigoma (21.0%) and Ifakara (12.0%) respectively. Males were more affected than females in Amani, Ruvuma and Tukuyu, and vice versa in Bwakira; there were no differences in Mahenge and Kilosa. The skin changes were observed in all age groups both in males and females in all foci.

(ii) Nodules

Nodules cases were found in Amani (1.3%), Bwakira (9.5%), Mahenge (5.8%), Ruvuma (7.9%), Tukuyu (2.0%) and Ifakara (1.4%); and none in Nguru and Kilosa. In Bwakira the nodules

Table 12. Age distribution of clinical manifestations

Focus	Age group	No. examined		Skin changes +ve						Nodules +ve					
				Males		Females		Both sexes		Males		Females		Both sexes	
		Males	Females	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Bwakira	0 - 9	30	13	5	13.0	5	38.5	8	22.2	4	13.3	1	7.7	5	13.9
	10 - 19	138	86	40	28.9	37	43.0	77	34.3	8	5.8	1	1.2	9	4.0
	20 - 39	47	101	17	22.0	37	36.6	54	30.3	5	10.6	6	5.9	11	7.4
	30 - 39	50	91	15	30.0	35	38.4	50	35.4	4	8.0	5	25.5	9	6.4
	40 - 49	40	71	8	47.0	26	36.6	34	38.6	5	12.5	11	15.5	16	14.4
	50+	74	36	23	33.7	22	61.1	47	42.7	16	21.6	8	22.2	24	21.8
	T o t a l	379	398	108	28.4	162	40.7	270	34.7	42	11.1	32	8.0	74	9.5
Mahenge	0 - 9	24	23	11	45.8	4	17.4	15	31.9	1	4.2	2	8.7	3	6.3
	10 - 19	53	91	27	50.9	22	24.2	49	34.0	4	7.5	0	0	4	2.8
	20 - 29	42	47	21	50.0	17	36.2	38	43.8	5	11.9	2	4.3	7	7.9
	30 - 39	30	32	12	40.0	16	50.0	28	45.2	6	20.0	0	0	6	10.0
	40 - 49	23	39	11	47.8	21	53.8	32	51.6	0	0	2	5.1	2	3.2
	50+	48	30	21	43.8	25	83.3	46	59.0	2	4.2	4	13.3	6	7.7
	T o t a l	220	262	103	46.8	105	40.1	208	43.2	18	8.2	10	3.8	28	5.8
Tukuyu	0 - 9	25	28	1	4.0	6	21.4	7	13.2	0	0	0	0	0	0
	10 - 19	75	65	5	6.6	11	16.9	16	11.4	1	1.3	0	0	1	0.7
	20 - 29	15	18	8	53.3	12	11.1	10	38.5	0	0	0	0	0	0
	30 - 39	17	20	7	41.2	11	55.0	18	48.6	0	0	0	0	0	0
	40 - 49	20	15	9	45.0	6	40.0	15	42.9	1	5.0	0	0	1	2.8
	50+	34	22	28	82.4	1	4.5	29	51.8	5	14.7	0	0	5	8.9
	T o t a l	186	168	58	31.2	37	22.0	95	26.8	7	3.8	0	0	7	2.0

Table 12. Age distribution of clinical manifestations

Focus	Age group	No. examined Males	No. examined Females	Skin changes +ve Males No.	Skin changes +ve Males %	Skin changes +ve Females No.	Skin changes +ve Females %	Both sexes No.	Both sexes %	Nodules +ve Males No.	Nodules +ve Males %	Nodules +ve Females No.	Nodules +ve Females %	Both sexes No.	Both sexes %
Bwakira	0 - 9	30	13	5	13.0	5	38.5	8	22.2	4	13.3	1	7.7	5	13.9
	10 - 19	138	86	40	28.9	37	43.0	77	34.3	8	5.8	1	1.2	9	4.0
	20 - 39	47	101	17	22.0	37	36.6	54	30.3	5	10.6	6	5.9	11	7.4
	30 - 39	50	91	15	30.0	35	38.4	50	35.4	4	8.0	5	25.5	9	6.4
	40 - 49	40	71	8	47.0	26	36.6	34	38.6	5	12.5	11	15.5	16	14.4
	50+	74	36	23	33.7	22	61.1	47	42.7	16	21.6	8	22.2	24	21.8
	T o t a l	379	398	108	28.4	162	40.7	270	34.7	42	11.1	32	8.0	74	9.5
Mahenge	0 - 9	24	23	11	45.8	4	17.4	15	31.9	1	4.2	2	8.7	3	6.3
	10 - 19	53	91	27	50.9	22	24.2	49	34.0	4	7.5	0	0	4	2.8
	20 - 29	42	47	21	50.0	17	36.2	38	43.8	5	11.9	2	4.3	7	7.9
	30 - 39	30	32	12	40.0	16	50.0	28	45.2	6	20.0	0	0	6	10.0
	40 - 49	23	39	11	47.8	21	53.8	32	51.6	0	0	2	5.1	2	3.2
	50+	48	30	21	43.8	25	83.3	46	59.0	2	4.2	4	13.3	6	7.7
	T o t a l	220	262	103	46.8	105	40.1	208	43.2	18	8.2	10	3.8	28	5.8
Tukuyu	0 - 9	25	28	1	4.0	6	21.4	7	13.2	0	0	0	0	0	0
	10 - 19	75	65	5	6.6	11	16.9	16	11.4	1	1.3	0	0	1	0.7
	20 - 29	15	18	8	53.3	12	11.1	10	38.5	0	0	0	0	0	0
	30 - 39	17	20	7	41.2	11	55.0	18	48.6	0	0	0	0	0	0
	40 - 49	20	15	9	45.0	6	40.0	15	42.9	1	5.0	0	0	1	2.8
	50+	34	22	28	82.4	1	4.5	29	51.8	5	14.7	0	0	5	8.9
	T o t a l	186	168	58	31.2	37	22.0	95	26.8	7	3.8	0	0	7	2.0

Table 12 (cont).

	0 - 9	7	4	2	25.0	3	75.0	5	41.6	0	0	0	0	0	0
	10 - 19	18	20	5	16.6	8	40.0	11	28.9	1	5.6	0	0	1	0
	20 - 29	13	30	9	69.2	7	25.3	16	37.2	0	0	0	0	0	0
Ruvuma	30 - 39	17	24	8	47.0	15	62.5	23	56.1	0	0	3	12.5	3	0
	40 - 49	12	20	7	33.3	13	65.0	17	53.1	2	16.7	3	15.0	5	0
	50+	46	16	22	47.8	11	68.8	33	53.2	4	8.7	5	43.8	9	0
	T o t a l	113	115	51	42.1	47	35.6	98	43.0	7	19.6	11	11.3	18	7.9
	0 - 9	113	104	15	13.3	6	5.7	21	9.6	0	0	0	0	0	0
	10 - 19	115	162	25	21.7	7	4.3	32	11.5	0	0	3	1.9	0	0
	20 - 29	69	82	35	50.7	14	17.1	49	32.4	0	0	1	1.2	0	0
Amani	30 - 39	48	44	22	45.0	16	36.4	38	42.3	2	4.2	1	2.3	0	0
	40 - 49	22	20	13	59.1	19	95.0	32	76.1	2	9.1	1	5.0	0	0
	50+	24	17	12	50.0	11	64.7	23	56.0	1	4.2	0	0	0	0
	T o t a l	391	429	122	31.2	73	17.0	1195	23.8	5	1.2	6	1.3	11	1.3
	0 - 9	2	4	0	0	0	0	0	0	0	0	0	0	0	0
	10 - 19	14	25	1	7.1	4	29.0	5	13.0	0	0	0	0	0	0
	20 - 29	4	5	1	25.0	4	80.0	5	55.5	0	0	0	0	0	0
Nguru	30 - 39	17	6	0	0	0	0	0	0	0	0	0	0	0	0
	40 - 49	4	13	2	50.0	9	69.2	11	64.7	0	0	0	0	0	0
	50+	13	7	9	69.2	6	85.7	15	75.0	0	0	1	14.3	0	0
	T o t a l	54	60	12	22.2	19	31.7	51	27.1	0	0	1	1.6	1	0.8
	0 - 9	34	20	13	38.2	8	40.0	21	38.8	0	0	0	0	0	0
	10 - 19	41	47	22	53.6	18	38.2	40	45.5	0	0	0	0	0	0
	20 - 29	12	28	9	75.0	13	46.4	22	55.0	0	0	0	0	0	0
Kilosa	30 - 39	23	32	11	47.8	12	37.5	23	41.8	0	0	0	0	0	0
	40 - 49	18	20	13	72.2	11	55.5	24	63.3	0	0	0	0	0	0
	50+	24	19	10	41.7	18	94.7	28	65.1	0	0	0	0	0	0
	T o t a l	152	166	78	52.7	80	45.2	158	49.7	0	0	0	0	0	0

were found in all age groups; whereas in other foci, the age distribution varied.

2. Development of the IHA test

a) Establishment of antigen bank

(i) Adult O.gutturosa and O.volvulus

A six months collection of ligamentum nuchae of cattle from Korogwe abbatoir, gave 1.1 gm of adult O.gutturosa. A total of 41 nodules were dissected and 31 (75.6%) of them were found to contain 0.52 gm of adult O.volvulus.

(ii) Feeding trial of S.vorax on the cow infected with O.gutturosa

In five feeding trials, a total of 310 flies were allowed to feed and 43 (13.8%) out of these, took the infective blood meal. These fed flies were identified by their distended abdomen (Wenk and Raybould, 1971); subsquently they were dissected and 8 (18.6%) out of them had picked up the microfilariae. Average number of microfilariae per fly was 2.3. The remaining 267 which did not take up the infective blood-meal; were also dissected and none of them had the microfilariae.

This average number 2.3 of the microfilariae per fly however, was considered to be too small for the subsquent recovery of sufficient amount of the third stage larvae, when taking into account of losses that might occur due to digestion and other refrectory mechanism, during the developing period of six days and above after the infective blood-meal.

b) Procedure for the IHA test

Haemagglutination was assessed as plus (+) or minus (-) sign as follows:-

+++ maximum)	
++ medium)	haemagglutination
+ low)	
+ - least)	
-)	no haemagglutination

The haemagglutination pattern from above assessment is illustrated in Plate 3. Table 13 shows the IHA titres (serum dilutions) which were obtained with sera for cross-reaction analysis using crude soluble antigens of both adult O.volvulus and O.gutturosa respectively. From this Table 13 it can be seen that a haemagglutinating maximum titre of 1/640 was obtained with serum of a person infected with W.bancrofti

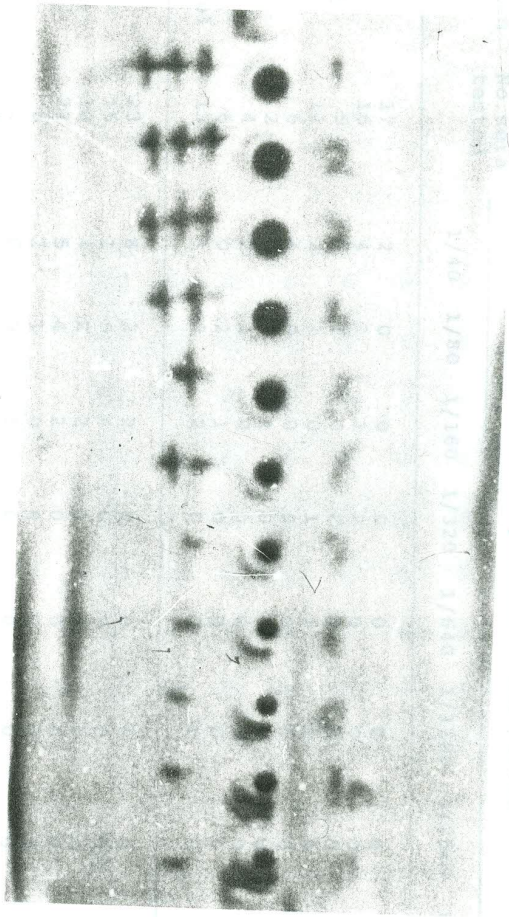


Plate 3. Haemagglutination pattern

Table 13. IHA titres with sera of people infected with S.haematobium A.duodenale, A.lumbricoides, W.bancrofti, D.perstans, T.trichiura, S.stercoralis and N.americanus using crude O.volvulus and O.gutturosa antigens

Antigen	Type of Infection	No.sera tested	No. sera within respective IHA titres							
			1/40	1/80	1/160	1/320	1/640	1/1280	1/2560	1/5120
O.volvulus	S.haematobium	17	2	0	0	0	0	0	0	0
	A.duodenale	16	4	1	1	2	0	0	0	0
	A.lumbricoides	5	0	1	0	0	0	0	0	0
	W.bancrofti	11	2	1	0	1	1	0	0	0
	D.perstans	6	4	0	0	0	0	0	0	0
	T.trichiura	2	0	0	1	0	0	0	0	0
	S.stercoralis	4	2	1	0	0	0	0	0	0
	N.americanus	6	0	2	1	0	0	0	0	0
	Negative control	13	1	3	2	0	0	0	0	0
O.gutturosa	S.haematobium	23	8	2	2	0	0	0	0	0
	A.duodenale	15	1	6	4	0	0	0	0	0
	A.lumbricoides	12	4	2	2	0	0	0	0	0
	W.bancrofti	21	5	4	2	0	0	0	0	0
	D.perstans	6	2	1	0	0	0	0	0	0
	T.trichiura	2	0	0	1	0	0	0	0	0
	S.stercoralis	4	0	2	0	0	0	0	0	0
	N.americanus	6	2	1	0	0	0	0	0	0
	Negative control	16	4	4	0	0	0	0	0	0

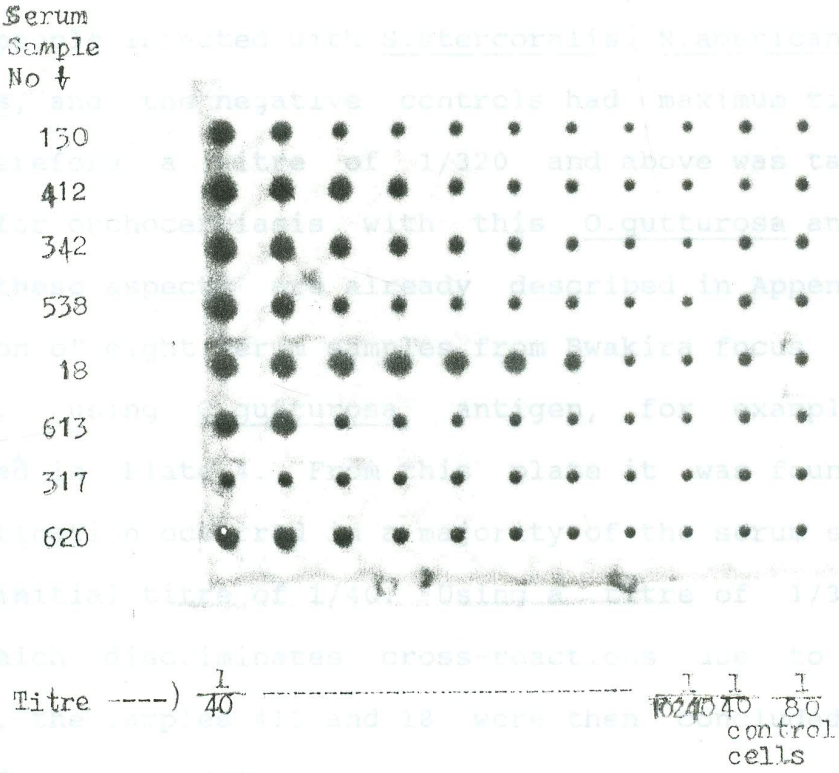


Plate 4. Examination of 8 serum samples by the IHA test

when tested with the O.volvulus antigen. Sera of people infected with other helminths however, had maximum titres between 1/40 and 1/320. With negative controls, a maximum titre of 1/160 was obtained. From these results with the O.volvulus antigen, a titre of 1/1280 and above was taken as positive for onchocerciasis. With O.gutturosa antigen, a titre of 1/160 was maximum with sera of people infected with S.haematobium; A.doudenale; A.lumbricoides, and W.bancrofti. Sera of people infected with S.stercoralis, N.americanus and D.perstans, and the negative controls had maximum titre of 1/80. Therefore a titre of 1/320 and above was taken as positive for onchocerciasis with this O.gutturosa antigen. Some of these aspects are already described in Appendix 5. Examination of eight serum samples from Bwakira focus by the IHA test, using O.gutturosa antigen, for example, is illustrated in Plate 4. From this plate it was found that haemagglutination occurred in a majority of the serum samples from the initial titre of 1/40. Using a titre of 1/320 and above, which discriminates cross-reactions due to other helminths, the samples 412 and 18 were then concluded to be positive for onchocerciasis.

The antigens of both worm species gave a maximum titre of 1/5120 with sera of people infected with the O.volvulus and the overall results of IHA test with batch of sera collected from the onchocerciasis areas of Bwakira, are shown in Table 14. There were 15 (26.7%) and 31 (63.2%) IHA positive sera

Table 14. IHA test with sera collected from people of Bwakira areas

Antigen	No. sera tested	No. sera *O.volvulus* positive	% *O.volvulus* positive
O.volvulus	56	15	26.8
O.gutturosa	49	31	63.3

with these O.volvulus and O.gutturosa antigen respectively.

3. Serological Observations

High IHA positive rates were obtained in Bwakira (89.0%), Kilosa (74.1%), Amani (70.1%) and Ruvuma (55.2%). In Mahenge, Tukuyu and Nguru lower rates of 27.4%, 20.1% and 8.5% were obtained respectively as shown in Table 15. The rates for males and females were nearly the same in each focus. The microfilaria rates in the population and in the IHA sample are presented in Table 16.

Relationship of the IHA test positivity and negativity with respect to skin snip positivity and negativity were assessed. High sensitivity (proportion of IHA positive among microfilaria positive) of the IHA test was detected in Bwakira, Amani, Kilosa and Ruvuma. Whereas low sensitivity was detected in Nguru, Tukuyu and Mahenge (Table 17). Significant association between the IHA test and skin snip method was observed in Bwakira, Amani, Kilosa, Ruvuma and Nguru; and not in Mahenge and Tukuyu (Tables 18-24).

Geometric mean of IHA reciprocal titres (GMRT) and corresponding geometric mean microfilarial counts (GMMF) per mg skin were found to be parallel to each other within respective reciprocal titres, as shown in Table 25. The corresponding GMRT and GMMF were found to be high at 1280 and

Table 15. IHA positive in males and females

Focus	Sera examined			Sera positives				Total Positives	
	Males No.	Females No.	Total	Males No.	%	Females No.	%	No.	%
Bwakira	80	74	154	71	88.8	66	89.1	137	89.0
Mahenge	52	45	97	13	25.0	11	24.4	24	24.7
Tukuyu	18	11	29	4	22.2	2	18.1	6	20.7
Amani	46	11	57	32	69.6	8	72.7	40	70.1
Kilosa	39	42	81	30	76.9	30	71.4	60	74.1
Ruvuma	51	45	96	27	52.9	26	57.7	53	55.2
Nguru	26	33	59	2	7.7	3	9.0	5	8.5

Table 16. Microfilaria (mf) in the IHA sample and in the population

Focus	% mf in IHA sample	% standardized mf in the population
Bwakira	95.5	63.6
Mahenge	70.1	58.6
Ruvuma	36.5	31.9
Tukuyu	58.9	22.8
Kilosa	40.7	20.6
Amani	49.1	22.4
Nguru	13.6	5.8

Table 17. Relationships of the IHA test positivity with respect to skin snip positivity and negativity in the IHA sample, in different foci

Focus	% mf+ve	% IHA+ve	IHA+ve/ mf-ve (x 100)	IHA-ve/ mf+ve (x 100)	*IHA+ve mf+ve (x 100)	IHA-ve/ mf-ve (x 100)
Bwakira	95.5	89.0	66.6	10.3	89.7	33.3
Amani	49.1	70.1	50.0	8.0	93.7	51.9
Kilosa	40.7	74.1	52.2	18.1	81.8	47.8
Ruvuma	36.5	55.2	46.3	25.0	75.0	55.7
Nguru	13.6	8.5	4.0	62.5	27.8	90.7
Tukuyu	58.6	20.7	10.0	72.2	27.8	90.0
Mahenge	70.1	27.4	20.7	73.5	26.5	79.5

*Sensitivity

Table 18. Association between IHA test and skin snip method in Bwakira focus

	Skin snip positive	Skin snip negative
IHA positive	70	4
IHA negative	8	2
T o t a l	78	6

X^2 = 2.89 degree of freedom = 1 p > 0.05

Significant

Table 19. Association between IHA test and skin snip method in Amani focus

	Skin snip positive	Skin snip negative
IHA positive	27	13
IHA negative	2	14
T o t a l	29	27

X^2 = 13.84 degree of freedom = 1 p < 0.0001

Significant

Table 20. Association between IHA test and skin snip method in Kilosa focus

	Skin snip positive	Skin snip negative
IHA positive	18	24
IHA negative	4	22
T o t a l	22	46

$X^2 = 5.54$ degree of freedom = 1 $p > 0.01$

Significant

Table 21. Association between IHA test and skin snip method in Ruvuma focus

	Skin snip positive	Skin snip negative
IHA positive	24	31
IHA negative	8	36
T o t a l	32	56

X^2 = 7.24 degree of freedom = 1 0.01 < p > 0.001

Significant

Table 22. Association between IHA test and skin snip method in Nguru focus

	Skin snip positive	Skin snip negative
IHA positive	3	2
IHA negative	5	48
T o t a l	8	50

Fisher's exact test: p = 0.015

Significant

Table 23. Association between IHA test and skin snip method in Tukuyu focus

	Skin snip positive	Skin snip negative
IHA positive	5	1
IHA negative	13	10

$X^2 = 1.45$ degree of freedom = 1 $p > 0.1$

Not significant

Table 24. Association between IHA test and skin snip method in Mahenge focus

	Skin snip positive	Skin snip negative
IHA positive	18	6
IHA negative	50	23
T o t a l	68	29

X^2 = 0.37 degree of freedom = 1 p > 0.1

Not significant

Table 25. Geometric mean of IHA reciprocal titres (GMRT) and respective geometric mean of microfilaria counts (GMMF) per mg skin

IHA reciprocal titre		Foci					
	Bwakira	Mahenge	Ruvuma	Tukuyu	Kilosa	Amani	Nguru
320	No.sera						
positive	17	6	7	3	10	6	4
GMRT	2.0	1.4	1.5	1.8	2.0	1.8	1.5
GMMF	1.4	1.2	1.2	1.2	1.3	1.2	1.1
640	No.sera						
positive	36	10	19	0	18	4	0
GMRT	4.5	2.0	3.5	0	4.2	1.6	0
GMMF	2.8	1.4	2.3	0	2.3	1.0	0
1280	No.sera						
positive	43	4	12	3	13	8	1
GMRT	7.4	1.3	2.4	2.1	3.2	3.9	1.1
GMMF	3.6	1.2	1.4	1.1	1.7	1.7	0
2560	No.sera						
positive	12	2	5	0	7	7	0
GMRT	1.8	1.2	1.5	0	2.0	2.6	0
GMMF	1.2	1.0	1.0	0	1.1	1.5	0
5120	No.sera						
positive	29	3	10	0	12	15	0
GMRT	4.1	1.1	2.4	0	3.5	9.5	0
GMMF	2.4	1.0	1.3	0	1.9	2.3	0

Table 26. Geometric mean of IHA reciprocal titres (GMRT) and geometric mean of Microfilaria counts (GMMF) per mg skin, within age groups

Focus	Age group (years)		IHA reciprocal titre 320	640	1240	2560	5120
Bwakira	0 - 19	No.sera positive	11	25	18	6	20
		GMRT	1.5	2.9	2.3	1.5	3.0
		GMMF	1.2	1.6	1.7	1.0	1.7
	20 - 39	No.sera positive	3	9	18	1	4
		GMRT	1.1	1.5	2.2	1.1	1.2
		GMMF	1.0	1.2	1.5	0	1.1
	40+	No.sera positive	4	4	7	3	3
		GMRT	1.2	1.2	1.4	1.2	1.2
		GMMF	1.0	1.1	1.2	1.0	1.0
Mahenge	0 - 19	No.sera positive	2	2	0	1	2
		GMRT	1.1	1.1	0	1.1	1.2
		GMMF	1.0	1.0	0	0	1.0
	20 - 39	No.sera positive	1	1	2	1	2
		GMRT	1.1	1.1	1.2	1.1	1.2
		GMMF	0	1.0	1.0	1.0	0

Table 26 (cont.)

	40+	No.sera					
		positive	2	6	2	1	0
		GMRT	1.1	1.4	1.2	1.1	0
		GMMF	1.0	1.3	1.0	1.0	0
Amani	0 - 19	No.sera					
		positive	1	1	1	1	1
		GMRT	1.1	1.1	1.1	1.1	1.1
		GMMF	0	0	0	0	0
	20 - 39	No.sera					
		positive	4	2	5	4	11
		GMRT	1.5	1.3	1.9	1.7	5.2
		GMMF	1.1	0	1.3	1.3	2.0
	40+	No.sera					
		positive	3	0	3	1	2
		GMRT	1.4	0	1.5	1.1	1.3
		GMMF	1.6	0	1.1	0	1.0
Kilosa	0 - 19	No.sera					
		positive	4	7	3	2	4
		GMRT	1.3	1.7	1.3	1.2	1.5
		GMMF	1.0	3.6	0	0	1.0
	20 - 39	No.sera					
		positive	3	7	8	1	2
		GMRT	1.2	1.7	2.0	1.1	1.2
		GMMF	1.1	1.7	2.0	0	0

Table 26 (cont.)

	40+	No.sera					
		positive	3	4	3	3	6
		GMRT	1.2	1.4	1.3	1.3	1.3
Ruvuma	0 - 19	No.sera					
		positive	2	3	5	3	4
		GMRT	1.1	1.2	1.4	1.3	1.4
		GMMF	0	1.0	1.1	0	1.0
	20 - 39	No.sera					
		positive	2	10	4	0	4
		GMRT	1.1	1.9	1.3	0	1.4
		GMMF	0	1.4	1.0	0	1.1
	0+	No.sera					
		positive	2	6	4	2	2
		GMRT	1.1	1.5	1.3	1.2	1.2
		GMMF	0	1.3	1.3	0	0

5120 reciprocal titres in Bwakira, Amani and Kilosa. Whereas in Ruvuma, they were high at 640 and 5120, in Mahenge, the GMRT and GMMF were high at 640 and then decreased as the reciprocal titres increased in the respective order of 1280, 2560 and 5120. The trend of GMRT and GMMF values within age groups is shown in Table 26. The corresponding values were generally high at the 0-19 years age group in Bwakira; and in Amani at the age group of 20-39 years specifically at 1280, 2560 and 5120 reciprocal titres.

In Bwakira, the proportions of people with mean microfilaria counts $\geq$ 6.3 per mg skin were found to increase with the IHA titres as shown in Table 27. Whereas the proportions of those with mean microfilaria counts < 6.3 per mg skin were decreasing as the titres were increasing.

The age distribution of microfilaria rates and respective IHA rates in the IHA samples, in the foci, is shown in Tables 28-37 and Figs. 13-17. Using this distribution, the foci could be classified into groups. In the first group, microfilaria rates are higher than IHA rates as observed in Bwakira and Mahenge. In the second group, microfilaria rates are lower than the IHA rates as observed in Ruvuma, Kilosa and Amani. The confidence intervals between the IHA and microfilaria rates in these foci (Table 38) include each other showing that they only differ in a multiplying factor implying that they are mirror images of one another. There

was a positive correlation between microfilaria and IHA rates with age in Bwakira, Mahenge, Kilosa and Ruvuma. In Amani however, where there was low and negative correlation(r = 0.24). The IHA and microfilaria rates were generally high in all age groups in Bwakira focus. In Mahenge, Kilosa and Ruvuma, the rates were found to increase with age. In Amani, the rates were showing a slight decline with age.

The association between the IHA titres and skin changes in Bwakira, Kilosa, Amani, Ruvuma and Mahenge are shown in Tables 39-43. There was significant association between IHA titres and the skin changes in Bwakira and Kilosa.

Table 27. IHA reciprocal titres and microfilaria (mf) counts in Bwakira focus

IHA reciprocal titres	Total examined	Those with mf counts < 6.3 per mg skin		Those with mf counts ≥ 6 per mg skin	
		No.	%	No.	%
320	13	8	61.5	5	38.5
320	19	10	52.6	9	47.4
640	35	16	45.7	19	54.3
1280	38	15	39.5	23	60.5
2560)) 5120)	34	13	38.2	21	61.8

Table 28. Age distribution of microfilaria (mf) rates in the IHA sample in Bwakira focus

Coded age (t)	Age group (years)	No. examined	No. mf positive	No. mf positive
1	0 - 9	8	8	100
2	10 - 19	79	76	96.2
3	20 - 29	20	16	80.0
4	30 - 39	21	21	100.0
5	40 - 49	10	10	100.0
7	50+	16	16	100.0
T o t a l		154	147	95.5

Linear regression estimates:-

Microfilaria rates = 95.16 - 0.04t

r = 0.60

Table 29. Age distribution of IHA test in Bwakira focus

Coded age (t)	Age group (years)	No. tested	No. positive	% positive
1	0 - 9	8	7	87.5
2	10 - 19	79	74	93.7
3	20 - 29	20	16	80.0
4	30 - 39	21	19	90.0
5	40 - 49	10	9	90.0
7	50+	16	12	75.0
T o t a l		154	137	89.0

Linear regression estimates:-

IHA = 92.82 + 1.83t

r = 0.55

Table 30. Age distribution of microfilaria (mf) rates in the IHA sample in Mahenge focus

Coded age (t)	Age group (years)	No. tested	No. mf positive	% mf positive
1	0 - 9	13	7	53.8
2	10 - 19	15	10	66.7
3	20 - 29	16	9	56.3
4	30 - 39	17	11	64.7
5	40 - 49	17	14	82.4
7	50+	19	17	89.5
T o t a l		97	68	70.1

Linear regression estimates:-

Microfilaria rates = 47.28 + 5.90t

r = 0.89

Table 31. Age distribution of IHA test in Mahenge focus

Coded age (t)	Age group (years)	No. tested	No. positive	% positive
1	0 - 9	13	1	7.7
2	10 - 19	15	4	26.7
3	20 - 29	16	4	25.0
4	30 - 39	17	3	17.6
5	40 - 49	17	7	41.2
7	50+	19	5	26.3
T o t a l		97	24	24.7

Linear regression estimates:-

IHA = 13.60 + 2.86t

r = 0.56

Table 32. Age distribution of microfilaria (mf) rates in the IHA sample in Ruvuma focus

Coded age (t)	Age group (years)	No. examined	No. mf positive	% mf positive
1	0 - 9	7	0	0
2	10 - 19	30	9	30
3	20 - 29	16	6	37.5
4	30 - 39	14	7	50.0
5	40 - 49	10	6	60.0
7	50+	19	7	36.8
T o t a l		96	35	36.5

Linear regression estimates:-

Microfilaria rates = 13.04 + 6.19t

r = 0.65

Table 33. Age distribution of IHA test in Ruvuma focus

Coded age (t)	Age group (years)	No. tested	No. positive	% positive
1	0 - 9	7	3	42.9
2	10 - 19	30	14	46.7
3	20 - 29	16	10	62.5
4	30 - 39	14	9	64.3
5	40 - 49	19	6	31.6
7	50+	19	11	57.9
T o t a l		96	53	55.2

Linear regression estimates:-

IHA = 46 + 2.59t

r = 0.64

Table 34. Age distribution of microfilaria (mf) rates in the IHA sample in Kilosa focus

Coded age (t)	Age group (years)	No. examined	No. mf positive	% mf positive
1	0 - 9	12	2	16.7
2	10 - 19	19	7	36.8
3	20 - 29	14	4	28.6
4	30 - 39	14	8	57.1
5	40 - 49	11	5	45.5
7	50+	11	7	63.6
T o t a l		81	33	40.7

Linear regression estimates:-

Microfilaria rates = 15.23 + 7.14t

r = 0.85

Table 35. Age distribution of IHA test in Kilosa focus

Coded age (t)	Age group (years)	No. tested	No. positive	% positive
1	0 - 9	12	6	30.0
2	10 - 19	19	14	73.7
3	20 - 29	14	10	71.4
4	30 - 39	14	11	78.6
5	40 - 49	11	9	81.8
7	50+	11	10	90.9
T o t a l		81	60	74.1

Linear regression estimates:-

IHA = 53.26 + 5.76t

r = 0.90

Table 36. Age distribution of microfilaria (mf) rates in the IHA sample in Amani focus

Coded age (t)	Age group (years)	No. examined	No. mf positive	% mf positive
1	10 - 19	6	3	50.0
2	20 - 29	25	13	52.0
3	30 - 39	15	7	46.7
4	40 - 49	6	3	50.0
6	50+	5	2	40.0
T o t a l		57	28	49.1

Linear regression estimates:-

Microfilaria rates = 54 - 2t

r = 0.81

Table 37. Age distribution of IHA test in Amani focus

Coded age (t)	Age group (years)	No. tested	No. mf positive	% mf positive
1	10 - 19	6	5	83.3
2	20 - 29	25	16	64.0
3	30 - 39	15	10	66.7
4	40 - 49	6	5	93.5
6	50+	5	4	80.0
T o t a l		57	40	70.1

Linear regression estimates:-

IHA = 71.73 + 1.16t

r = 0.24

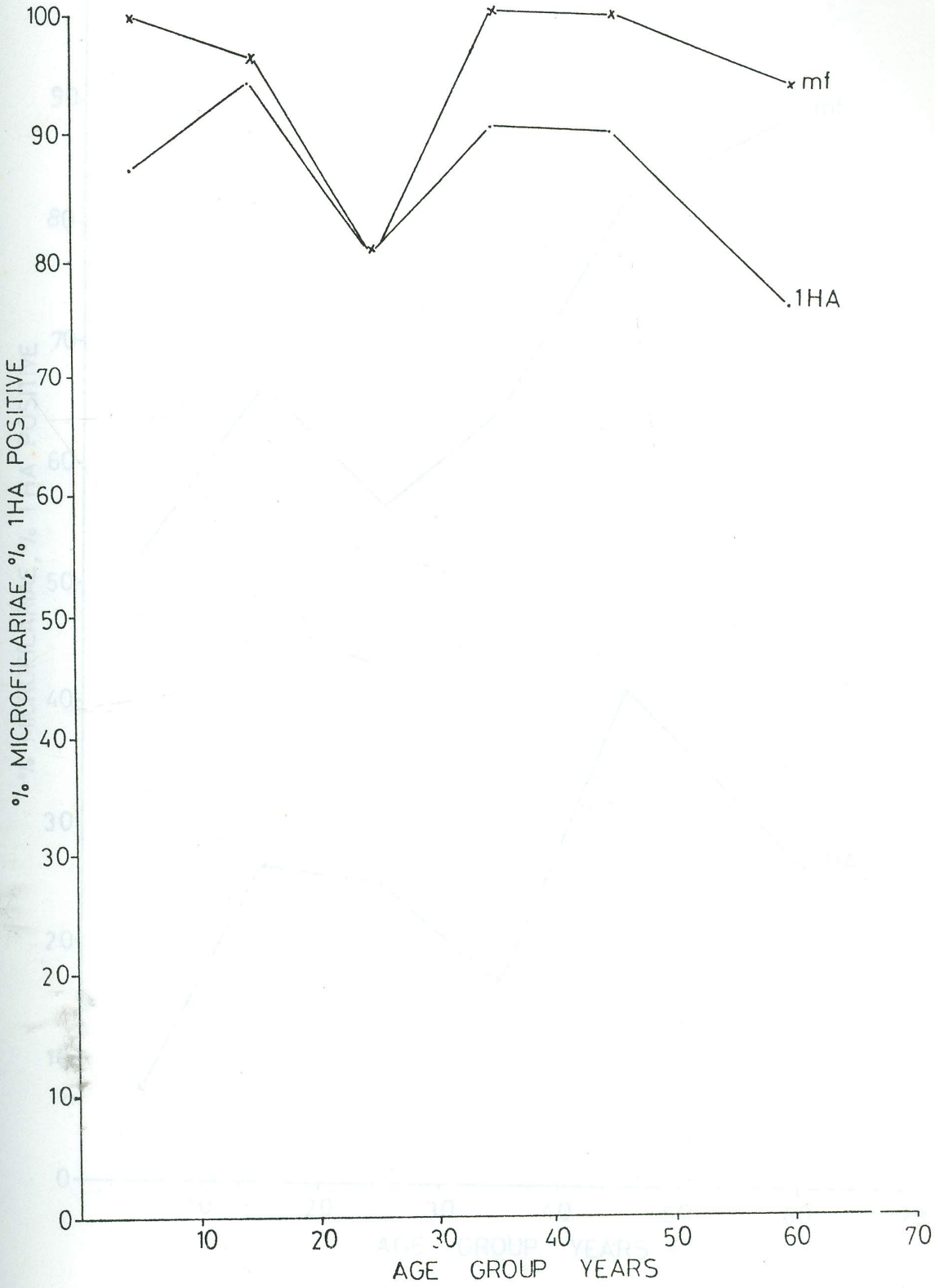


Fig.13. Age distribution of microfilaria rates in the IHA sample in Bwakira focus

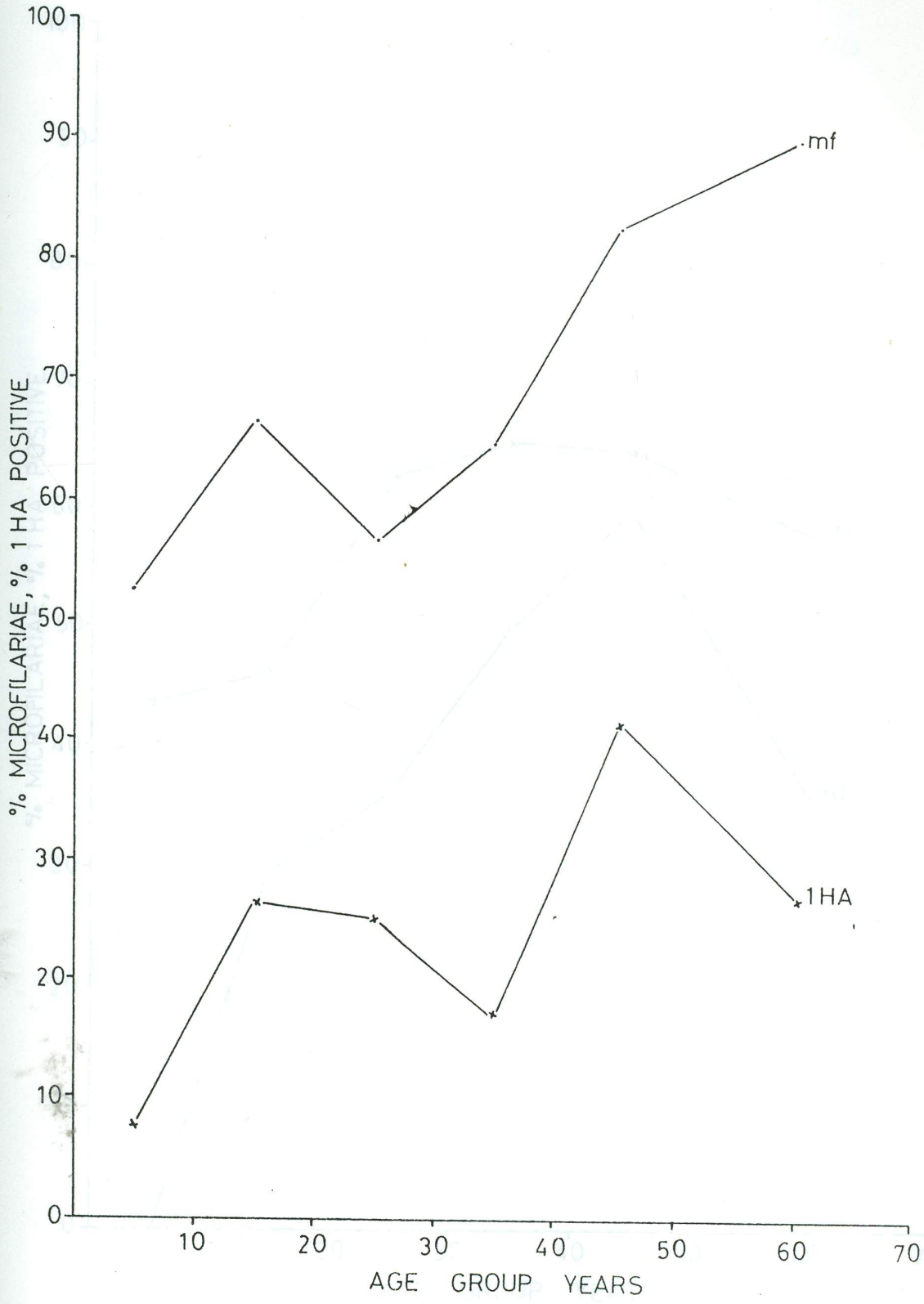


Fig.14. Age distribution of microfilaria rates in the IHA sample in Mahenge focus

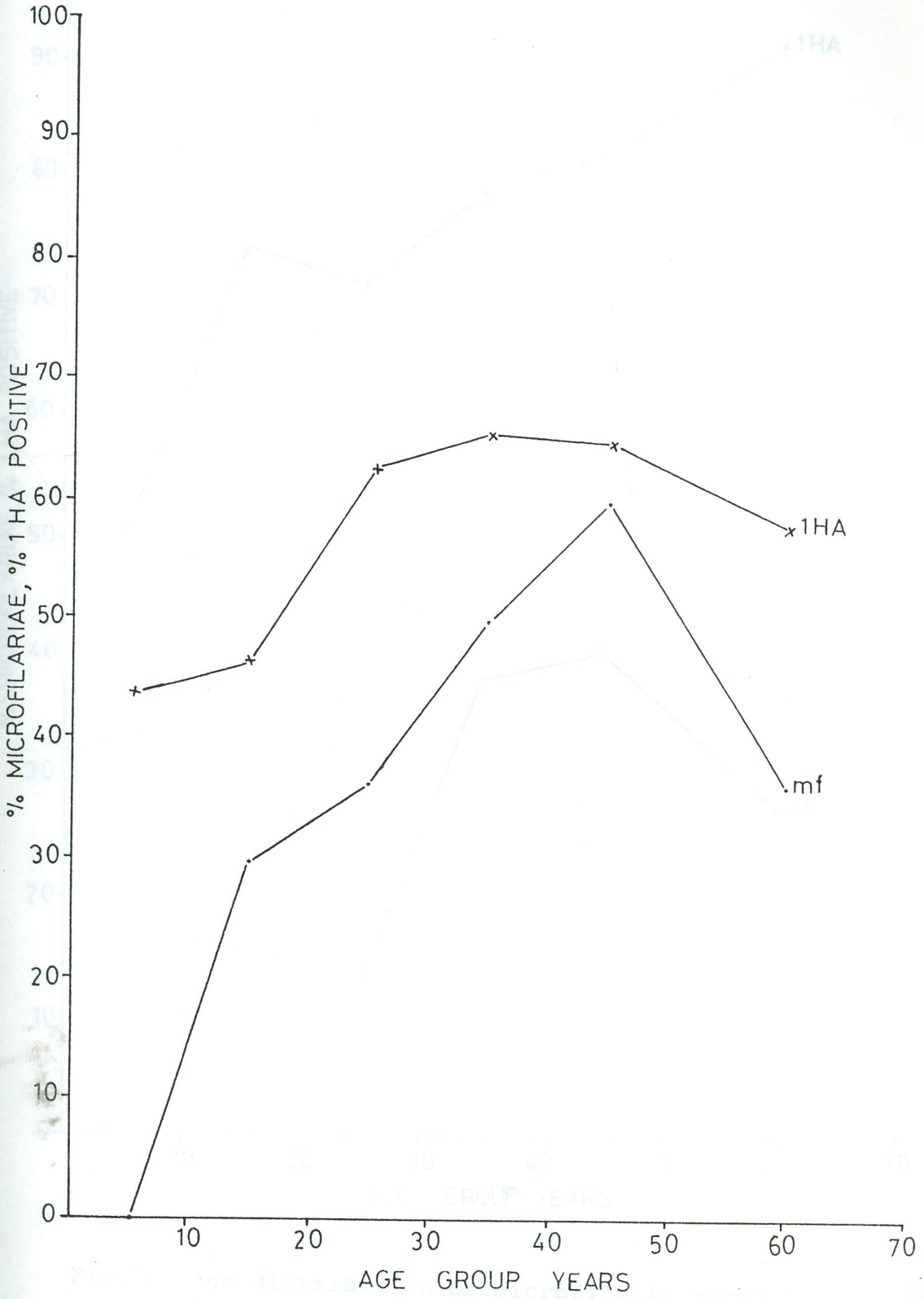


Fig.15. Age distribution of microfilaria rates in the IHA sample in Ruvuma focus

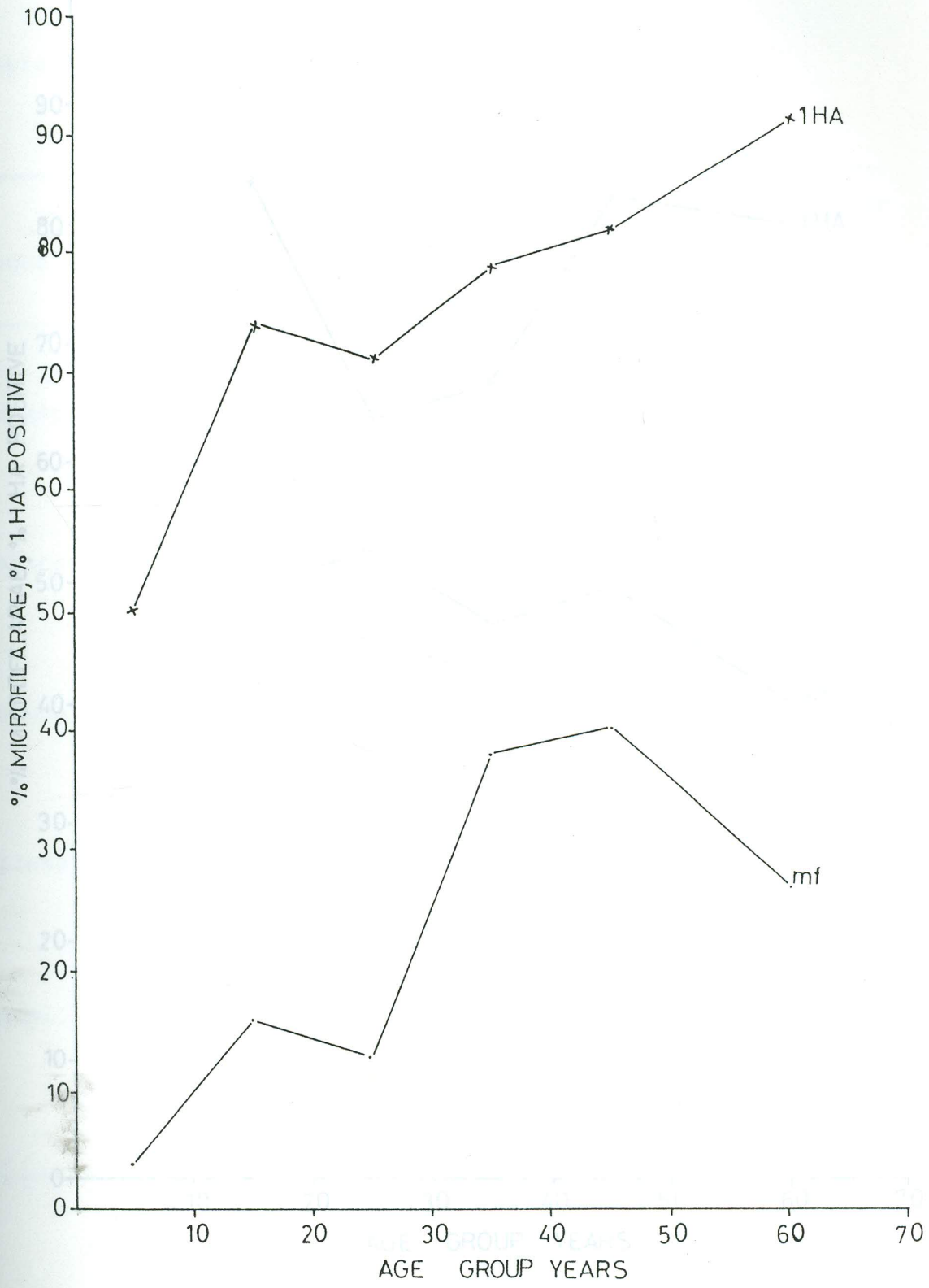


Fig.16. Age distribution of microfilaria rates in the IHA sample in Kilosa focus

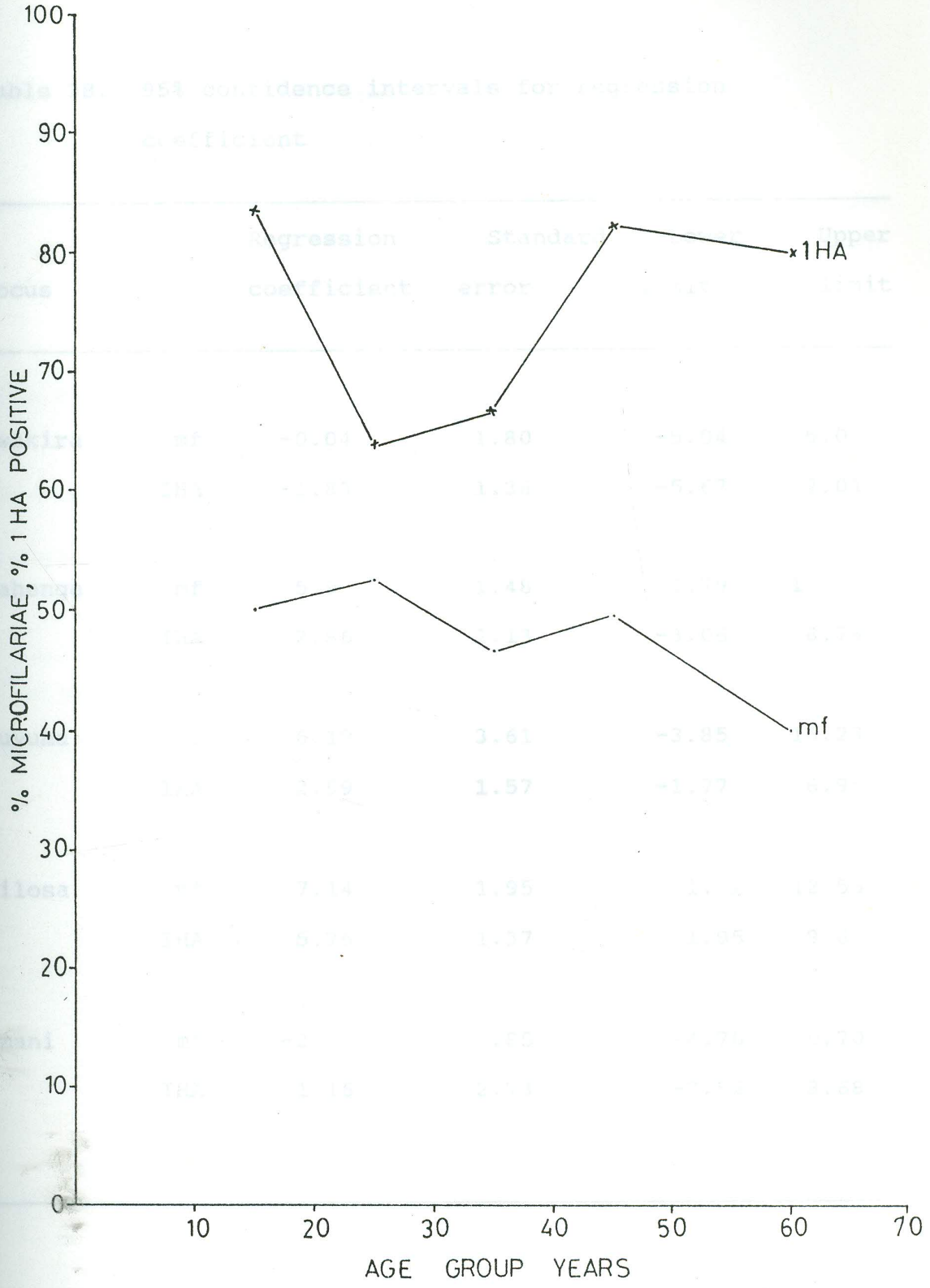


Fig.17. Age distribution of microfilaria rates in the IHA sample in Amani focus

Table 38. 95% confidence intervals for regression coefficient

Focus		Regression coefficient	Standard error	Lower limit	Upper limit
Bwakira	mf	-0.04	1.80	-5.04	5.0
	IHA	-1.83	1.38	-5.67	2.01
Mahenge	mf	5.9	1.48	1.79	10.01
	IHA	2.86	2.13	-3.06	8.78
Ruvuma	mf	6.19	3.61	-3.85	16.23
	IHA	2.59	1.57	-1.77	6.95
Kilosa	mf	7.14	1.95	1.72	12.56
	IHA	5.76	1.37	1.95	9.6
Amani	mf	-2	.85	-4.70	0.70
	IHA	1.16	2.73	-7.52	8.68

Table 39. Association between IHA reciprocal titres and skin changes in Bwakira focus

	Reciprocal titres 1280, 2560, 5120	Reciprocal titres 320, 640
Skin changes positive	24	5
Skin changes negative	30	76
T o t a l	54	81

X^2 = 28.13 degree of freedom = 1 $p < 0.001$

Significant

Table 40. Association between IHA reciprocal titres and skin changes in Kilosa focus

	Reciprocal titres 1280, 2560, 5120	Reciprocal titres 320, 640
Skin changes positive	19	9
Skin changes negative	9	19
T o t a l	28	28

X^2 = 7.14 degree of freedom = 1 $0.01 < p > 0.001$

Significant

Table 41. Association between IHA reciprocal titres and skin changes in Amani focus

	Reciprocal titres 1280, 2560, 5120	Reciprocal titres 320, 640
Skin changes positive	1	10
Skin changes negative	8	29
T o t a l	9	39

Fischer's exact test: p = 0.21

Not significant

Table 42. Association between IHA reciprocal titres and skin changes in Ruvuma focus

	Reciprocal titres 1280, 2560, 5120	Reciprocal titres 320, 640
Skin changes positive	3	3
Skin changes negative	22	13
T o t a l	25	16

Fischer's exact test: p = 0.43

Not significant

Table 43. Association between IHA reciprocal titres and skin changes in Mahenge

	Reciprocal titres 1280, 2560, 3120	Reciprocal titres 320, 640
Skin changes positive	7	4
Skin changes negative	8	5
T o t a l	15	9

Fischer's exact test: p = 0.7

Not significant

V. DISCUSSION

1. Parasitological and clinical observations

The parasitological data obtained in this study, provides information on the general distribution of onchocerciasis in the country particularly in Kigoma region where the presence of the disease was still doubtful; and along the Kilombero areas which appear to form a connection between Kilosa and Mahenge foci. The microfilaria rates (Tables 3 and 4) were found to be much higher in Bwakira, Mahenge and Ruvuma, and lower in Amani when compared with those reported by Wegesa (1969, 1970a). These high or low microfilaria rates indicated the increase or reduction of transmission of onchocerciasis in these respective foci. These are significant observations for those who advocate the need for control of the disease in the country. Causes for the increase on the transmission are speculative. In Bwakira, Mahenge and Ruvuma, S.damnosum is the main vector and S.neavei in small localities especially in much or very dense woodland areas (Raybould, 1968a; Raybould and White, 1979). It is known that forests retard the movement of S.damnosum and extensive forest and bush clearing which is going on like any other parts of Africa (Raybould, 1968b) is known to increase transmission because the breeding sites become much more exposed; and possibly therefore, to account for the higher microfilaria rates in these three foci of Bwakira,

Mahenge and Ruvuma. In Amani focus, S.woodi a member of S.neavei complex is the local vector and the breeding sites are confined to small streams in thick forest (Raybould, 1968b). Raybould (1975-76) found that the numbers of biting S.neavei females in eastern Usambara mountains, had fallen to about 5.0%. Deforestation and subsquent exceptionally dry periods (Raybould 1975-76) were concluded to be the most possible causes for the fall in black fly population and therefore, transmission was probably interrupted to account for the low microfilaria rate of 22.4% in this Amani focus, reported in this study.

Microfilaria rates and intensity of infection in the foci and other areas varied. Acquired or inherited human activities in relation to the transmitting behaviour of the respective vectors in these foci, are usually the factors which account for the variations (Buck, et.al., 1969). In Amani focus (Wegesa and Chimtawi, 1971b), it was found that the microfilaria rates in 1970 were much higher than those in 1963-64. Whereas there might be other factors influencing the microfilaria rates in the area, it was believed that the increase of cultivation of cadamon (Ellitaria cardomomum) at that time, had contributed the most by simply raising the exposure factor. Age factor is also important; people of much higher age groups are usually more infected because they have been exposed to Simulium bites for much longer period (Buck, et.al., 1969; Abaru and Van Dam, 1972-73), as

observed in Bwakira, Mahenge, Ruvuma, Tukuyu, Kilosa and Amani foci (Tables 6-11, Figs. 3-8).

Duration of infection, and maintanance of high parasites density have a positive influence on the formation of nodules and the appearance of the skin changes (Woodruff, et.al., 1977). Any differences in such factors that might exist in the respective foci and other reported areas in this study, should then explain the differences in the observed pattern of these clinical manifestations (Table 12).

2. Development of the IHA test

In principle, homologous antigen should be given a high priority in serological tests because it is more specific and sensitive. On the contrary, the results of the present study were found to impose some limitations on the use of this homologous O.volvulous antigen. First this crude O.volvulus antigen had high cross-reactions with sera of people infected with helminths other than the O.volvulus to a titre of 1/640 (Table 12). It is thought that this high cross-reaction was due to contamination of the worm with human tissue extract which might have reacted with antihuman proteins of the sera tested (Bartlett, et.al., 1975). The effect of this high cross-reaction was to raise a positive titre for onchocerciasis to 1/1280 which was exclusively high to miss those sera which could be positive at lower titres; the

percentage of sera positive for onchocerciasis was far less when compared to the results which were obtained with the crude O.gutturosa antigen which showed less reactivity with negative control sera and those of people infected with other helminths (Table 13).

The antigens of both O.gutturosa and O.volvulus gave a maximum titre of 1/5120 with sera of people infected with O.volvulus. These observations could suggest antigenic similarity in the two worm species; these worm species are closely related morphologically (Wegesa, 1968).

From the results therefore, overall assessment on the choice of parasite for antigen, was found to lie with the O.gutturosa whose antigen in the IHA test, gave a titre of 1/320 which was found to discriminate cross-reaction effects due to other helminths. Using the antigen, the IHA test was therefore considered to be sufficiently valid for diagnosis of human onchocerciasis. More important aspects of this test with this antigen are discussed in Appendix 5.

In five feeding trials, 13.8% of the wild caught S.vorax fed on ears of a cow infected with O.gutturosa. This 13.8% was not far from that of 18.0% which was obtained by Raybould (1969) when the same fly species was fed on ears of rabbit in two feeding trials using this technique. However, the average number of 2.3 microfilariae per fly, ingested by the S.vorax

was considered to be small for a significant yield of the infective stage larvae. Probably blood feeding habits of the fly species and behaviour of the parasite in cattle, need to be studied more intensively for subsquent refinement of the feeding technique, to obtain higher microfilaria intake by the S.vorax .

3. Serological observations

The IHA test was developed for serodiagnosis of onchocerciasis. The test was found to be complementary to parasitological and clinical diagnosis. The immunological response detected by the IHA test, included also people who did not show microfilariae in the skin snips as shown in Tables 17-24. To a clinician such response would probably be not important because he emphasizes absence of the parasite in the host as a total cure of the disease. But on the other hand the positive immunological response gave a clear indication of the people who had actually experienced onchocerciasis and therefore, a more accurate method for the detection of the disease.

Association of the IHA test with skin snip method was observed in Bwakira, Amani, Kilosa, Ruvuma and Nguru foci. Furthermore, the sensitivity of the IHA test varied within the foci with high levels in Amani (93.7%), Bwakira (87.7%), Kilosa (81.8%), and Ruvuma (75.0%) as shown in Table 17. Such

variations of the sensitivity indicated differences in antigen sensitisation and ability to produce antibodies in those people infected; and these usually depend upon the frequency of exposure of a person to the O.volvulus infection and the frequency of Simulium bites. Differences in antigen sensitization could also indicate different strains of O.volvulus existing in these respective foci.

A high degree of onchocerciasis challenge and a well established balance between the parasite and the host existed in Bwakira as indicated by the high corresponding IHA and the microfilaria rates in the IHA sample 89.0% and 95.5% respectively; higher IHA titres in those people with high microfilaria counts (Table 27) and high GMRT and GMMF (Tables 25 and 26).

Correlation between IHA and microfilaria rates within age groups was observed (Tables 28-37; Figs. 13-17). In Bwakira the IHA and microfilaria rates were relatively high at all age groups. Whereas in Mahenge, Kilosa and Ruvuma, the rates were relatively low and were found to increase with age to indicate that the IHA test developed, became an additional tool for assessing period prevalence of onchocerciasis to indicate time-related trends in the status of the disease in the collective group.

Significant association between IHA titres and skin

changes was observed in Bwakira and Kilosa (Tables 39 and 40), consequent upon a sustained onchocerciasis challenge. These observations formed an important link between the pathological manifestations and the immunological phenomena and therefore the IHA test was useful not only in detecting but also ascertaining the disease in those people showing clinical symptoms; the skin changes in this case.

VI. CONCLUSIONS

Onchocerciasis causes health and social economic problems in those areas affected. It is a disease which affects about 20 million people in the world particularly in Africa, Central and South America. The disease causes blindness as the main adverse effect which has been clearly seen in parts of West Africa with subsquent depopulation and therefore to emphasize the urgent need for pilot control programme which is being carried out there in the Volta River Basin.

There have been attempts to compare onchocerciasis endemicity and severity between West and East Africa with little evidence that the disease is acute in the east as it is in the west. The results of the present study in Tanzania, now indicate that the disease may attain high endemicity levels equal to those areas in West Africa as judged from the prevalence found in Bwakira (63.6%) and Mahenge (58.6%).

In this study, conventional technique (skin snip) and the IHA test were complementary in diagnosing onchocerciasis and it was possible to assess the disease age distribution and ascertain the skin changes which are advanced stages of the disease in the foci. However, there is still need to continue the study on the homologous O.volvulus antigen, particularly into more attempts to isolate and purify that part of the antigen complex which is most reactive and specific in serological test, in order to improve serological testing. Observations on the clinical manifestations were restricted to skin changes and nodules. It is then suggested that ocular complications should also be studied together with the social economic aspects of the disease in order to assist making adequate decision on the need for control of the disease especially in Bwakira where all diagnostic parameters used in this study showed greater involvement.

VII. SUMMARY

Prevalence of onchocerciasis in Tanzania was studied. The study was within the research policies on the morbidity problem of onchocerciasis in the country undertaken by the National Institute for Medical Research, Amani Medical Research Centre. Also it was in line with WHO's policy on onchocerciasis which was intended to improve knowledge on the natural history of the disease generally. It was important to carry out surveys on the disease in several parts of the

country and assess its prevalence before the control strategies could be envisaged. During the surveys, the presence of the microfilariae of Onchocerca volvulus was detected microscopically in skin snips taken from people living in those affected areas. With this skin snip method, it was found that the disease was on the increase in Bwakira, Mahenge and Ruvuma with prevalence of 63.6% , 58.60% and 31.9% respectively. Whereas in Amani, the prevalence had fallen to 22.4% when compared to the data of the previous surveys. In those affected areas, onchocerciasis was detected in both males and females from the age of three years onwards. People of much older age groups were found to be much more infected than younger ones because they were exposed to Simulium bites for a much longer period of their life time. The disease was not encountered in other previously suspected areas like Kagera, Ngara, Kibondo, Kasulu and Sumbawanga on the western side of Tanzania.

Diagnosis of onchocerciasis using the skin snip method was considered to be inadequate in some cases in which microfilariae could not be detected in those skin snips. In such cases, immunodiagnostic approach towards the disease was considered to be important. The IHA test was found to be sufficiently sensitive and specific for the serological testing using adult Onchocerca gutturosa antigen.

In principle, homologous antigen of adult O.volvulus was

to be given high priority in the serological testing because it was supposed to be more sensitive and specific. On the contrary, high cross-reactivity was encountered with this antigen when sera of people infected with various other human helminths were tested to check the degree of specificity. These high cross-reactivity effects were thought to be due to the contamination of the O.volvulus adult worms with human tissue extracts which might have reacted with proteins of the sera tested.

The serological data obtained with IHA test was complementary to the skin snip and clinical diagnostic parameters. Sensitivity was highly variable in the foci with highest levels in Amani (93.7%), Bwakira (87.7%), Kilosa (81.8%) and Ruvuma (75.0%). This was an indication of high degree of antigen sensitization and ability of the infected people to produce antibodies. In some of these foci, the antibodies detected were associated with the skin changes due to onchocerciasis infection. These were important observations which provided evidence for a link between the immune response and the pathological manifestations of the disease which require more investigation to find out the significance of the relationship between the two parameters.

The study was concluded by the general observation that it was possible to assess the prevalence and general distribution of onchocerciasis in Tanzania using

parasitological, clinical and serological methods and the results indicated that the disease may attain high endemicity levels equal to those found in areas of West Africa as judged from the prevalence found in Bwakira (63.6% and Mahenge (58.6%). However, it is suggested that in addition to this, ocular complications and the social economic aspects of the disease should be studied in order to assist in making adequate decision on the need for control especially in Bwakira where the diagnostic parameters used in this study showed greatest involvement.

The wild caught S.vorax were successfully fed on a cow infected with O.gutturosa. But the average number of 2.3 microfilariae ingested per fly was considered to be small for a significant yield of the infective larvae whose antigen could be useful in detecting prepatent infection. The blood feeding habits of this fly species and the behaviour of O.gutturosa parasite in the infected cattle need to be investigated more intensively for subsquent improvements on the methodologies that will enable these parasites to be ingested in large numbers by the fly while taking the infective blood meal from the infected cow. The infected cow was obtained from Lushoto District, one of the areas in which the prevalence of O.gutturosa was studied. It was of considerable importance to study the presence of O.gutturosa in cattle in localities in which the presence of O.volvulus was established because there was evidence that the two

parasite species could be transmitted by the S.vorax as a common vector found in the respective localities.

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