# BABOONS AS MAINTENANCE HOSTS OF HUMAN

SCHISTOSOMIASIS IN KENYA

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of the University of Liverpool for the
Degree of Doctor of Philosophy

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DEDICATION

This thesis is dedicated to my Mother and Father

and all those who have contributed towards my education.

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ABSTRACT

A parasitological survey of 131 free-ranging baboons from Dwa Sisal Estate revealed that 32 (24%) were infected with S. mansoni. The highest prevalence (100%) and intensity (176 worms per baboon) of infection was demonstrated in baboons which used the Thange River for drinking and frequently slept in trees above the river.

Some of the worm burdens represent the highest natural infections recorded in wild baboons, and their faeces contained viable eggs of S. mansoni.

In contrast, lower levels of infection were recorded in baboons using the Kibwezi River, and schistosome eggs were not detected in faeces.

Surveys of primary school children revealed higher prevalence of S. mansoni in Thange and Nzavoni Schools (82.4% and 85.6% respectively) compared to Dwa Primary School (38.4%). The intensities of infection were higher in Thange and Nzavoni Schools (geometric means 55 eggs/gm and 120 eggs/gm respectively) and lower in Dwa Primary School (geometric mean 5 eggs/gm).

These differences and possible relationships have been discussed, including the potential of baboons in the area to act as maintenance hosts of S. mansoni.

Laboratory experiments have shown that although percutaneous infection with schistosome cercariae is the most efficient method, oral exposure can lead to viable S. mansoni infections. It has been shown also that S. mansoni originating from Thange baboons can be maintained in captive baboons.

Preliminary isoenzyme electrophoresis of human and baboon adult S. mansoni from Thange failed to reveal differences in isoenzyme profiles.

The need for further investigation of the role of baboons and other animals in transmission of S. mansoni, and the genetic relationship between parasites originating from baboons and humans in the study area, have been discussed.

ACKNOWLEGEMENTS

CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

1.1   Schistosomiasis

Schistosomiasis is caused by flukes of the genus, *Schistosoma*, the adult stages of which are found in the blood vessels of the vesical plexus or the hepatic portal system.

The parasites were first described in 1851 by Theodor Bilharz, a German pathologist, who found the adult worms in the mesenteric veins of an Egyptian in Cairo during a post-mortem examination.

The disease has a long history, particularly in Africa where Sir Armand Ruffer (1910) found typical calcified schistosome eggs in the kidneys of two Egyptian mummies dating from between 1250-1000 BC.   In Japan, Daijiro Fujii had described "Katayama" disease in 1847, now recognised as acute schistosomiasis japonicum.   In 1904, Fujiro Katsurada discovered *S. japonicum* in the mesenteric veins of a cat in Japan.

There are 18 species of schistosomes known to infect humans or animals (Rollinson and Southgate, 1987). The 5 principal species that infect humans fall into one of the three groups that are characterised by the type of egg produced (WHO, 1985):

a.   eggs with a lateral spine (*S. mansoni*)

b.   eggs with a terminal spine (*S. haematobium* and *S. intercalatum*)

c.   eggs that are round and minutely spined (*S. japonicum* and *S. mekongi*)

Two other species, *S. bovis* and *S. mattheei*, that are normally found in domestic and wild animals, occasionally infect humans in Africa (Raper, 1951; Pitchford, 1961; MacConnel *et al.*, 1974).

1.2   Geographical distribution of human schistosomiasis

Schistosomiasis is known to be endemic in 76 countries of the world. It is estimated that 200 million persons are infected and up to 600 million exposed to infection because of poverty, ignorance, poor housing, sub-standard hygiene practices and inadequate sanitary facilities (WHO, 1985). It is one of the most widespread of all human parasitic diseases, ranking second only to malaria in terms of its socio-economic and public health importance in tropical and sub-tropical areas (WHO, 1990).

It is also the most prevalent of the water-borne diseases and one of the greatest risks to health in rural areas of developing countries (WHO, 1990).

The global distribution of human schistosomes shown in Figure 1.1 is summarised below.

a.   *S. mansoni* is found in Africa, S.W. Asia, the Caribbean and S. America.

b.   *S. haematobium* mainly occurs in Africa and S.W. Asia.

c.   *S. japonicum* is found in S.E. Asia.

d.   *S. mekongi* is found along the Mekong River in Cambodia and Laos.

e.   *S. intercalatum* occurs in six countries of Central and West Africa.

S. bovis and *S. mattheei* are primarily parasites of domestic and wild ungulates but have been reported in humans in Eastern and Southern Africa.

1.3   Schistosomiasis in Kenya

Both *S. mansoni* and *S. haematobium* are endemic in Kenya. In the 1960's, 1 million persons were estimated to be infected but, with the increasing human population and water resource developments, this figure has now risen to 3 million (Doumenge *et al*, 1987). It is one of the diseases that causes considerable morbidity as evidenced by hospital attendance records (Ouma, 1987).

Distribution of the parasites is shown in Figure 1.2 which identifies 3 main regions:

1.   The coastal strip and Tana River basin (*S. haematobium*)

2.   Some parts of Eastern, Central and Rift Valley Provinces (both *S. mansoni* and *S. haematobium*)

3.   The shores of Lake Victoria (*S. mansoni* and *S. haematobium*).

Other isolated foci of infection occur throughout the country, the most important being the Lake Jipe area of Taita-Taveta District of the Coast Province near the border with Tanzania (Figure 1.2).

Recent surveys for schistosomiasis seem to indicate that the disease is spreading and there are now foci of infection in every Province (Doumenge *et al*, 1987). Spread of this infection seems to be related to development of water utilisation projects for hydroelectric, irrigation and water conservation schemes. It has been observed in Kenya and other parts of Africa that irrigation development enhances snail breeding and increases prevalence and intensity of schistosomiasis (Choudhry, 1975). An example is the Mwea/Tebere Irrigation Scheme in Central Province where neither *S. mansoni* nor *S. haematobium* infections could be found by Teesdale (1962) before development of the irrigation project but, by 1970, there was a 60% prevalence of *S. mansoni* (Kenya Ministry of Health Report quoted in Doumenge *et al*, 1987).

The distribution of the parasites follows closely that of the snail intermediate hosts; *Biomphalaria* spp. for *S. mansoni* and *Bulinus* spp. for *S. haematobium*. There are no *Biomphalaria* spp. at the coast which explains the absence of *S. mansoni* in the Coastal plain and Tana River basin (Highton, 1974).

Biomphalaria sp. is found in many areas of Kenya. Four main species are identified; *B. pfeifferi*, found in nearly all districts at altitudes between 600m and 1800m, is the main snail host for *S. mansoni*. *B. sudanica* is found on the shores of Lake Naivasha while *B. choanomphala* and *B. angulosa* are found in Lake Victoria and L. Jipe respectively.

Bulinus globosus, the main intermediate host for *S. haematobium*, is found along the Kenya Coastal plain and Tana River basin at altitudes that rarely exceed 1250m. Of lesser importance are: *B. africanus* found at altitudes between 0 and 1800m in Kitui, Machakos, parts of Central Province and in Western Kenya and *B. nasutus* in Nyanza and Western Provinces.

Schistosomiasis is one of the diseases targeted for control by the Kenya Ministry of Health (Ouma, 1987).

1.4 Life cycle of schistosomes

All schistosomes have a similar life cycle involving a specific intermediate host snail (*Biomphalaria* sp. for *S. mansoni*, *Bulinus* sp. for *S. haematobium*, *Oncomelania* sp. for *S. japonicum* and *Tricula aperta* for *S. mekongi*).

The schistosome life cycle is complex, involving alternating parasitic and free-living stages: the egg, miracidium, first and second stage sporocysts, cercaria, schistosomulum, and adult worm (see Jordan and Webbe, 1982, and Rollinson and Simpson, 1987; for general reviews).

1.4.1   The egg

The eggs are yellowish, non-operculate and have a spine the position and prominence of which forms the basis of classification of various species as outlined in Section 1.1. The embryo (miracidium) develops inside the egg over a period of 6 days after oviposition (Jordan and Webbe, 1982).

Eggs either pass through the bladder or intestinal wall (depending on species) or remain in the tissues where they die and are destroyed over a period of weeks or months depending on whether calcification has taken place (Warren, 1973). The eggs passed into urine or faeces hatch when they come into contact with fresh water. Hatching is thought to be influenced by warmth (10-30°C) and light, which stimulate the miracidia to become active, and emerge being partly facilitated by osmotic effects and activity of the miracidia (Jordan and Webbe, 1982).

1.4.2   The miracidium

Once in the water, the ciliated miracidia swim actively until they find and penetrate a suitable snail host. Location of the intermediate host is thought to be aided by chemical substances emitted from snails and other aquatic organisms called "miraxones" (Chernin, 1970).

When contact is made with the snail, the miracidium attaches itself to the body surface (usually the head-foot region) by secretions from the apical gland cells. Penetration then occurs with the help of the papilla, and lytic enzymes from the miracidium "gut".

Miracidia will attempt to penetrate a variety of natural and synthetic materials but only develop in the appropriate snail. They remain infective for 8-12 hours.

Once inside an appropriate snail, the miracidium loses the ciliated surface and transforms into a mother sporocyst. This takes place near the point of penetration. Eight days after penetration, the mother sporocyst has grown into a non-motile convoluted tube coiled into a globular shape from which germ cells are budded off internally from the epithelial lining. These germ cells then form daughter sporocysts which migrate in the snail, primarily through loose connective tissue, to the digestive gland (Jordan and Webbe, 1982).

Inside the daughter sporocysts, through a process of asexual multiplication, the final larval stage, the cercaria, is formed.

Cercariae escape from the daughter sporocyst and emerge from the snail sometimes in large numbers. Production of mature cercariae from the time of miracidial penetration takes 4-5 weeks in *S. mansoni*, 5-6 weeks in *S. haematobium* and 7 weeks or longer in *S. japonicum* (Jordan and Webbe, 1982).

1.4.3   The cercaria

Cercariae produced from one miracidium are all of the same sex. As they emerge from the snail, some get trapped in the tissues and die, leading to proliferative changes in the intermediate host. This may cause a high mortality in the snails, particularly in the first 2 weeks of cercarial emergence.

Each cercaria has a head which will ultimately develop into the mature adult worm and a forked tail which propels it. The head (or anterior organ) has six pairs of cephalic glands: two pre-acetabular and four post-acetabular. One pair of pre-acetabular glands facilitates emergence from the snail (Smyth, 1966). The primary function of pre-acetabular secretion is probably enzymatic, while that of post-acetabular glands is thought to be mainly adhesive (Jordan and

Webbe, 1982). Post-acetabular secretions swell in water to provide a sticky mucus for attachment of the oral and ventral suckers during penetration.

The principal stimulus for cercarial emergence is associated with light and water temperatures ranging from 10-30°C (McClelland, 1965). Cercariae of *S. mansoni* and *S. haematobium* are abundant in natural water bodies during the day, particularly between 1000 to 1400 hours while those of *S. japonicum* are most numerous in the early part of the night with a peak at 2300 hours (Webbe and Jordan, 1966; Pesigan *et al*, 1958).

Although their lifespan is shorter at higher temperatures (20-24°C), cercariae may survive for up to 48 hours. There is no evidence that chemotaxis is involved in host location by cercariae although mechanical stimuli such as agitation and touch increase their swimming activity.

Once in contact with human (or animal) skin, cercariae adhere to it using their sucker, aided by mucus secretions from the post-acetabular glands. The oral sucker penetrates the horny layer of skin into underlying tissues, releasing enzymatic secretions from the pre-acetabular glands. Fatty acids in the skin are thought to provide the signal for this process (Sturrock, 1987).

Enzymatic secretions disrupt the ground cement of skin cells while penetration is achieved through muscular contractions of the cercarial body, shedding the tail and transforming into a schistosomulum which may remain in the dermis for up to 3 days.

Cercariae often attempt to penetrate any object with which they come into contact.

1.4.4   The schistosomulum

The schistosomulum develops into the adult worm through a gradual transformation process in which the tegumental membrane changes from a single to a double lipid bilayer.

Numerous investigations have been done in mice to see how the schistosomulum migrates from the skin and finally reaches the liver where maturation and pairing of male and female worms takes place (Miller and Wilson, 1978; Wilson *et al*, 1978; Wilson and Coulson, 1986; Wilson *et al*, 1986). The evidence suggests that migration is via the circulating system to the right heart and lungs. From the lungs they are thought to delay as they transverse the alveolar capillary beds, from where they are carried on in the blood to the left heart, the systemic circulation and through anastomoses around the mesentery to the hepatic portal system.

The total worm burden found in the hepatic portal system may require 2-3 circuits of blood circulation (Wilson and Coulson, 1986). Other routes of migration have been recorded, such as retrograde migration of schistosomula from the heart down the vena cava to the hepatic veins in cattle (Nelson, personal communication). Georgi *et al* (1986) suggest that migration may occur from the lungs through intervening tissues or vessels to the liver.

It is thought that those schistosomula that do not reach the liver within three circuits, die (Wilson and Coulson, 1986; Wilson *et al*, 1986). Once in the liver, schistosomula grow to adult male and female worms, which then pair.

1.4.5 The adult worm

Most of the schistosomes infecting man leave the liver and migrate to either the veins of the vesicle plexus and pelvis (*S. haematobium*) or the mesenteric veins (other species) where most of the egg laying by females takes place. The eggs are either trapped in the tissues or pass out of the blood vessels and migrate through the tissues aided by the peristaltic motion of the gut, into the lumen of the intestines or urinary bladder. Many eggs in the portal venous system reach the liver and with systemic circulation to the lungs. Eggs that are trapped die within 21 days after oviposition and are surrounded by a granulomatous reaction (Warren, 1973). The trapped eggs are the main cause of pathology in schistosomiasis (Warren, 1987).

For human schistosomes, the prepatent period is 30-40 days or more and although the adult worms may live for 20-30 years, the average life span is 3-8 years (Jordan and Webbe, 1982).

The life cycle of the parasites in baboons and the transmission from snails follows the above pattern in most respects but observations made in the present study suggest that there are major differences in the way in which baboons acquire and disseminate the infection.

1.5   Taxonomy of the baboon

Baboons belong to the Order of Primates, defined in 1758 by Linnaeus, and which includes: the prosimians, (Tupaidae, Lorises, Tarsiers and Lemurs), the American or platyrrhine monkeys, the Old World or catarrhine monkeys, the anthropoid apes and man (Chiarelli, 1973).

The word "primate" when used in small case often refers to primates other than man (Ruch, 1959). Alternative terms in common use include "simian primates" and "non-human primates".

In his description of the Order, Chiarelli (1973) divides it into 3 suborders, 15 families and 52 genera. Figure 1.3 summarises the taxonomic classification of primates with particular emphasis on Kenya.

Prosimians are primitive, with characteristics close to those of insectivores. They are primarily arboreal and unlikely to be exposed to schistosomiasis. The other two infra-orders, platyrrhine (New World) and catarrhine (Old World) monkeys, are more likely to be exposed since some of the species are terrestrial.

There is still some disagreement among taxonomists regarding the classification of various species of primates. Nowhere is this more apparent than in the classification of the baboon.

The baboon belongs to the family Cercopithecidae, subfamily Papinae and genus *Papio*. Problems of classification arise at the species level. The most widely accepted classifications are those of Hill (1967) and Kingdon (1971). Hill (1967) classifies the genus *Papio* into 2 super species thus:

Genus *Papio*

Super species 1.		*P. hamadryas* Linnaeus 1758
Super species 2.	a.	*P. papio* Desmarest 1820
	b.	*P. anubis* F. Cuvier 1825
	c.	*P. cynocephalus* Linnaeus 1766
	d.	*P. ursinus* Kerr 1792

P. anubis, *P. cynocephalus* and *P. ursinus* have overlapping geographical ranges. *P. anubis* is synonymous with *P. doguera* (Hill, 1967).

Kingdon (1971) classifies the genus *Papio* into 2 distinct species as follows:

Genus *Papio*

Species	1.		*P. hamadryas*
	2.	a.	*P. cynocephalus cynocephalus*
		b.	*P. c. anubis*
		c.	*P. c. papio*
		d.	*P. c. ursinus*

Apart from the slight differences in nomenclature these two classifications are similar. Eley (1989) divides the genus into 5 species based on the above classifications, of which 2 are found in Kenya. These are *P. anubis* (Olive baboon) and *P. cynocephalus* (Yellow baboon). Olive baboons are found in Central and Western parts of Kenya, while yellow baboons range

In the Eastern and Southern parts. For the purposes of this study baboons found in Amboseli National Park and Kibwezi were recognised as *P. cynocephalus* (Eley, 1989).

1.6 Zoonotic aspects of schistosomiasis

The joint FAO/WHO Expert Committee defined zoonoses as "those diseases and infections which are naturally transmitted between vertebrate animals and man (WHO, 1959).

Although schistosomiasis due to *S. japonicum* has always been regarded as a zoonosis, it is only in the recent past that animals have been seriously considered as possible reservoir hosts of *S. mansoni, S. haematobium* and *S. intercalatum* (Nelson, 1975). Though these infections have been found in animals, the main difficulty has been to decide on the direction of transmission and to discover whether animals were true maintenance hosts or merely incidental hosts of these parasites.

1.6.1 Animal hosts of *S. japonicum*

As stated above many animal reservoirs of *S. japonicum* have been found particularly in China and the Philippines (Cheng, 1971; Mao Shu Pai, 1982; Pesigan *et al*, 1958). High prevalence rates were reported in various species by Pesigan *et al* (1958) in the Philippines while in China 13 species of domestic and at least 31 species of wild mammals are known to have natural infection (Cheng, 1971; Mao Shu Pai, 1982).

1.6.2 Animal hosts of *S. mansoni*

A checklist of animal species found to have natural *S. mansoni* infections in various African countries has been compiled by Ouma and Fenwick (1991). From the list, it appears that apart

from a few ungulates, insectivores and the domestic dog, non-human primates and rodents form the bulk of species found to have natural *S. mansoni* infections.

Natural infections in rodents have been reported in Egypt (Kuntz, 1952), Sudan (Karoum and Amin, 1985), East Africa (Teesdale and Nelson, 1958; Nelson, 1960; Kawashima *et al*, 1978; Ouma, 1987) and South Africa (Pitchford, 1959; Pitchford and Visser, 1960; Pitchford and Visser, 1962).

It is still not known whether rodents play any part in the transmission of *S. mansoni* in Africa. Much higher prevalence rates have been found in South America (Jordan and Webbe, 1982). In Guadeloupe, *Rattus rattus* and *R. norvegicus* were found infected but their role in transmission is not established (Theron *et al*, 1978; Imbert-Establet, 1982; Rollison *et al*, 1986).

Most non-human primates are thought to be susceptible to *S. mansoni* and successful laboratory infections have been obtained by various investigators (Martins, 1958; Meisenhelder and Thompson, 1963; Jordan and Goatly, 1966; Gear, 1967; Ritchie *et al*, 1967; Obuyu, 1972a; Sturrock *et al*, 1976).

Natural infections in primates have been reported in various species. Cameron (1928) found vervet monkeys (*Cercopithecus sabaeus*) infected in St. Kitts Islands in the Caribbean while Miller (1959, 1960), Nelson (1960) and Strong *et al* (1961) found baboons (*Papio doguera*) infected in Kenya and Uganda. McQuay (1952) reported infection in a Guinea baboon (*P. papio*) imported into the New Orleans Zoo while Fenwick (1969) found baboons (*P. anubis*) infected in Lake Manyara National Park, Tanzania. Recently, Else *et al* (1982) found various species of *Cercopithecus* monkeys having natural infections in Kenya while McGrew *et al* (1989) reported infection in baboons (*P. papio*) in Senegal.

1.6.3   **Animal hosts of S. haematobium**

There are several reports of natural *S. haematobium* infections which are mainly incidental and unlikely to play any part in the transmission of the parasite (Ouma and Fenwick, 1991).

Most of these infections have been reported in non-human primates apart from a few ungulates and rodents. Hill and Onabamiro (1960) reported infection of a domestic pig (*Sus scrofa*) in Nigeria while Basson *et al* (1970), Mackenzie (1979) and Pitchford (1959) found infections in a cape buffalo (*Syncerus cafer*), sheep (*Ovis aries*) and rodents respectively in South Africa. Mansour (1973) found rodents infected in Egypt.

In non-human primates, Nelson (1960) and Else *et al* (1982) found baboons (*Papio* sp.), vervets (*C. aethiops*) and Sykes monkeys (*C. mitis*) infected in Kenya. Purvis *et al* (1965) reported infection of baboons (*Papio* sp.) in Zimbabwe while De Paoli (1965) and Taylor *et al* (1972) reported infection in a chimpanzee (*Pan satyans*) and a baboon (*Papio* sp.) in Sierra Leone and Senegal respectively.

Laboratory infections have been successfully carried out in various primate species (Kuntz and Malakatis, 1955; Smithers and Terry, 1965; Jordan and Goatly, 1966; Foster *et al*, 1968; Obuyu, 1972b; Sulaiman *et al*, 1982).

1.7   **The primate pest problem in Kenya**

In Kenya and many other African countries, there exists a growing conflict between wildlife conservation and agricultural activities. This is partly due to a rapidly expanding human population which has encroached on well established wildlife habitats.

Some primate species, particularly baboons, cause serious crop damage in parts of East Africa because of their ability to adapt to a wide range of diets (Kingdon, 1971). In some cases, the crop raiding can be serious enough to threaten human subsistence. Such was the case in Gilgil near Nakuru, in the Rift Valley Province, where 3 troops of baboons (*P. anubis*) that were under long term sociological studies had to be relocated to a private ranch in Laikipia, near Mount Kenya (Strum, 1987). In addition to raiding crops from the surrounding peasant farms, the baboons were scavenging at a garbage dump near the Gilgil army barracks.

Similar crop raiding problems have been observed in the Del Monte Pineapple Plantation, Thika; Kibwezi Division in Machakos District; Kilifi, Lamu and Kwale Districts in the Coast Province (author's unpublished observations).

Baboons and vervet monkeys also become a nuisance in National Park tourist lodges when they scavenge in the garbage disposal pits (Eley, 1989; author's unpublished observations) (Figure 1.4). Crop raiding and scavenging behaviour brings these animals into contact with human settlements and water resources. Nelson (1960) states that baboons could be possible reservoirs of human schistosomiasis because they are gregarious, visit the water regularly and often sleep in trees near water, which they can easily contaminate with their faeces and urine.

1.8   Objectives of this study

It is well known that baboons can be infected with human schistosomiasis. What is not clear is their role in the transmission and maintenance of the parasites, particularly in the absence of human hosts. The only case where this apparently occurred is that reported by Fenwick (1969) where humans were infected with a baboon-maintained *S. mansoni*. However, the origin of that infection may have been human since infected workers were found in the area. Furthermore there were people living upstream from the baboon sleeping site although the distance was considerable.

It is still necessary to establish whether infected baboons can maintain the parasite in their troop through a baboon-snail-baboon transmission cycle.

Thus, the present study was set out with the following objectives:

1. To examine free-ranging baboons from areas endemic for human schistosomiasis, for their susceptibility to the human infection.

2. To determine the potential route(s) of infection in baboons by:

    a. Field observations of baboons at water contact sites.

    b. Laboratory experiments to investigate the efficiency of different routes of infection.

3. To investigate the prevalence and intensity of infection in humans, using adjacent water sources.

4. To establish the human water contact pattern and snail infections in the affected rivers or water bodies.

5. To investigate the potential of baboons to maintain and transmit the infection within a troop.

6. To investigate the relationship between the human and baboon *S. mansoni* strains in the study area.

These objectives are addressed in the following Chapters as outlined below:

Chapter 2   :   Epidemiological survey of schistosomiasis in free-ranging baboons in Kibwezi.

Chapter 3   :   Epidemiological survey of schistosomiasis in school children from Kibwezi.

Chapter 4   :   Water contact and snail sampling studies.

Chapter 5   :   Laboratory investigations of *S. mansoni* infections in baboons.

Chapter 6   :   General discussion and conclusions.

Fig. 1.1a   Global distribution of schistosomiasis due to *Schistosoma haematobium, S. japonicum,* and *S. mekongi*

Reproduced, by kind permission, from *The control of schistosomiasis*. Report of a WHO Expert Committee. Geneva, World Health Organization, 1985 (WHO Technical Report Series, No. 728), pp. 17-18.

S. haematobium

S. japonicum

S. mekongi

WHO 851002

Fig. 1.1b   **Global distribution of schistosomiasis due to** *Schistosoma mansoni* **and** *S. intercalatum*

Reproduced, by kind permission, from *The control of schistosomiasis.* Report of a WHO Expert Committee. Geneva, World Health Organization, 1985 (WHO Technical Report Series, No. 728), pp. 17-18.

S. *mansoni*

S. *intercalatum*

WHO 851003
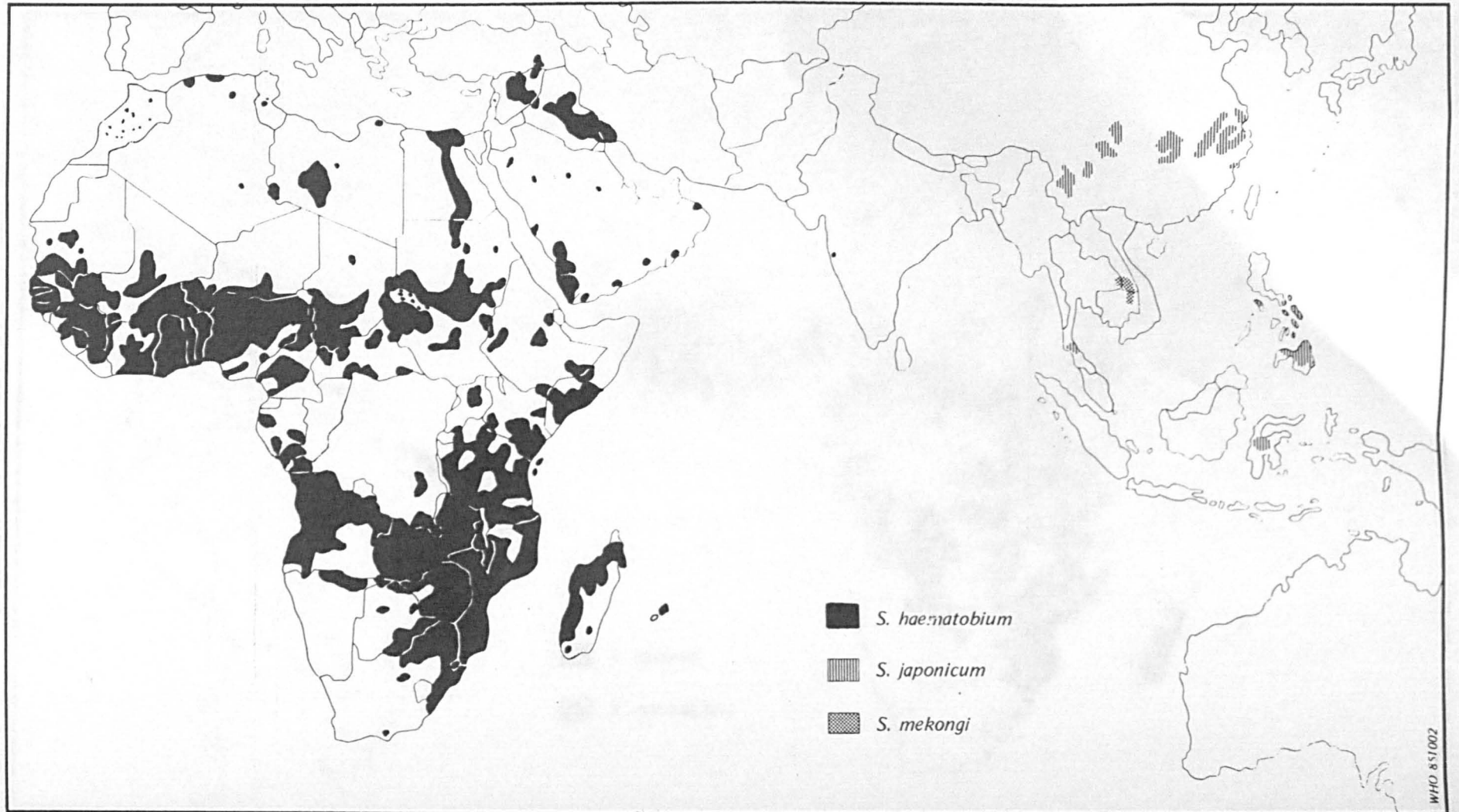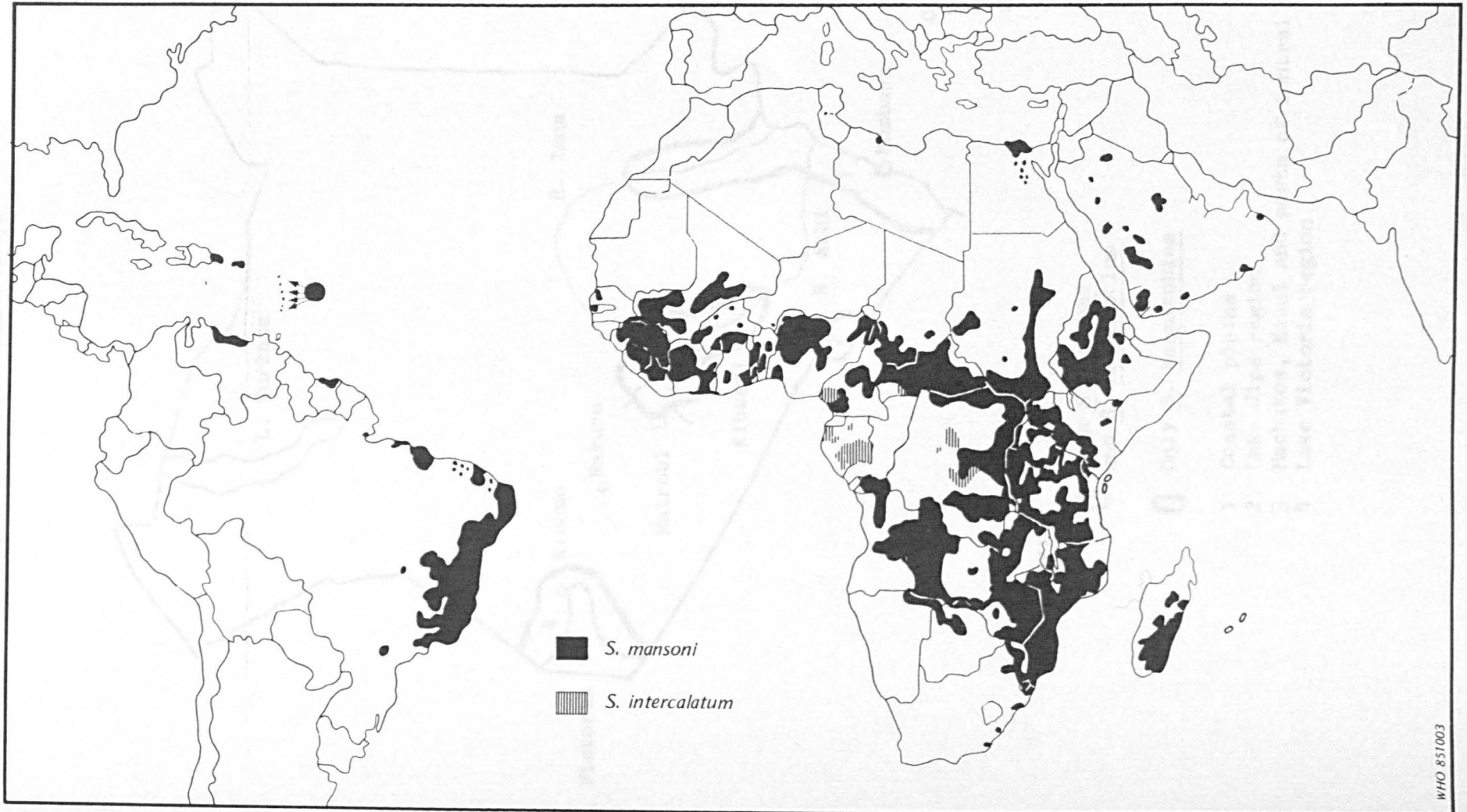
^aThe original version of this map was prepared by Ch. Cheung.

Fig. 1.2   **Distribution of schistosomiasis in Kenya**

L. Turkana

N

R. Tana

4 ○ Kisumu

L. Victoria

○ Nakuru

Nairobi □

3

Kibwezi ○

R. Athi

2

1

○ Mombasa

0   200 km

Scale

Key:
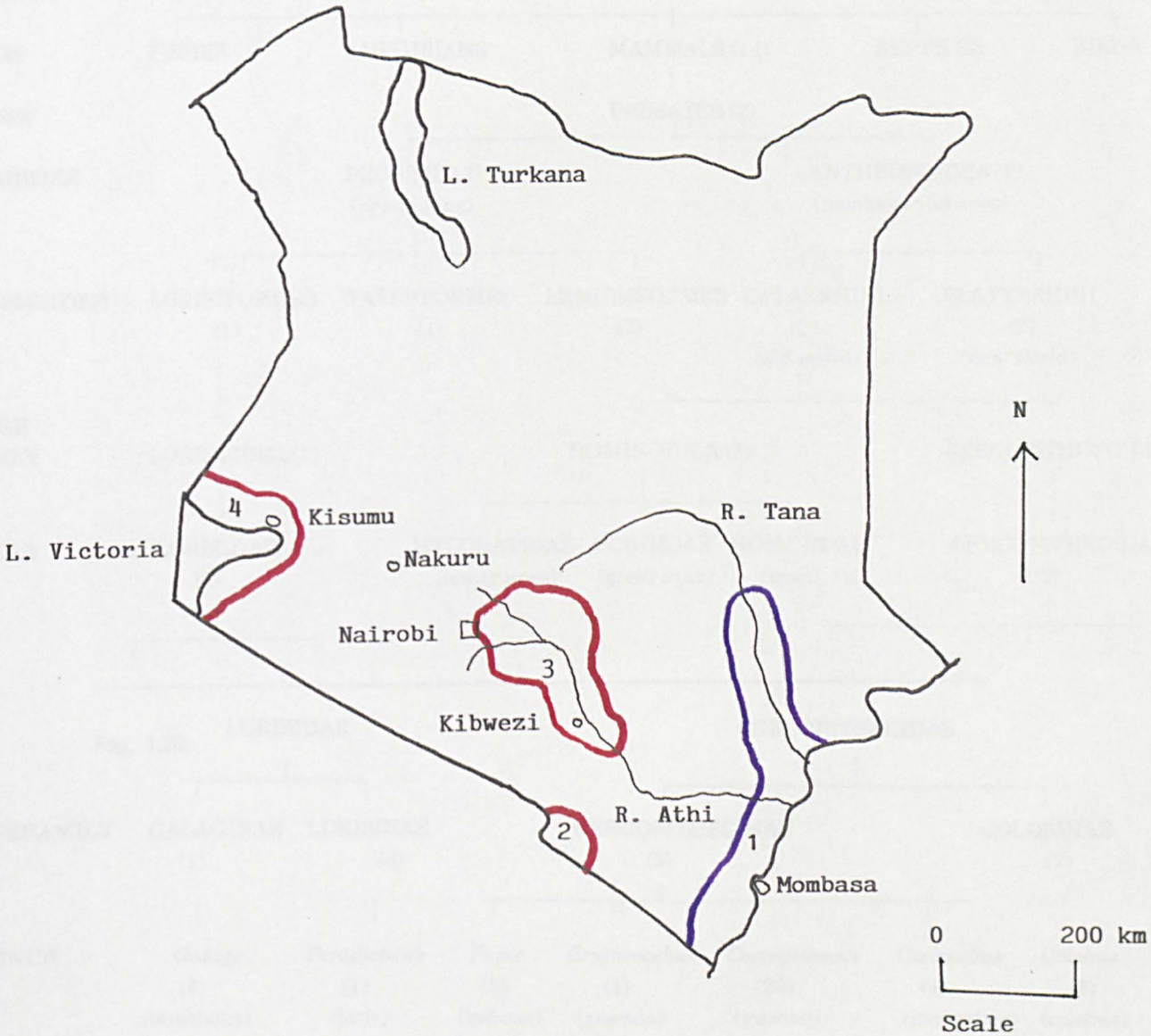
◘ Both S. mansoni
   and S. haematobium

◘ Only S. haematobium

1   Coastal plains
2   Lake Jipe region
3   Machakos, Kitui and parts of Central Province
4   Lake Victoria region

Fig. 1.3a   **Classification of Kenyan primates**
Reproduced, with permission, from Eley (1989)

KINGDOM   PLANT (12)   ANIMAL (27)

PHYLUM   CHORDATA (5)

CLASS   FISHES   AMPHIBIANS   MAMMALS (14)   REPTILES   BIRDS

ORDER   PRIMATES (2)

SUBORDER   PROSIMII (3)   ANTHROPOIDEA (2)
(prosimians)   (monkeys and apes)

INFRAORDER   LORISIFORMES   TARSIIFORMES   LEMURIFORMES   CATARRHINI   PLATYRRHINI
(1)   (1)   (2)   (1)   (2)
(old world)   (new world)

**SUPER
FAMILY**   LORISOIDEA (1)   HOMINOIDEA (3)   CERCOPITHECOIDEA (1)

FAMILY   LORISIDAE   HYLOBATIDAE   PONGIDAE   HOMINIDAE   CERCOPITHECIDAE
(2)   (lesser apes)   (great apes)   (man)   (2)

Fig. 1.3b   LORISIDAE   CERCOPITHECIDAE

SUBFAMILY   GALAGINAE   LORISINAE   CERCOPITHECINAE   COLOBINAE
(1)   (4)   (9)   (7)

GENUS   Galago   Perodicticus   Papio   Erythrocebus   Cercopithecus   Cercocebus   Colobus
(8)   (1)   (5)   (1)   (20)   (4)   (6)
(bushbaby)   (loris)   (baboon)   (guenon)   (guenon)   (mangabey)   (colobus)

**KENYA
SPECIES**   garnettii   potto   anubis   patas   aethiops   galeritus   guereza
greater   potto   olive   patas   vervet   crested   black & white
bushbaby   baboon   monkey   mangabey   colobus

zanzibaricus   cynocephalus   neglectus   angolensis
lesser   yellow   DeBrazza's   black & white
bushbaby   baboon   monkey   colobus

senegalensis   mitis   badius
lesser   mitis   red
bushbaby   monkey   colobus

ascanius
redtail
monkey

Fig. 1.4   Baboons at a garbage dump in Keekorok Lodge, Masai Mara National Park

CHAPTER 2

AN EPIDEMIOLOGICAL SURVEY OF SCHISTOSOMIASIS IN

BABOONS IN SELECTED SITES IN KENYA

2.1   Introduction

The first report of schistosomiasis in non-human primates was that by Cameron (1928) who found five out of eight vervets (Cercopithecus sabaeus) infected with S. mansoni in the St. Kitt's Islands in the West Indies. Since then there have been several other reports, mainly in African primates (Ouma and Fenwick, 1991).

Most of these reports are from incidental findings apart from those of Miller (1959), Nelson (1960), Strong et al (1961), Nelson et al (1962) and Else et al (1982) who examined various species of primates in Kenya and Uganda. Fenwick (1969) found baboons infected with S. mansoni in Lake Manyara National Park in Tanzania while Cheever et al (1970) reported finding the same parasite in vervets (C. aethiops) in Tanzania and Ethiopia.

Most investigations took place in the 1960's and even though the authors highlight the possibility that non-human primates may be potential reservoirs of human schistosomiasis, no serious follow-up of this work has been carried out. Indeed Ouma and Fenwick (1991) in their review observe that the true significance of primates and rodents in the epidemiology of human schistosomiasis in Africa still remains obscure and merits further study.

In order to establish whether a potential transmission cycle exists in an area, it is necessary to identify the water contact source for the primates and also ascertain whether infection is present in the local snail population. Fenwick (1969) showed that faeces from infected baboons using a cliff face as a sleeping site were contaminating a rock pool in which susceptible snails

(*Biomphalaria pfeifferi*) were found infected. A group of tourists who swam in the rock pool became heavily infected with *S. mansoni*.

The methods used in most of these early investigations were qualitative and did not give an indication of the parasite burden or the viability of the eggs released in the faeces.

The main objective of this study was to examine free-ranging baboons for infection with human schistosomes, in areas where the parasite is endemic in the human population. This would establish their susceptibility to the human parasites under natural conditions. The relationship between their water contact sites, their closeness to human settlement and the presence of infection in the local snail population was also considered.

2.2   The study area

2.2.1   Choice of the study area

In order to fulfil the objectives set out in section 2.1, an ideal study area should:

1.   Present a high baboon population

2.   Be endemic for human schistosomiasis

3.   Reveal baboon interference with agriculture or human settlement.

4.   Be easily accessible by road and have facilities for setting up a field laboratory for processing trapped animals.

In section 1.7 the primate pest problem was shown to be an important hindrance to agriculture in certain parts of Kenya. Damage to crops is sometimes so extensive that farmers ask for compensation from the Kenya Wildlife Service, the Government department charged with the responsibility for wildlife conservation and management. Such was the case when Dwa Sisal Estate in Kibwezi Division of Machakos District in Kenya's Eastern Province submitted a

compensation claim for Ksh. 1.9 million in 1984 (at that time equivalent to US dollars 95,000). This compensation was requested for damage to sisal plants by baboons (Dwa Estate Records). Dwa Sisal Estate (DSE) is owned by Sulmac Company, a subsidiary of Brooke Bond, the multinational conglomerate. DSE management had also approached the Institute of Primate Research (IPR), (the home institution of this author), to assist in trapping baboons in order to reduce their population and thus crop damage.

Machakos District is reported to have one of the highest prevalence rates of human infection with *S. mansoni* in Kenya with Kibwezi Division having a prevalence of 30% or more (Mutinga and Ngoka, 1971; Ouma, 1987). DSE is next to Kibwezi town on the Nairobi-Mombasa highway (Fig. 2.1) and has an extensive network of plantation roads between the sisal fields. It was in this area where Nelson (1960) and others found baboons infected with *S. mansoni*. For these reasons, DSE was chosen as the major study area.

2.2.2　　　　Description of the study area

DSE is a large scale sisal estate covering over 8,000 hectares (Fig. 2.3). It is situated in an area with a low agricultural potential with an annual rainfall of 500-750mm, most of which falls in April and November (DSE records). The main natural vegetation is bushy woodland (Ojany, 1974). The main water supply for domestic and factory use comes from a dam constructed at the source of Kibwezi River, although there are a few seasonal streams which are dry for most of the year. Kibwezi River and all the other streams in the region drain into the Athi River, the second largest river in Kenya after Tana River (Fig. 2.2).

The estate is divided into numerous plantations in which sisal is grown on a rotational basis, leaving some land fallow for several years. The uncultivated land becomes bushy, providing an ideal habitat for various wildlife species including primates.

2.2.3 Primate species in DSE

The main primate on the estate is the baboon. The taxonomy of the baboons in this area is confusing although it is generally agreed that the yellow baboon (*Papio cynocephalus*), is the most common (Hill, 1967; Maples and McKern, 1967; Kingdon, 1971). Some authors suggest that there may be an overlap of geographical ranges between *P. cynocephalus* and the olive baboon (*P. anubis*). Maples and McKern (1967) suggested that there may be hybrids between the two species in this region. The current author has encountered only *P. cynocephalus*.

Small numbers of vervet (*Cercopithecus aethiops*) and Sykes monkey (*C. mitis*) are also found in DSE.

2.2.4 Sisal damage by primates in DSE

Baboons do most of the damage to the sisal crop. This happens when they break the main-stem at the centre of the plant in order to chew the soft part at the base (Fig. 2.4). This interrupts further growth and hence the production of leaves from which sisal fibre is produced. Baboons can cause damage to the sisal at any stage of growth. Vervets and rarely Sykes monkeys only damage young seedlings because they are not strong enough to break the bigger plants.

The estate employs vermin control guards who keep watch over the plantations and discourage the primates.

2.2.5 History of *S. mansoni* infections in Kibwezi baboons

The first report of *S. mansoni* infection in Kenyan baboons was from Miller (1959) who, with his Kenyan and American colleagues, trapped baboons for arteriosclerosis research sponsored by

the South West Foundation of Research and Education of Texas, USA. Baboons were trapped near the confluence of Kibwezi and Athi Rivers and, using faecal examination and histological techniques they found 32 out of 134 baboons (*P. anubis*) infected with *S. mansoni*. Nelson (1960) followed up the investigation and captured 64 baboons in the same area, 35 of which were found infected with the same parasite species. No further work on *S. mansoni* has been carried out in this area prior to the present study. None of the previous studies established whether baboons could serve as reservoir hosts for human schistosomiasis in the Kibwezi area. Information was not available on the source and intensity of infection in the baboons, and there was only limited information on the closeness of the baboon water contact sites to those of humans. Local snail populations were examined for infection but with negative results. No baboons were found infected in the neighbouring uninhabited Tsavo National Park and this led to the conclusion that the baboons near Kibwezi had been infected from man (Nelson, personal communication).

2.3   The Study Plan

As previously discussed, DSE is a large estate covering over 8,000 hectares. In order to get a representative sample of baboons from the whole estate, the study was divided into three phases.

2.3.1   The pilot study

This was carried out in 1986 with two main objectives:

1.   To locate the baboon troops on the estate.

2.   To assess the status of schistosomiasis infection in the baboon troops.

2.3.2   The main study

This was carried out in 1988 and was based on the findings of the pilot study. It was an extensive survey of schistosomiasis in the baboon troops located on the estate and was aimed at establishing the prevalence and intensity of the infection.

2.3.3   Follow-up studies

These were carried out in 1989 and 1990. They were aimed at specific baboon troops in which the pattern of infection seemed to have some significant relationship with the water contact source.

2.4   Materials and Methods

2.4.1   Capture of baboons from the wild

It is a legal requirement in Kenya that any person wishing to collect wildlife specimens obtains permission from the Kenya Wildlife Service. This was requested by IPR and DSE and subsequently granted for the author to trap baboons from DSE.

2.4.1.1   Selection of a holding site

Prior to trapping the animals, a holding site had to be established. Selection of a holding site was based on:

1.   Availability of roofed shelter and surrounding shade in order the keep the animals cool.

2.   Presence of a hard floor to facilitate easy cleaning and collection of faecal specimens.

3.   Easy access by vehicle in order to facilitate delivery of animals and then food, water and other supplies.

4.   At least 500 metres from human habitation to prevent onlookers disturbing the animals.

A disused estate building (Fig. 2.5) was used for this purpose. One of the rooms served as a field laboratory and post-mortem room while the rest were used for holding animals. To provide electricity, a portable generator was used.

2.4.1.2   Location of baboon troops

This was probably the most difficult part of the exercise. Estate vermin control employees were interviewed to give information on where baboons were damaging sisal plants. Visits to the areas identified were made to search for evidence of the baboons activity. This involved listening for baboon vocalisation, looking for footprints, presence of urine and faeces on the paths, or signs of feeding such as broken branches of shrubs. This was helped by use of a pair of standard binoculars to examine the surrounding area (Fig. 2.6). Once a troop was located, its movements were then studied.

2.4.1.3   Study of troop movements

To follow baboon troops it was necessary to use a small four-wheel drive vehicle (Suzuki[R], Japan) to facilitate off-the-road driving. It was sometimes necessary to follow on foot in thick bush or rocky outcrops. Once a troop was located, it was followed for the rest of the day to its sleeping site, normally in trees or rock cliffs, usually reached by 1800 hours. A sleeping site was visited early the next morning (0600 hours), before the troop started moving away. The animals were then followed from a distance and observed using binoculars. In most cases it was possible to identify the preferred sleeping sites, watering points and regular resting sites within 3 days. The selection of a trapping site was made on the basis of this information. The average baboon troop consisted of 40-50 animals.

2.4.1.4   Selection of a trapping site

A trapping site was selected along the most regular route used by the baboons. The criteria for its selection were:

1.   Proximity to a plantation road, but not so close that passers by could interfere with the procedures. A distance of 200-300 metres from a road was found to be convenient.

2.   Ease of access of vehicles. This was necessary for delivery of the baiting materials and traps.

3.   At least 300 metres from a sisal plantation to avoid damage to the crops.

2.4.1.5   Baiting at the trapping site

The common bait used was maize grain either shelled or on the cob. This was particularly suitable because baboons in the study area raided peasant farms bordering the estate on which the main crop was maize. Also maize had been successfully used previously for trapping baboons, at Gilgil near Nakuru (Strum, 1987) and Kibwezi (Nelson, personal communication).

In each case and prior to introduction of traps, shelled maize and a few cobs were scattered along a path which the baboons were known to favour. Some cobs were placed in branches of surrounding bushes to further attract the animals. This was carried out in the early morning and repeated daily for 2-3 weeks until the baboons became conditioned. By this time the animals would routinely visit the site expecting to find food.

2.4.1.6   Introduction of traps

The traps used were large strong, steel-mesh collapsible structures measuring 1.5m high x 0.9 and 0.9m at the base with a weighted trap door (Fig. 2.7). Designed by IPR staff for obtaining breeding stock for the primate colony, the traps had been successfully used for relocation of baboons from Gilgil to Chololo Ranch in Laikipia near Nanyuki in 1984 for the Gilgil Baboon Project (Strum, 1987). The present author participated in that exercise and so had first hand experience of the traps (Eley *et al*, 1989).

Traps were introduced gradually in batches of 10 to 15 at the baiting site in order to get the baboons accustomed to their presence. They were erected and scattered around the trapping site, with the trap doors either removed or fixed in the open position with steel wire to prevent accidental trapping. There was then a gradual change in the baiting over 2 weeks during which maize outside the traps was discontinued and replaced with bait placed inside the traps. During the same period remaining traps were erected about 2 to 3 metres apart. In this way, up to 40 traps were introduced at a trapping site over a period of 2 to 3 weeks. This period was also long enough for the baboons to be fully conditioned to visiting the site on a daily basis and accepting the bait from the traps.

2.4.1.7   Trapping of baboons

An appropriate time for trapping was chosen which in addition to factors related to the above was dependent on the availability of a lorry and up to 6 DSE employees to help in moving the trapped animals. Traps were set in the evening after the animals had settled in their sleeping site with the aim of capturing them early the following day. It is crucial to avoid trapping one or two animals in the evening as they would have to remain in the traps overnight and could alert others to the danger. Traps were set using a platform made of two sticks supporting a piece of timber (10 x 15 cm) with a hole drilled through the middle (Fig. 2.8). A maize cob was

tied with sewing thread which, in turn was tied to a stronger cotton string (Fig. 2.9). The string was threaded through the hole in the timber board, taken outside the trap and around it to be tied onto the trap door (Fig. 2.10). When a baboon walked into the trap and broke the thread tied to the maize cob, the trap door was released and the exit blocked.

The following morning, traps were initially checked from a distance using binoculars; only when it was clear that most or all the animals in the immediate vicinity had been captured, were the traps approached. These procedures could result in up to two thirds of a troop being trapped in one morning with another 10% over the next 2 days. Remaining animals were rarely trapped beyond this period.

The baboons were then transferred from the traps into smaller holding cages (0.8m high and 0.6 x 0.6m at the base) by aligning the doors and guiding each primate into the smaller cages (Fig. 2.11) before transfer to the holding site by lorry.

2.4.1.8   The holding site

The animals were unloaded in their individual holding cages which were arranged on the floor of the holding rooms at least 0.5 m apart to prevent animal contact between adjacent cages through the heavy gauge wire mesh. They were provided with food and water and left to settle for at least 24 hours before sampling. The food provided was usually shelled maize or formulated monkey chow (Unga Ltd, Kenya) supplemented with fresh fruit. To ensure the animals were not disturbed by either people or potential predators, a 24 hour guard was posted at the entrance.

2.4.2 **Collection and preservation of specimens at the holding site**

2.4.2.1 **Collection and processing of faecal samples**

Faeces were the only samples collected before sedation. This was done usually a day after trapping and was facilitated by raising the holding cages a few centimetres above the floor with wooden or stone blocks and laying plastic sheets underneath, for 24 hours. Each faecal sample thus collected was divided into two portions and processed as follows:

1. The first was used immediately for a miracidial hatching test (described in Appendix 2.1).

2. The second was placed in a cool box prior to processing by the modified Kato technique (Appendix 2.2).

2.4.2.2 **Sedation of baboons for physical measurements**

Before handling, baboons were sedated with an anaesthetic mixture containing 2% xylazine (Rompun (R), Bayer, Leverkusen) and ketamine (Ketaset (R), Bristol Laboratories, Syracuse, New York), 6mg Rompun: 70mg ketamine per millilitre (ml) respectively.

A rough estimate of baboon weight, based on previous experience with captive animals, was made and an intramuscular injection at a dosage rate of 1 ml per 10 kg of body weight administered whilst it was confined to one side of the holding cage. If an animal was particularly excited, a blow gun or pole syringe was used to deliver the injection. Effective sedation occurred within 5 minutes; the animal was removed from the holding cage and then transferred to the post mortem room.

A physical examination was performed and information entered onto a recording form (Appendix 2.5). The weight was determined using a portable weighing balance hung from a beam in the roof while the animal was held in a sacking material. Sex of the animal was recorded; nutritional status (body condition) assessed, the skin examined for ectoparasites and external injuries and the musculoskeletal system examined for evidence of past injuries, etc. When the number of animals was small, dentition was also monitored. Prior to further processing, each animal was assigned to an age group as outlined in Section 2.4.2.3.

2.4.2.3   Ageing of baboons

Using a weight classification based on the observations of Snow (1967) who studied baboons from Kibwezi, the animals were grouped as follows:

1.   Infants: - less than 3 kg body weight
2.   Juveniles: - males above 3 kg but less than 20 kg

                     - females above 3 kg but less than 10 kg
3.   Adults: - males above 20 kg

                     - females above 10 kg

Since it was not always possible to perfuse all animals for schistosomes, due to the number trapped, available reagent supplies and manpower, some selection was necessary. In most cases all the juveniles and adults were perfused but infants were excluded partly due to difficulties encountered in adapting the perfusion equipment for this age group.

2.4.2.4   Euthanasia of baboons

Following physical examination, each animal was placed on its back and the limbs secured to a table using strong cotton string (Fig. 2.13). For animals not selected for further processing,

10 ml of blood was taken from the femoral vein for the IPR serum bank followed by a lethal injection of pentobarbitone sodium (Euthatal (R), May and Baker Ltd, UK) administered at a dosage rate of 100 mg/kg of body weight. The carcass was then donated to the National Museums of Kenya (NMK) for research purposes. For those animals to be perfused, 2500 (for those below 5 kg) to 5000 I.U. (for those above 5 kg) of heparin were administered into the femoral vein before the pentobarbitone injection.

2.4.2.5   Perfusion technique for recovery of adult schistosomes

The perfusion method devised by Smithers and Terry (1965), with some modifications, was used in this study.

Following the lethal injection of Euthatal (R), with the animal secured to the table (ventral side uppermost), a mid-line incision was made from the neck to the groin. The skin was undermined to expose the ribs and abdominal muscles. The abdomen was opened and the rib cage cut at the costochondral junctions leaving the diaphragm intact. The intercostal muscles were severed to allow for clamping of the aorta and the posterior vena cava. The abdominal aorta was clamped immediately prior to the bifurcation into the iliac arteries, in order to exclude the lower limbs from the circulation of perfusion fluid. The posterior vena cava was clamped to exclude the heart.

A small incision was made into the abdominal aorta just after it leaves the heart and a canula connected to the perfusion equipment, inserted. Another incision was made into the hepatic portal vein. Perfusion fluid (0.85% sodium chloride and 1.5% sodium citrate) was introduced into the abdominal aorta via a standard perfusion pump (Masterflex (R), Cole Palmer Instrument Company, USA). This means that a collateral circulation excluding the heart and lower limbs was created. Thus the mesenteric veins, the liver and other abdominal organs were perfused.

The perfusate was collected at the incised hepatic portal vein by creating a depression between the liver and abdominal muscles and then using plastic tubing connected to a standard vacuum pump (Millipore, Bedford, Massachussets, USA). The perfusate was aspirated into a 10 litre bell jar. Perfusion was continued until the mesenteric veins and liver contained no more blood or schistosome worms if they were present. This required about 2 to 3 litres of perfusion fluid. When efficiently perfused, these organs became cream in colour.

The perfusate was then poured through a 105-mesh sieve (Arthur H Thomas Company, Philadelphia, USA) and any worms transferred into a urine jar for counting and preservation.

2.4.2.6   Preservation of adult schistosomes

The worms were transferred from the urine jar to petri dishes containing normal saline and counted under the low power of a dissecting microscope (x 40 magnification).

They were then transferred to a universal bottle containing 70% ethanol prior to staining and morphological assessment. In some cases and where more than 50 worms were recovered, they were divided into two groups:

1.   Preserved as above

2.   Placed in plastic cryopreservation tubes (Nunclon (R), Intermed, Denmark) and kept in a portable freezer at -20°C. These worms were later used for isozyme electrophoresis assays (described in Chapter 5).

2.4.2.7   Collection and preservation of tissue samples

After perfusion, the lungs, liver, small intestine and large intestine were removed separately and their total wet weights taken using a portable laboratory balance (accurate to 1 gm).

From each organ, a sample representing about 10% of the wet weight was taken, placed in a labelled plastic bag and then stored in a portable freezer prior to digestion in potassium hydroxide (Cheever, 1968) for recovering schistosome eggs trapped in the tissues.

In some cases, portions of tissue were preserved in 10% formalin for histology. The carcass was donated to NMK for research purposes.

2.5   Results

2.5.1   Pilot study

2.5.1.1   Observations and trapping

Fifteen baboon troops were located in various parts of the estate. Although it would have required a more detailed study to get accurate numbers in each troop, most were estimated to have between 30 and 50 members.

Four troops were selected, based on ease of access to their sleeping and resting sites and their sources of drinking water, a factor crucial in the transmission of schistosomiasis. The troops were identified according to the most prominent feature in their foraging area (Fig. 2.2) as follows:

1.   The Dwa Rock (DR) troop: Members of this troop foraged at a factory and domestic waste dump near the Dwa Rock, an important landmark in the area, from which DSE takes its name. The troop obtained drinking water from Kibwezi River except during the wet season when pools formed around the rock and surrounding bushes.

2.   The Manoni Springs (MG) troop: foraged in the area near Manoni Springs in the eastern part of the estate. Their preferred drinking site was on Kibwezi River probably because of the thick vegetation around the Springs which could conceal predators.

3.   The Tisia Rock (TR) troop: Near the Tisia Rock, another important landmark in the southern part of the estate. Members of this troop drank from the Wanduli seasonal stream at a site which had water throughout the year due to the fact that it received the continuously flowing sisal effluent from the factory.

4.   The Kikumbulyu (KK) troop: Close to the Kikumbulyu Railway Station on the Nairobi-Mombasa railway. Except during the wet season when members of this troop drank from rain pools and seasonal streams, they had to travel 5 km to the Thange River for water. This river forms the south eastern boundary of the estate.

During the dry season, food was scarce; extensive foraging meant that these baboons occasionally had to go without water for up to two days. Sisal leaves were chewed, for moisture.

2.5.1.2   Perfusion

A total of 106 baboons were trapped from the four troops although only 25 were perfused because the field laboratory was not fully operational. This led to the transportation of baboons to the IPR laboratories. An attempt to perfuse the baboons using a 50ml plastic syringe was unsuccessful.

Table 2.1 shows the number of baboons trapped per troop and those selected for perfusion while Table 2.2. shows a breakdown of age and sex of the animals selected.

Of the 25 baboons perfused, 10 (40%) were found to have S. mansoni infections as shown in Table 2.3. The number of worms recovered per baboon were counted and sexed as shown in Table 2.4. They were later stained with Fast Red B as described in Appendix 2.3. This revealed that the female worms had fully developed vitellaria with single lateral-spined eggs in utero thus positively identifying them as S. mansoni (Fig. 2.14).

2.5.2   Main study

2.5.2.1   Observations and trapping

Survivors of three troops sampled in the pilot study (DR, TR and KK) and other members of three new troops were located and trapped. The new troops are described below.

1.   Kibwezi (KB) troop: Foraging in the outskirts of Kibwezi town to the north of DSE. The troop made no contact with Kibwezi River and relied mainly on a seasonal pond for water. When this pond dried up, water was obtained from a sewage pond near the Kibwezi Shell/BP petrol station on the Nairobi-Mombasa highway (Fig. 2.2).

2.   New Garbage (NG) troop: originating from the bush surrounding a new waste dumping site in the north-eastern part of DSE, the water contact site for these baboons was not identified due to the thick bush surrounding the area. It was assumed that the Kibwezi River, about 1 km away from the dumping site, was utilised.

3.   Manoni Dam (MD) troop: Found close to Manoni Dam (Fig. 2.2) which receives sisal effluent from the factory via the Wanduli seasonal stream. The troop obtained water from the dam throughout the year.

2.5.2.2   Perfusion

A total of 129 baboons were trapped from the six troops and, since a fully operational field laboratory had by then been established, a higher proportion of animals were perfused than had been possible previously (Table 2.5).

A total of 76 animals were perfused for schistosomes. The remaining 53 were euthanised without further processing.

Of the 76 baboons perfused, 13 were found to have infections morphologically similar to *S. mansoni* (Table 2.5). The numbers of worms recovered from each animal is shown in Table 2.6. The highest worm counts were recorded in baboons from the KK troop, in contrast to the very low worm numbers in those from the DR troop. No infections were found in KB, NG and MD troops and only one animal was infected in the TR troop.

2.5.3   Follow-up studies

As stated in section 2.5.2.2 baboons from the KK troop were found to have a significantly high *S. mansoni* worm burden. Since they were the only baboons at the time known to use the Thange River, an attempt was made to locate and trap other baboons using Thange River as a water source.

2.5.3.1   Observations and trapping

Several baboon troops were thought to use the river although only two were clearly identified thus:

1.   Kikumbulyu (KK) troop: previously described.

2.   Thange River (TH) troop: This troop foraged near the south eastern corner of the estate along Thange River (Fig. 2.2). The baboons regularly used a large semi-permanent rock pool on Thange River for water. They also regularly used the overhanging trees as a sleeping site. Baboons using some of the trees deposited urine and faeces directly into the water. Figure 2.15 shows the TH troop sleeping/drinking site.

2.5.3.2   Perfusion

Efforts to trap the survivors of the Kikumbulyu troop were unsuccessful due to human and domestic livestock interference at the trapping site.

Only seven members of the Thange troop were trapped; on perfusion, all were found to be heavily infected with *S. mansoni* (Table 2.7 and 2.8 respectively). More baboons from DR, NG and MD troops were also trapped and 2 members of the DR troop found lightly infected while no infections were found in the NG and MD troops (Tables 2.7 and 2.8 respectively).

2.5.4   Summary of *S. mansoni* infections in Kibwezi baboons

A total of 8 baboon troops were sampled over a 4-year period. Three of those (NG, KB and MD) were schistosome negative whilst the remaining five revealed varying levels of infection with *S. mansoni*. Out of a total of 265 baboons captured, 131 were perfused and 32 (24%) found positive (Table 2.12). The infection status could be classified into 3 categories thus:

1. Baboon troops using Kibwezi River as a source of water had very low *S. mansoni* parasite loads (Table 2.9) with a mean worm count of 10.2 per baboon and a range of 1 to 28.

2. Kikumbulyu troop had moderate levels of infection (Table 2.10) except one adult male (KK2) that had a very heavy parasite load. Excluding KK2 they had a mean worm count of 24.5 per baboon and a range of 4 to 62.

3. Thange River troop had heavy parasite loads (Table 2.11) with a mean of 176.3 worms per baboon and a range of 51 to 449, despite the fact that some spillage of perfusate occurred before counting of worms from baboon numbers TH1 and TH2.

2.5.5 Screening of faecal samples by Kato technique

Faecal samples collected from baboons trapped in the main and follow-up studies were processed using the Kato technique described in Appendix 2.2. They were examined under the low power (x 10 objective) of the light microscope for schistosome eggs.

Samples from troops where no adult schistosomes were recorded on perfusion (KB, NG and MD) were negative for schistosome eggs. No schistosome eggs were found from the single animal in TR troop which had adult worms.

No positive results were recorded from the DR and KK troops (Tables 2.13 and 2.14 respectively).

All the seven baboons from TH troop were positive for *S. mansoni* eggs. Two had low while 5 had moderate to high egg loads (Table 2.15) based on the WHO (1985) classification for human schistosomiasis.

2.5.6 Miracidial hatching tests

Attempts to carry out miracidial hatching tests on faecal samples collected at the holding site were abandoned due to delays caused to other sampling procedures.

Subsequently, fresh faecal samples were collected at the sleeping and resting sites of the baboon troops at the time of baiting in the main and follow-up studies. The samples were processed as outlined in Appendix 2.1.

No positive results were recorded for baboons from DR, TR, NG, KB and MD troops.

Two collections were made at the TH troop sleeping site on the Thange River (Figure 2.15). In June 1989, 6 out of 9 samples were found positive for *S. mansoni* by the Kato technique while 3 were positive for miracidia (Table 2.16). At another collection in August 1989, 8 out of 12 samples were positive on Kato technique and 5 were positive for miracidia (Table 2.17).

Of 8 samples collected at the KK troop sleeping site in August 1989, 6 were positive on Kato technique and 5 were positive for miracidia (Table 2.18). Except for two samples, positive miracidial hatching coincided with positive Kato technique for the rest of the samples (Tables 2.16, 2.17 and 2.18 respectively).

2.5.7   Tissue digests

Tissue samples from baboons perfused in the main and follow-up studies were collected and digested with 10% KOH as described in Section 2.4.2.7 and Appendix 2.4.

Positive results were recorded from one baboon from the DR troop, 3 from the KK troop and all 7 from the TH troop as shown in Tables 2.19, 2.20 and 2.22 respectively.

There was no correlation between the number of mature female worms recovered at perfusion and the tissue egg load from digestion (Tables 2.19, 2.20 and 2.22 respectively).

The distribution of the total egg count from the organs sampled followed a general pattern in which the large intestine had the highest percentage, followed by liver and small intestine in decreasing order. No eggs were found in any of the lung tissues examined (Figure 2.16).

2.6   Discussion

An estimated 15 baboon troops were located in various sites in DSE. The presence of large tracts of uncultivated land was thought to encourage the increase in baboon troops on the estate. Baboons were trapped from four troops in the pilot study.

Although it was not possible to perfuse many animals in the pilot study due to problems encountered in the field laboratory, the main objectives of that study were achieved. It highlighted the large baboon population in DSE and the presence of *S. mansoni* in 40% of the 25 animals perfused.

In the main trapping operation of 1988, three of the original four troops in the pilot study were retraced. These were the DR, TR and KK troops. The MG troop could not be traced because the bush surrounding their home range had been cleared for sisal cultivation. They had probably moved to other parts of the estate.

In order to get a broader picture of the distribution of schistosomiasis in the estate, three more troops were sampled.

Once again, only the three troops sampled in the pilot study were found infected. There was a definite pattern in the intensity of infection with KK troop having a relatively heavier parasite load than DR troop (Table 2.6). One male from the KK troop (KK2) had an unusually large number of worms compared with other members of this troop. It is possible that he had migrated from another more heavily infected troop. Male baboons are known to migrate from one troop to another, particularly in search of better social status (Strum, 1987; Eley, 1989).

It is also interesting to note that the only two baboons found infected in the Tisia troop were males (Tables 2.4 and 2.6 respectively). The TR troop was drinking water from the sisal effluent

which is known to be toxic to *B. pfeifferi* snails (author's unpublished observation). In Tanzania, sisal effluent was found to contain saponins and was used as a molluscide (Nelson, personal communication). The infection in the two males might have come from another source or they found water elsewhere.

Failure to find infection in the large number of baboons perfused from the KB troop may be explained by the fact that they were not observed to make contact with potential sources of infection. The seasonal pond they drank from dried up after the rains and could not support snails. Casual examination of the sewage pond which they used in the dry season did not reveal any snail populations.

The same argument can be applied to the MD troop. Efforts by the author to collect snails from the Manoni Dam only yielded empty shells possibly due to the toxic nature of the sisal effluent. Consequently the baboons in this troop were not exposed to infection.

The NG troop presents a problem. Although its water contact site(s) could not be identified, the only water source in the vicinity was the Kibwezi River. It is therefore unclear as to why they had no infection in contrast to DR and MG troops using the same River.

In the follow-up studies of 1989 and 1990 DR, NG, MD and TH troops were sampled. Only 2 members of the DR troop were found to have very low *S. mansoni* infections. No infection was found in NG and MD troops.

However, very heavy infections were found in TH baboons. These baboons regularly used a rock pool on the Thange River as a water source and the trees overhanging it as a sleeping site (Figure 2.15). The heavy parasite burden might have been due to:

1. The contaminative behaviour of the baboons which dropped infected faeces known to contain viable *S. mansoni* eggs into the water (Tables 2.16 and 2.17 respectively).

2. The large *B. pfeifferi* snail population in the pool known to be susceptible to and infected with *S. mansoni* (see Chapters 4 and 5).

These factors led to a build-up of infection in the rock pool providing a constant source of infection for TH baboons, hence the heavy worm burdens.

There was a remarkable difference in the intensity of infection between baboons using Kibwezi River and those using Thange River (Tables 2.8, 2.9 and 2.10 respectively). The KK baboons fall somewhere in the middle presumably because they used Thange River for only part of the year when their seasonal sources dried out. Furthermore they visited Thange River irregularly since they sometimes got moisture from chewing sisal leaves.

The Kato technique has been widely used for the diagnosis of *S. mansoni* and *S. japonicum* in humans (WHO, 1985; Barreto *et al*, 1990).

Although the method is quite specific, the relatively small amount of faeces examined reduces its sensitivity, particularly in light infections (Brinkmann *et al*, 1988; Barreto *et al*, 1990).

The method has also been adopted for the diagnosis of experimental and natural infections in baboons (Sturrock *et al*, 1976; Sturrock *et al*, 1985; Sturrock *et al*, 1988; Harrison *et al*, 1990). However, certain drawbacks have been encountered when examining baboon faeces, particularly due to the variability in consistency of the faeces and the failure to detect light infections (Sturrock *et al*, 1976).

The negative Kato results obtained when baboons from the DR troop were examined can be explained by the light worm burdens (Table 2.13). However, it is unclear why baboons from the

KK troop with worm burdens similar to those of positive animals from the TH troop were negative (Tables 2.14 and 2.15 respectively).

There is a possibility that the negative findings were due to human error, particularly because samples collected at the KK sleeping site were positive (Table 2.18).

Faeces from wild baboons have a variable consistency ranging from hard and dry to soft and fibrous. It was found in this study that Kato preparations made from these samples were deeply pigmented, with a lot of fibrous debris, making the differentiation between artefacts and *S. mansoni* eggs difficult. This problem is less marked in preparations made from captive baboons, since their diet is less fibrous. This may explain the preference of Kato technique by Sturrock *et al* (1976).

Other methods may be considered for diagnosis of *S. mansoni* in baboon faeces. Taylor *et al* (1973) used the Bell method (Bell, 1963) which was reported to be accurate for the diagnosis of six African schistosomes including *S. mansoni*. McGrew *et al* (1989) used the formol-ether concentration method for the diagnosis of *S. mansoni* and other helminth infections in baboons (*Papio* sp.) and chimpanzee (*Pan* sp.). Pitchford and Visser (1975) devised a method for diagnosis of helminth infections including schistosomiasis in human and animal faeces which was successfully used by Majid *et al* (1980) for the diagnosis of *S. bovis* in cattle in the Sudan. Marshall *et al* (1989) devised a potassium hydroxide digestion method for diagnosis of *S. mansoni* in human faeces. The main problem with these alternative methods is the time involved in performing them and the requirement of bulky equipment and reagents which reduces their usefulness in field diagnosis of the infection.

Improvement of the Kato method may still be the solution to field diagnosis of *S. mansoni* in baboons. Using less material as in the quick Kato technique devised by Peters *et al* (1980) and examining more preparations per sample would be recommended.

The positive miracidial hatching of faecal samples from TH and KK troops was significant. The TH troop used trees above a rock pool on the Thange River as a sleeping and drinking site (Figure 2.15). The pool was found to have large numbers of *B. pfeifferi* snails that were shedding schistosome cercariae (see Chapter 4). There were human settlements downstream from the site.

In some samples, Kato results were negative but miracidial hatching positive (Tables 2.17 and 2.18). This is likely due to the larger sample (one to two grammes) processed for miracidial hatching. Kato samples are only 50mg in weight. The finding of a positive Kato and negative miracidial hatching is more difficult to explain. The time given for hatching of eggs may have been too short (two hours). Ouma (1987) allowed up to 48 hours before recording a miracidial hatching test as negative. The possibility of the eggs not being viable also exists.

There was no direct relationship between eggs recovered in the faeces, the number of adult females recovered at perfusion or the number of eggs recovered in the tissues on digestion. This phenomenon has been observed by various investigators although the explanation for it has not been clearly understood. Cheever and Powers (1969) using rhesus monkeys (*Macaca* sp.) found that the number of eggs per worm pair passed in the faeces, and those recovered in the tissues (per worm pair) tended to decrease in prolonged infections. They suggested that this was due to: (a) egg suppression in female worms; (b) shift of worms from the colon to the venules of the small intestine or (c) egg destruction in the tissues.

Sturrock *et al* (1976) attributed the higher recoveries of male worms in experimentally infected baboons (*Papio anubis*) to the greater difficulty of recovering female worms using the perfusion method. They noted that the inability of the Kato technique to detect eggs in faeces in light infections, unisexual infections and in heavy bisexual infections with suppressed egg production invalidates any attempt to relate egg counts quantitatively to adult worm burdens.

Sturrock et al (1984) obtained similar findings in vervet monkeys (*Cercopithecus aethiops*) where total egg recoveries in faeces and tissues varied substantially within groups experimentally infected with the same cercarial dose.

In the present study, the situation is made even more complex by the fact that these were natural infections of unknown duration and unknown cercarial dose(s). Repeated examinations of faecal samples from the same animal would be recommended as suggested by Miller (1960).

The distribution of tissue eggs was similar to that reported by Sturrock *et al* (1976) and Sturrock *et al* (1988) in experimental infections. Atypical distributions where the liver had more *S. mansoni* eggs than the large intestines (Tables 2.21 and 2.23 respectively) were encountered in the natural infections found in this study. This finding is similar to that of Sturrock *et al* (1976) in experimental animals and has not yet been clearly understood.

Tissue digestion could be used as a diagnostic method in postmortem cases where other alternative methods are not available, since samples could be frozen and examined later.

The overall prevalence of infection for baboons perfused in this study was 32 out of 131 (24%). This figure is misleading because 51 of the animals came from troops with no infection (Table 2.12). When only animals from positive troops are considered the prevalence rises to 32 out of 80 (40%).

This overall prevalence of infection compares with that reported by Miller (1960) who found infection in 32 out of 134 (24%) baboons (*P. doguera*) from the Kibwezi area.

Nelson (1960) followed up that investigation and found 35 out of 64 (55%) baboons from Kibwezi and other parts of East Africa infected with the same parasite. This clearly indicates that *S. mansoni* has persisted in Kibwezi baboons for at least 30 years.

Thange baboons had the highest recorded intensity of natural infection with the parasite. Nelson (1960) found that the majority of the baboons he autopsied had light infections of less than 10 worms per animal while Fenwick (1969) found 12-40 worm pairs in the baboons he autopsied in Lake Manyara National Park in Tanzania. Their results were based on inspection of the mesentery and portal veins. It is likely that they would have recorded heavier worm burdens if they had used the perfusion technique as in the present study.

The significant findings in this investigation could be summarised as follows:

a. Baboons from Kibwezi were still infected with *S. mansoni*, 30 years after the first report by Miller (1959).

b. Baboons using the Kibwezi River as a source of drinking water had a lower intensity of *S. mansoni* infection than those using the Thange River.

c. Members of the TH troop using a rock pool on the Thange River as a drinking and sleeping site were releasing viable *S. mansoni* eggs into the pool which had large

numbers of susceptible *B. pfeifferi* snails. Since there were humans living downstream, the possibility existed of the baboon parasite infecting humans.

In order to expand on these findings, investigations were carried out to determine the pattern of infection in humans using the Kibwezi and Thange Rivers (Chapter 3); comparative water contact patterns in humans and baboons using the two rivers (Chapter 4) and comparison of the baboon and human *S. mansoni* (Chapter 5) were also carried out.

The ability of baboons to maintain the *S. mansoni* infection from TH troop was also investigated (Chapter 5).

Table 2.1   Baboons trapped from Dwa Sisal Estate, Kibwezi, in the pilot study

Baboon troop	Number trapped	Number perfused
Dwa Rock	38	2
Manoni Springs	23	9
Tisia Rock	12	11
Kikumbulyu	33	3
Total	106	25

Table 2.2   Age and sex of baboons selected for perfusion in the pilot study

Age of baboon	Sex		Total
	Male	Female	
Juvenile	11	7	18
Adult	3	4	7
Total	14	11	25

Table 2.3   S. mansoni infections from baboons perfused in the pilot study

Baboon troop	Number perfused	Number positive	Percent positive
Dwa Rock	2	0	0
Manoni Springs	9	6	66.7
Tisia Rock	11	1	9.1
Kikumbulyu	3	3	100
Total	25	10	40

Table 2.4   *S. mansoni* **adult worm counts from baboons perfused in the pilot study**

Tally	Baboon number	Troop	Age	Sex	*S. mansoni* adults recovered		
					Male	Female	Total
1	PCY 48	Manoni	J	F	2	2	4
2	PCY 44	Springs	A	F	3	2	5
3	PCY 59	"	J	M	9	1	10
4	PCY 60	"	J	F	ND*	ND	ND
5	PCY 62	"	J	F	2	2	4
6	PCY 63	"	J	M	14	14	28
7	PCY 28	Tisia Rock	J	M	2	3	5
8	PCY 73	Kikumbulyu	J	M	3	1	4
9	PCY 65	"	A	F	7	6	13
10	PCY 70	"	A	F	8	4	12

Key: Age   :   J = Juvenile
                                 A = Adult

           Sex   :   M = Male
                                   F = Female

           ND*   :   not done

Table 2.5   **Baboons trapped from DSE in the main study**

Baboon troop	Number trapped	Number perfused	Number (+ ve) for *S. mansoni*	Percent positive
Dwa Rock	12	12	6	50
Tisia Rock	12	8	1	12.5
Kikumbulyu	18	18	6	33.3
Kibwezi	43	20	0	0
New Garbage	25	8	0	0
Manoni Dam	19	10	0	0
Total	129	76	13	17.1

Table 2.6 **Recovery of adult S. *mansoni* from baboons trapped in DSE in the main study**

Tally	Baboon number	Troop	Age	Sex	Adult S. *mansoni* recovered		
					Male	Female	Total
1	DG6	Dwa Rock	J	F	2	0	2
2	DG7	"	J	F	0	1	1
3	DG3	"	A	F	5	1	6
4	DG5	"	A	M	1	0	1
5	DG10	"	A	M	3	4	7
6	DG2	"	J	M	0	1	1
7	TR1	Tisia Rock	A	M	10	12	22
8	KK1	Kikumbulyu	A	M	10	9	19
9	KK2	"	A	M	220	127	347
10	KK4	"	J	M	32	30	62
11	KK8	"	A	M	4	3	7
12	KK10	"	A	F	22	28	50
13	KK11	"	A	F	20	11	31

Key: Age : J = Juvenile
                A = Adult

       Sex : M = Male
                F = Female

Table 2.7   **Baboon perfusions in DSE in the follow-up studies**

Baboon troop	Number trapped	Number perfused	Number positive for *S. mansoni*	Percent positive
Dwa Rock	10	10	2	20
New Garbage	3	3	0	0
Manoni Dam	10	10	0	0
Thange River	7	7	7	100

Table 2.8   **Recovery of adult *S. mansoni* from baboons trapped in DSE in the follow-up studies**

Tally	Baboon number	Troop	Age	Sex	*S. mansoni* recoverd		
					Male	Female	Total
1	KD4	Dwa Rock	A	F	3	0	3
2	KD9	"	A	F	1	0	1
3	TH1	Thange River	A	F	114	25	169*
4	TH2	"	J	M	90	60	150*
5	TH3	"	A	F	46	26	72
6	TH4	"	J	F	276	173	449
7	TH5	"	A	F	29	29	58
8	TH6	"	A	F	21	30	51
9	TH7	"	A	F	160	125	285

Key: Age   :   J = Juvenile
                          A = Adult

          Sex   :   M = Male
                          F = Female

          *   =   lower number due to accidental spillage of perfusate before counting

Table 2.9 **Intensity of infection with S. mansoni for baboons using Kibwezi River**

Tally	Baboon number	Troop location in DSE	Age	Sex	Adult S. mansoni worms recovered		
					Male	Female	Total
1	DG6	Dwa Rock	J	F	2	0	2
2	DG7	"	J	F	0	1	1
3	DG3	"	A	F	5	1	6
4	DG5	"	A	M	1	0	1
5	DG10	"	A	M	3	4	7
6	DG2	"	J	M	0	1	1
7	KD4	"	A	F	3	0	3
8	KD9	"	A	F	1	0	1
9	PCY48	Manoni Springs	J	F	2	2	4
10	PCY44	"	A	F	3	2	5
11	PCY59	"	J	M	9	1	10
12	PCY60	"	J	F	ND	ND	ND
13	PCY62	"	J	F	2	2	4
14	PCY63	"	J	M	14	14	28

Key: Age : J = Juvenile
              A = Adult

       Sex : M = Male
              F = Female

       ND = not done

Note: Mean worm count (excluding PCY60) = 10.2 for all the animals

Table 2.10 **Intensity of infection with** *S. mansoni* **for baboons from the Kikumbulyu troop**

Tally	Baboon number	Age	Sex	Adult *S. mansoni* worms recovered		
				Male	Female	Total
1	PCY73	J	M	3	1	4
2	PCY65	A	F	7	6	13
3	PCY70	A	F	8	4	12
4	KK1	A	M	10	9	19
5	KK2	A	M	220	127	347
6	KK4	J	M	32	30	62
7	KK8	A	M	4	3	7
8	KK10	A	F	22	28	50
9	KK11	A	F	20	11	31

Key: Age   :   J = Juvenile
                 A = Adult

         Sex   :   M = Male
                 F = Female

Note: Mean worm count per animal = 60.5 (excluding baboon number KK2) = 24.5

Table 2.11 **Intensity of infection with** *S. mansoni* **for baboons from Thange River troop**

Tally	Baboon Number	Age	Sex	Adult *S. mansoni* worms recovered		
				Male	Female	Total
1	TH1	A	F	144	25	169*
2	TH2	J	M	90	60	150*
3	TH3	A	F	46	26	72
4	TH4	J	F	276	173	449
5	TH5	A	F	29	29	58
6	TH6	A	F	21	30	51
7	TH7	A	F	160	125	285

Key: Age   :   J = Juvenile
                 A = Adult

         Sex   :   M = Male
                 F = Female

         *   =   Lower number due to accidental spillage of perfusate before counting

Note: Mean worm count per animal = 176.3

Table 2.12   Summary of the trapping and perfusion of baboons in Dwa Sisal Estate, Kibwezi

Year of trapping	Number trapped	Number perfused	Number positive for S. mansoni	Percent positive
1986	106	25	10	40
1988	129	76	13	17
1989	10	10	0	0
1990	20	20	9	45
Total	265	131	32	24

Table 2.13   Recovery of S. mansoni eggs, expressed as eggs/gm of faeces, from the Dwa Rock troop

Tally	Baboon number	S. mansoni e.p.g.	Adult S. mansoni		
			Male	Female	Total
1	DG1	0	0	0	0
2	DG2	0	0	1	1
3	DG3	0	5	1	6
4	DG4	0	0	0	0
5	DG5	0	1	0	1
6	DG6	0	2	0	2
7	DG7	0	0	1	1
8	DG8	0	0	0	0
9	DG9	0	0	0	0
10	DG10	0	3	4	7
11	DG11	0	0	0	0
12	DG12	0	0	0	0
13	KD2	0	0	0	0
14	KD3	0	0	0	0
15	KD4	0	3	0	3
16	KD8	0	0	0	0
17	KD9	0	1	0	1
18	KD10	0	0	0	0

Table 2.14   Recovery of *S. mansoni* eggs, expressed as eggs/gm of faeces, from the Kikumbulyu troop

Tally	Baboon number	*S. mansoni* e.p.g.	Adult *S. mansoni*		
			Male	Female	Total
1	KK1	0	10	9	19
2	KK2	0	220	127	347
3	KK3	0	0	0	0
4	KK4	0	32	30	62
5	KK5	0	0	0	0
6	KK6	0	0	0	0
7	KK7	0	0	0	0
8	KK8	0	4	3	7
9	KK9	0	0	0	0
10	KK10	0	22	28	50
11	KK11	0	20	11	31
12	KK12	0	0	0	0
13	KK13	0	0	0	0
14	KK14	0	0	0	0
15	KK15	0	0	0	0
16	KK16	0	0	0	0
17	KK17	0	0	0	0
18	KK18	0	0	0	0

Table 2.15   Recovery of *S. mansoni* eggs, expressed as eggs/gm of faeces, from the Thange River troop

Tally	Baboon number	*S. mansoni* e.p.g.	Adult *S. mansoni*		
			Male	Female	Total
1	TH1	330	144	25	169*
2	TH2	1280	90	60	150*
3	TH3	30	46	26	72
4	TH4	320	276	173	449
5	TH5	100	29	29	58
6	TH6	30	21	30	51
7	TH7	250	160	125	285

* = spillage of perfusate occurred before counting, so less than true count.

Table 2.16 Screening of faecal samples collected from the Thange troop sleeping site, for *S. mansoni*, using Kato and miracidial hatching techniques (June 1989)

Sample number	Kato method: eggs/gm of faeces	Miracidial hatching test
A1	100	-
A2	0	-
A3	20	+
A4	0	-
A5	20	-
A7	30	+
A8	100	+
A9	0	-
A10	10	-

Table 2.17 Screening of faecal samples collected from the Thange troop sleeping site, for *S. mansoni*, using Kato and miracidial hatching techniques (August 1989)

Sample number	Kato method: eggs/gm of faeces	Miracidial hatching test
T1	0	+
T4	150	+
T8	130	-
T9	70	-
T11	40	+
T14	70	-
T16	10	+
T27	0	-
T34	180	-
T36	0	-
T43	70	+
T22	0	-

Table 2.18 Screening of faecal samples collected from Kikumbulyu troop sleeping site, for *S. mansoni*, using Kato and miracidial hatching techniques (August 1989)

Sample number	Kato method: eggs/gm of faeces	Miracidial hatching test
K1	180	+
K3	100	+
K4	10	-
K6	0	+
K7	20	-
K8	10	+
K9	0	-
K10	10	+

Table 2.19 Recovery of *S. mansoni* eggs from tissues of baboons perfused in the Dwa Rock troop

Baboon number	Total number of eggs per organ				Adult female *S. mansoni*
	Liver	Large intestine	Small intestine	Lung	
DG3	0	0	0	0	1
DG5	0	0	0	0	0
DG7	0	0	0	0	1
DG10	0	63756	23850	0	4
KD4	0	0	0	0	0
KD9	0	0	0	0	0

Note: Distribution of tissue eggs from the four organs as percentage of the total number of eggs recovered from DG10.

Liver   = 0%
Large intestine = 72.8%
Small intestine = 27.2%
Lung   = 0%

Table 2.20 Recovery of *S. mansoni* eggs from tissues of baboons perfused in the Kikumbulyu troop

Baboon number	Total number of eggs per organ				Adult female *S. mansoni*
	Liver	Large intestine	Small intestine	Lung	
KK1	0	0	0	0	9
KK2	566291	1072000	0	0	127
KK3	0	0	0	0	0
KK4	66000	453420	35000	0	30
KK5	0	0	0	0	0
KK6	0	0	0	0	0
KK7	0	0	0	0	0
KK8	4307	21285	0	0	3
KK9	0	0	0	0	0
KK10	0	0	0	0	28
KK11	0	0	0	0	11
KK12	0	0	0	0	0
KK13	0	0	0	0	0
KK14	0	0	0	0	0
KK15	0	0	0	0	0
KK16	0	0	0	0	0
KK17	0	0	0	0	0

Table 2.21   Distribution of the total tissue egg count from the four organs, in positive baboons from Kikumbulyu troop

Baboon number	% of the total number of eggs from the four organs			
	Liver	Large intestine	Small intestine	Lung
KK2	34.6	65.4	0	0
KK4	11.9	81.8	6.3	0
KK8	16.8	83.2	0	0

Table 2.22   Recovery of *S. mansoni* eggs from tissues of baboons perfused in the Thange River troop

Baboon number	Total number of eggs per organ				Adult female S. mansoni
	Liver	Large intestine	Small intestine	Lung	
TH1	531000	944794	18000	0	25*
TH2	181500	605250	41557	0	60*
TH3	29725	11944	875	0	26
TH4	82154	181157	2625	0	173
TH5	12343	285000	0	0	29
TH6	6837	1951	0	0	30
TH7	20706	64000	0	0	125

* = less than true number due to spillage of perfusate before counting

Table 2.23   Distribution of the total tissue egg count from the four organs, in baboons from Thange troop

Baboon number	% of the total number of eggs from the four organs			
	Liver	Large intestine	Small intestine	Lung
TH1	35.5	63.2	1.2	0
TH2	21.9	73.0	5.0	0
TH3	69.9	28.1	2.0	0
TH4	30.9	68.1	1.0	0
TH5	30.2	69.8	0	0
TH6	77.8	22.2	0	0
TH7	24.4	75.6	0	0

Fig. 2.1   Map of Kenya showing Kibwezi and Amboseli study sites

N
↑

Kisumu

Nakuru

R. Tana

Nairobi

Kibwezi

Amboseli
N.P.

R. Athi

Mombasa

0   200 k

Scale

Key:

☐ Study site

▬ River

── Nairobi-Mombasa Road
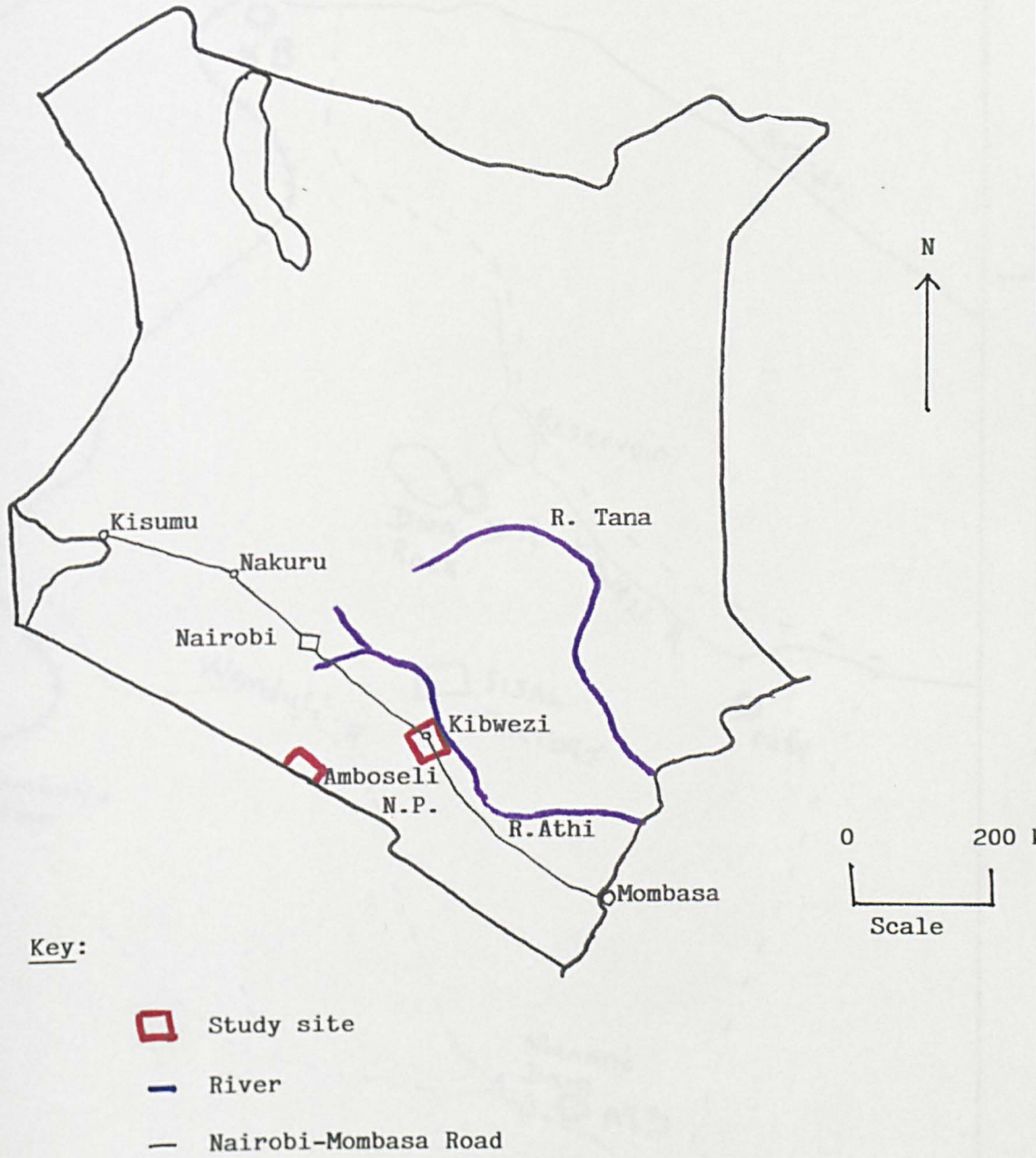
Fig. 2.2   **Map of Dwa Sisal Estate showing major features and location of baboon troops**

Key:
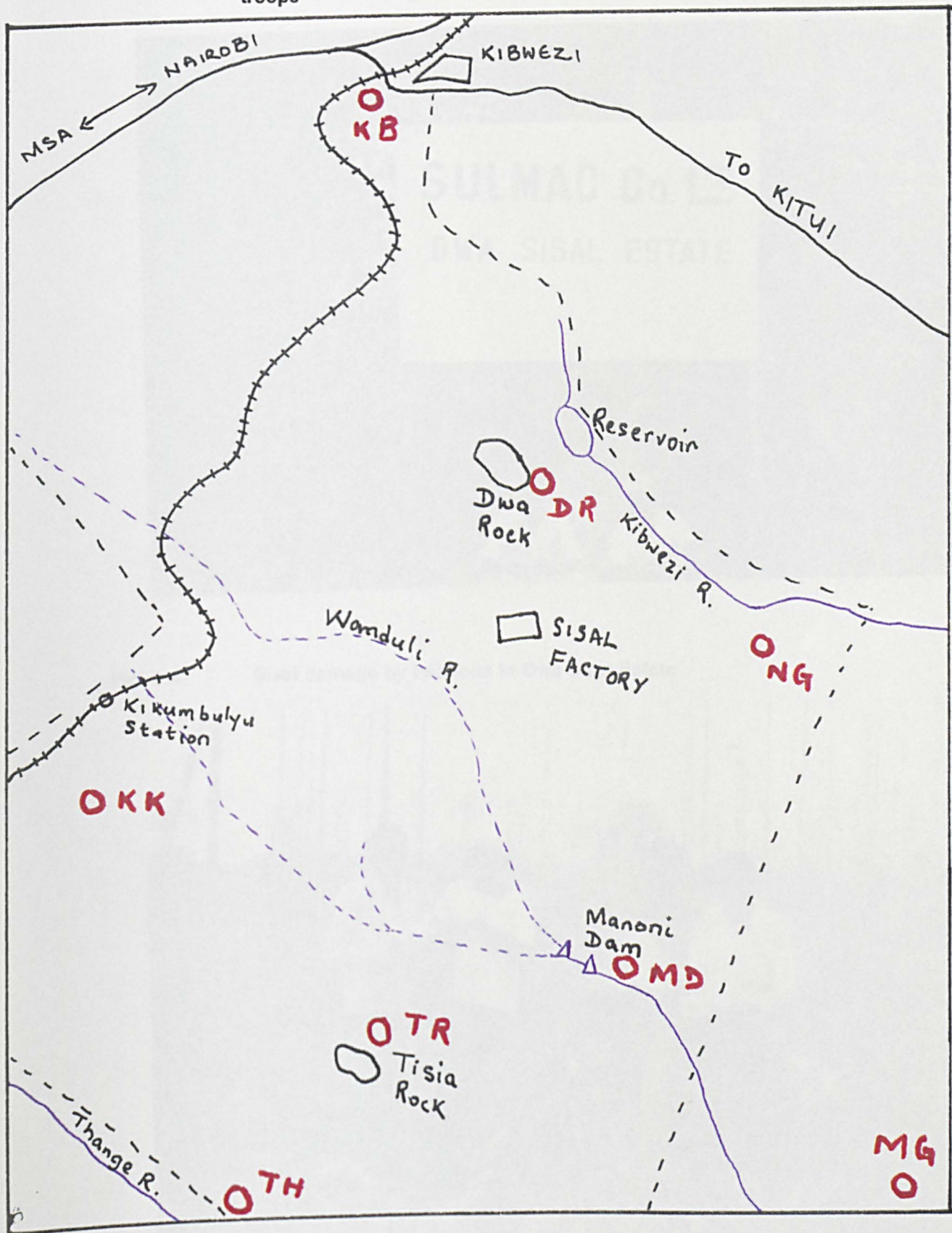
- – – – DSE boundary
- O Baboon troop
- — Road
- +++ Railway
- — River
- - - - Seasonal stream

Scale 0

Fig. 2.3   Dwa Sisal Estate, Kibwezi

Fig. 2.4   Sisal damage by baboons in Dwa Sisal Estate

Fig. 2.5   Disused Dwa Sisal Estate building that was used as a field laboratory
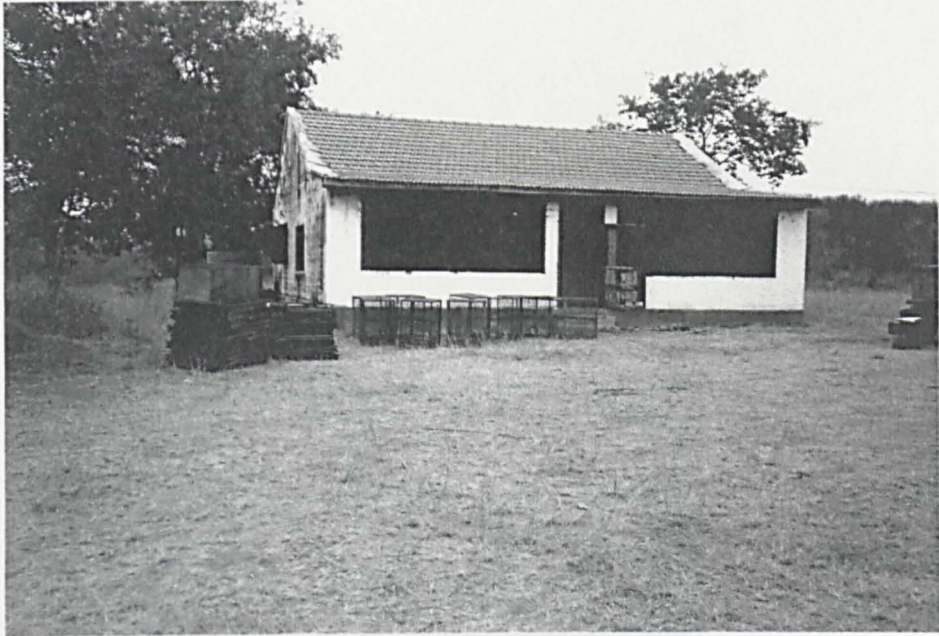
Fig. 2.6   Looking for baboons in Dwa Sisal Estate
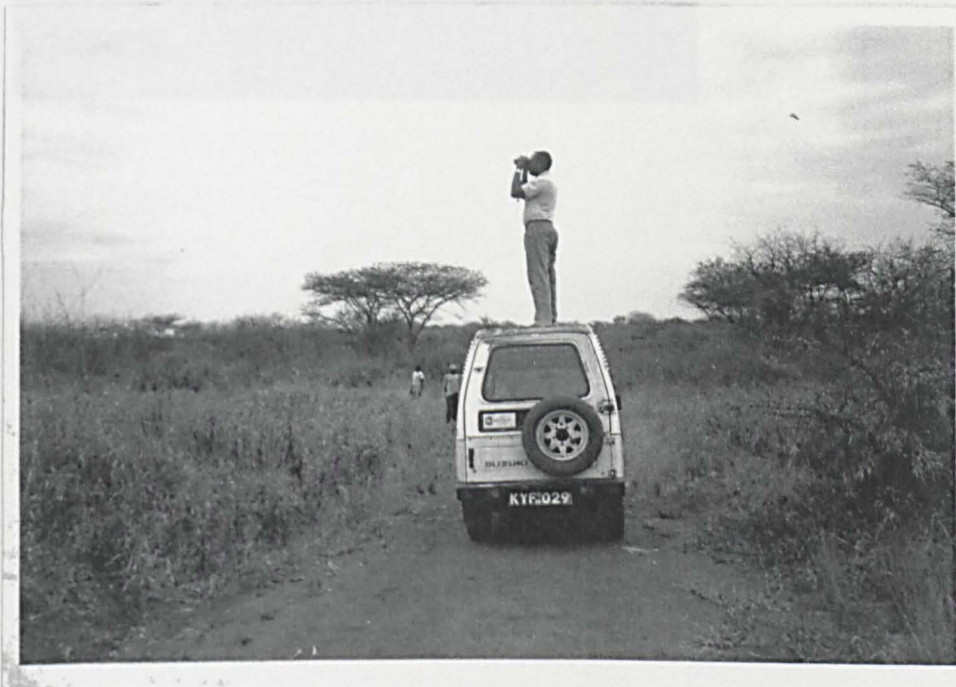
Fig. 2.7   Baboons inside traps

Fig. 2.8   Diagram of a trapping timber board

Holes for attachment to platform

Hole for threading string
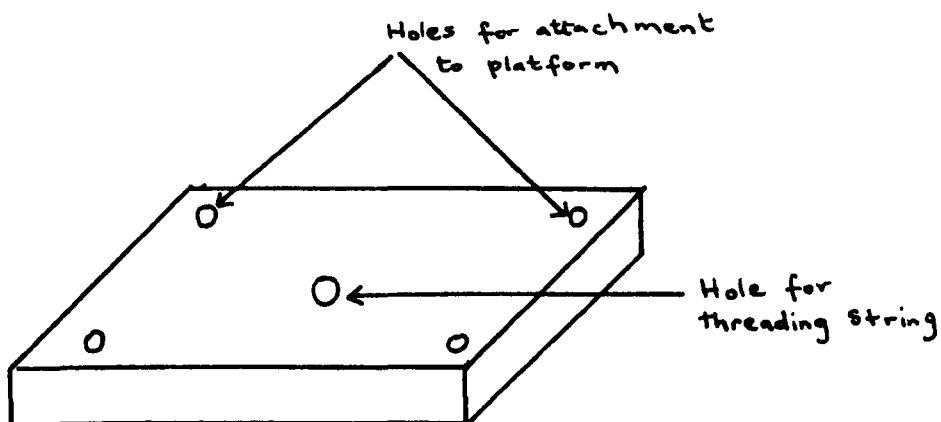
Fig. 2.9   Diagram of a maize cob attached to a string on the trapping board

Sewing thread

MAIZE COB

TIMBER BOARD

Cotton String
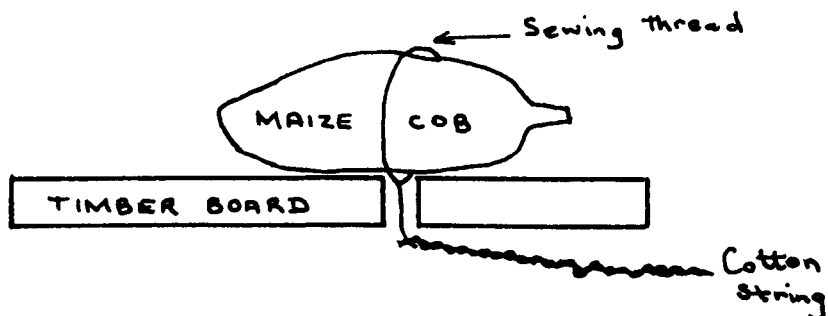
Fig. 2.10   Diagram showing the setting of a baboon trap

Cotton String

Maize

Platform

Weighted trap door

Entrance →

Ground
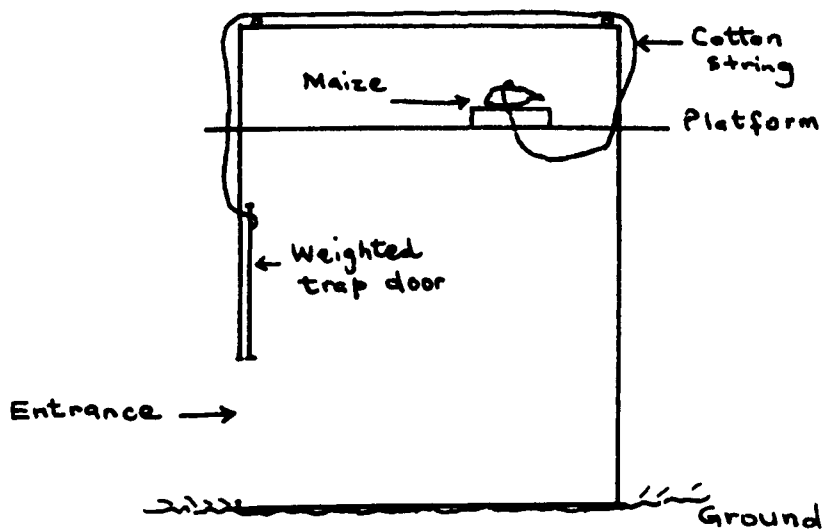
Fig. 2.11a   Transferring a baboon from a trap to a holding cage

Fig. 2.11b   Baboon inside a holding cage

Fig. 2.12   Equipment used in the Kato faecal smear technique.
Clockwise from top: cellophane tape, 105-mesh sieve with handle and a
Kato-Katz template
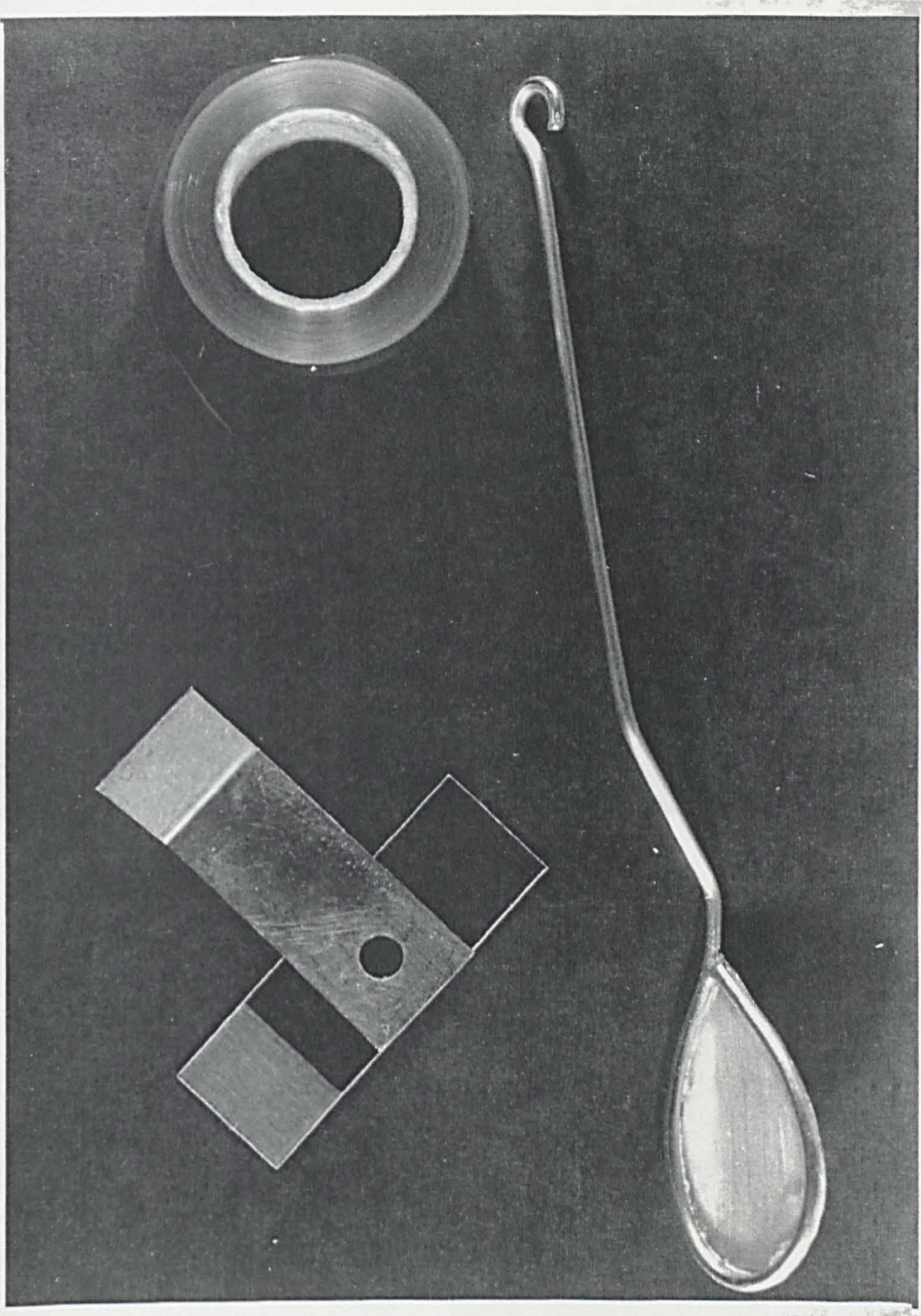
Fig. 2.13   Physical examination of a baboon

Fig 2.14a   Mature *Schistosoma mansoni* female stained with Fast Red B with single egg *in utero*

Fig. 2.14b   Kato preparation of *S. mansoni* egg in faeces
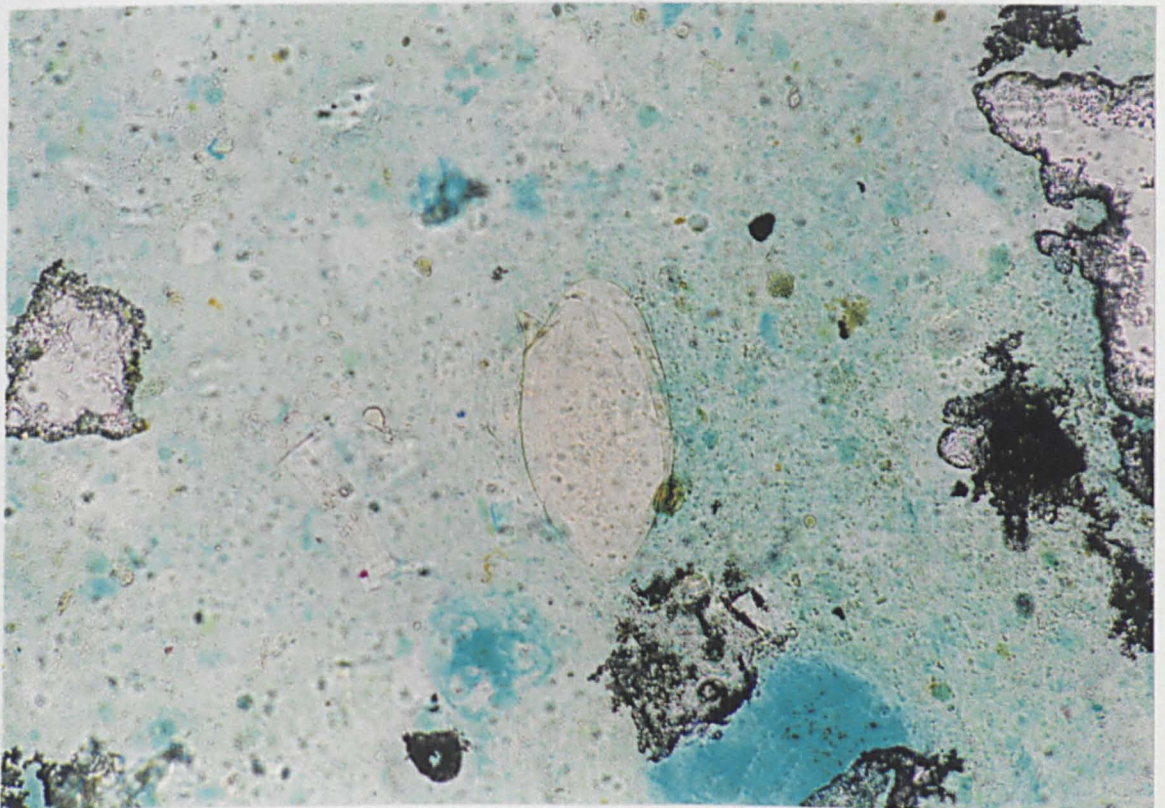
Fig. 2.15   Thange baboon troop drinking and sleeping site

Fig. 2.16   Percent distribution of the total tissue egg count in the liver (LV), large intestine (LI) and small intestine (SI) in baboons from DSE
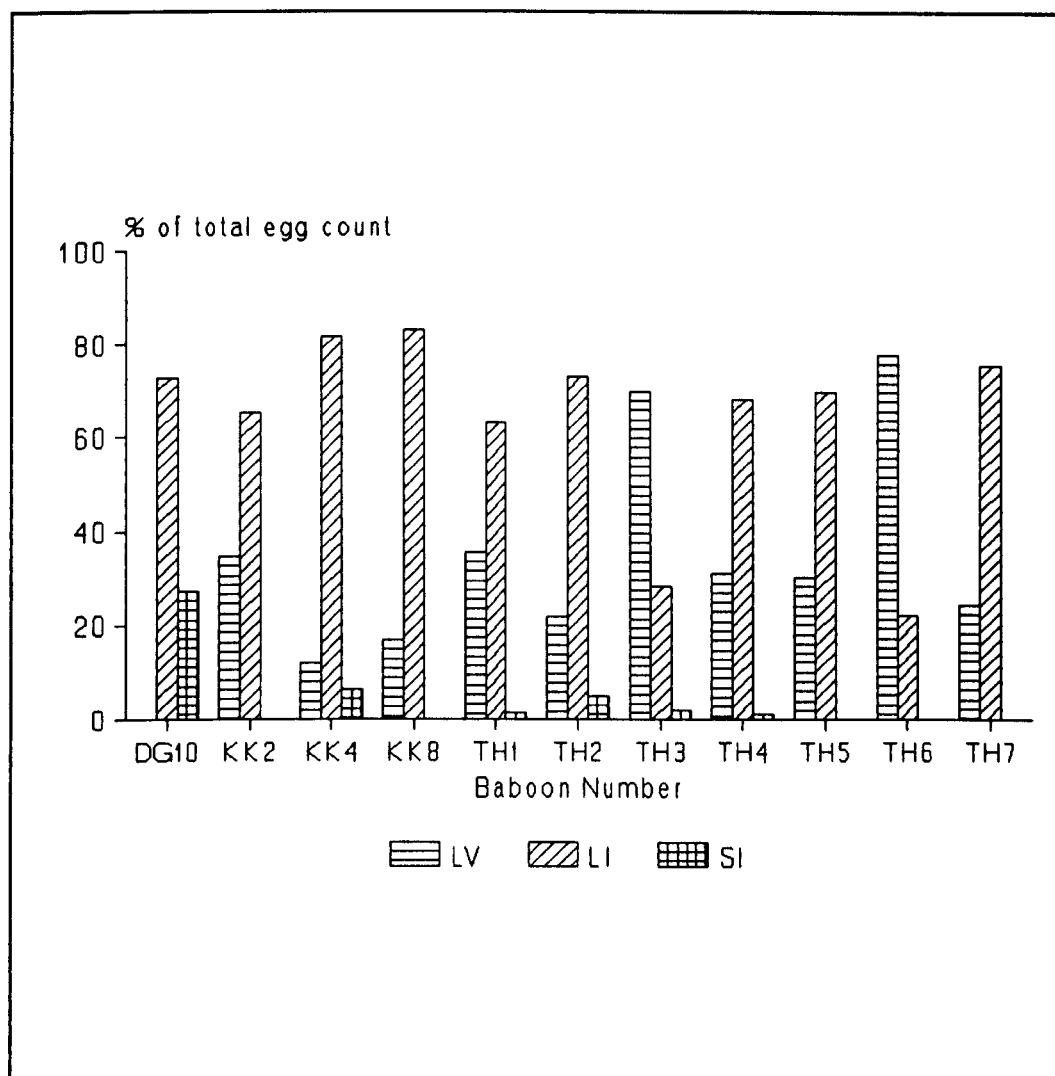
CHAPTER 3

AN EPIDEMIOLOGICAL SURVEY OF *S. MANSONI* IN SCHOOL CHILDREN

FROM SELECTED SITES IN KIBWEZI DIVISION

OF MACHAKOS DISTRICT, KENYA

3.1   Introduction

Machakos District has been the focus of *S. mansoni* prevalence studies for the past 20 years and has been reported to have high levels of infection in some areas (Mutinga and Ngoka, 1971; Butterworth *et al*, 1984; Ouma *et al*, 1985; Ouma, 1987; Butterworth, 1990).

In their survey of schoolchildren in the district, Mutinga and Ngoka (1971) reported a prevalence of 30.7% *S. mansoni* infection in Kibwezi Division. Recent reports indicate high prevalence and morbidity rates in schoolchildren from Kambu location about 20km south of Kibwezi town (Butterworth, 1990).

Several reports of *S. mansoni* infections have been made by clinical staff at Dwa Sisal Estate and Kibwezi Health Centre but no formal study of the prevalence and intensity of the parasite in the local population had been carried out.

The need to establish the status of the infection in the local population was made necessary by the finding of varying levels of the parasite in baboons from DSE (Chapter 2). The baboon infection was related to their source of water; those using Kibwezi River having low parasite burdens and those using Thange River harbouring high levels of infection.

Various investigators have found schoolchildren the most appropriate group for epidemiological studies, partly because they are easy to organise, and also constitute the age most susceptible

to schistosomiasis due to their increased water contact (Mutinga and Ngoka, 1971; Ouma, 1987; Wiselka et al, 1988; Chandiwana and Christensen, 1988).

Primary schoolchildren were chosen for this investigation.

3.2 Study sites

Three primary schools were selected because their sources of water were either Kibwezi River (Dwa Primary School) or Thange River (Thange and Nzavoni Primary Schools) (Figure 3.1).

3.2.1 Dwa Primary School (DPS)

This is the only primary school in DSE. Most of the children who attend are from families working and living on the estate. A few come from the neighbouring peasant farms.

Like other primary schools in Kenya, DPS has 9 classes of pupils as follows:

a.   Pre-primary or nursery class: this has the youngest pupils usually aged 3-6 years. It is the preparatory class for entrance to the National Primary School Curriculum.

b.   Primary school classes: there are 8 classes generally referred to as Standard (Std) 1 to 8. Pupils in Std 8 sit for the Kenya Certificate of Primary Education (KCPE) whose pass is necessary for entrance into the High School Curriculum.

DPS is supplied with piped and chlorinated water from an estate reservoir on Kibwezi River (Figure 2.2).

At the time of sampling there were 400 pupils attending DPS.

3.2.2   Thange Primary School (TPS)

This school is situated about 1km from Thange Market on the Nairobi-Mombasa highway (Figure 3.1). It is less than 1km from the main source of Thange River. Children who attend this school come from the surrounding peasant farms and generally have to wade across the river to get to school.

Thange River is semi-permanent, with a continuous flow only in the wet months of the year (March-May and October-November). In the dry months, water is found in rock pools along the river bed. It is the only source of water for the school and residents of surrounding areas. Since there is no piped supply, all water requirements are fulfilled by drawing from the river with containers (Figure 3.6).

There were 700 children attending the school at the time of sampling (Figure 3.5).

3.2.3   Nzavoni Primary School (NPS)

This school is about 3km downstream from TPS (Figure 3.1). It is next to Nzavoni Village and about 500m from Thange River.

Most of the children come from Nzavoni Village, although a few come from near TPS. They also have to wade across the Thange River to get to school.

Water requirements are satisfied by drawing from the River in containers.

There were 300 pupils at the time of this investigation attending the school (Figure 3.7).

3.3 Materials and Methods

3.3.1 Sampling of schoolchildren

In order to ensure maximum cooperation from the people, local administrators, elders and schoolteachers were approached and the need for sampling explained to them (Figure 3.8). After getting the necessary consent from all the parties involved, lists were prepared by teachers from each school with details of class, sex and age of each child. In most cases the age was accurate to the nearest year although at times estimates had to be made if parents were illiterate. A convenient date was chosen for sampling. The month of September was found to be the most appropriate because the weather was dry and road communication to the schools possible. It was also the beginning of the third school term, convenient for both teachers and pupils.

Samples were collected from all pupils present and willing to co-operate.

3.3.2 Collection and processing of faecal samples

Small plastic containers with tight-fitting lids (polypots) labelled for each pupil according to the lists were handed out. To make the handling of faecal samples as clean as possible for the pupils, a piece of wooden stick about 10cm long was provided together with a piece of tissue paper. Pupils were shown how to take a sample weighing approximately 10gm. They were asked to provide a sample as soon as possible without taking the polypot home.

The samples provided were kept in a coolbox and transported to a temporary laboratory in DSE (for DPS samples) or Kibwezi Health Centre (for TPS and NPS samples). They were then processed using the modified Kato technique (Appendix 2.2) and the prepared slides kept in wooden or plastic boxes for transportation to the IPR Parasitology laboratory.

The slides were examined under the low power (x 10 objective) of the light microscope for the presence of *S. mansoni* eggs.

3.3.3 Treatment of infected schoolchildren

For ethical reasons, all children found infected were treated. This was done by staff from the Ministry of Health and University of Nairobi Medical School. The drug used was praziquantel (Biltricide[R]) donated by Bayer[R], Leverkusen, Company of Germany. It was administered at a single oral dose of 40mg/kg of body weight (Figures 3.9 and 3.10).

Pupils from DPS provided another faecal sample, 5 months post-treatment. The samples were processed as described in Section 3.3.2 and examined for re-infection.

3.3.4 Analysis of data

The results from Kato technique were recorded as eggs/gram of faeces and the information entered into a personal computer using the SPSS/PC statistical programme. In order to compare the intensity of infection in the three schools, egg loads were classified according to WHO (1985) as follows:

1 to 100 eggs per gram	= low intensity
101 to 800 eggs per gram	= moderate intensity
over 800 eggs per gram	= high intensity

The results were statistically analysed using the Chi-Square test.

3.4 Results

A total of 1094 pupils were sampled from the 3 schools (Table 3.1), which gave a compliance rate of 78%. Out of these, 780 (71.3%) were positive for *S. mansoni* infection (Table 3.1). There was a significant difference (p < 0.01) in the prevalence rates of infection according to school (DPS = 38.4%, TPS = 82.4% and NPS = 85.6%).

There was no significant difference in the prevalence of infection according to sex of the pupil (Table 3.2) but prevalence according to age of the pupil increased gradually for all schools (Table 3.3). The lowest levels were recorded in DPS (Figure 3.2).

The intensity of infection in the 3 schools was calculated using arithmetic and geometric ($\log_{10}$ [eggs/gm + 1]) mean egg counts. There were significant differences (p < 0.01) between the schools. The arithmetic and geometric means rose gradually with age for all the 3 schools (Tables 3.4 and 3.5 and Figures 3.3 and 3.4).

Of the 113 pupils found infected in DPS, 82 were treated with praziquantel (Biltricide [R]) at a single oral dose of 40mg/kg of body mass.

Faecal samples were collected from the group, five months after treatment and assessed for re-infection. Sixteen (19.5%) of these pupils were found to have very low intensity (less than 100 eggs/gm of faeces) infections.

3.5 Discussion

Prevalence of infection significantly differed between the 3 schools. Although it was not obvious why this difference existed, it was partly due to the fact that DPS pupils had access to piped and chlorinated water from DSE. This, however, raises the question of what the likely source

of their infection was. One likely factor contributing to infection was that the pupils went to visit friends and relatives outside the estate thus coming into contact with contaminated water sources. There is an ongoing transmission of *S. mansoni* on the estate as evidenced by the re-infection of pupils within 5 months of treatment.

Apart from the local ethnic group, the Kamba, DSE employed workers from other parts of Kenya, including Lake Victoria, where they may have become infected during visits to their relatives.

Pupils in TPS and NPS obtained water from Thange River which is known to be contaminated as evidenced by the infection in Thange Troop of baboons (Chapter 2). The high prevalence rate of infection in these 2 schools might be explained by the fact that pupils had to wade across the river daily to get to school. Despite the fact that Thange River is semi-permanent the segment of river between these two schools always had some water during the time of the study.

The prevalence of infection increased according to age (Figure 3.2), presumably because of repeated exposure of older pupils over the years. This led to a build-up of infection that was detectable by faecal examination. It has been observed in other studies (Butterworth *et al*, 1984; Butterworth *et al*, 1988; Butterworth, 1990; Hagan, 1992) that cumulative re-infection occurs in young children and it is only in the adolescent and young adult stages that age-dependent acquired immunity starts to develop.

Older children also make more water contact particularly age groups between 10-19 years (Ouma, 1987; Chandiwana and Christensen, 1988).

The differences in arithmetic and geometric mean egg counts between TPS and NPS for similar age groups were probably due to different levels of contamination of their water contact

sources. TPS pupils make contact with the river close to the source while NPS pupils make contact 3km downstream. Since the river is the only source of water in the area, residents using it between the two schools would have increased contamination, thus leading to a higher level of infection in NPS pupils.

It is interesting to note that humans using Thange River had a higher prevalence of infection than those using Kibwezi River. This observation is similar to that in baboons using the two rivers, indicating a heavier contamination of Thange River than Kibwezi River.

The lower prevalence of infection in humans using the Kibwezi River is most likely due to the availability of treated piped water from the DSE supply. This reduces contact with contaminated water sources, particularly for domestic chores which form the bulk of water contact activity (Chapter 4; Ouma, 1987; Gryseels, 1991).

The high prevalence of infection in humans using the Thange River close to its source poses a problem for both the humans and animals, particularly baboons, using it downstream.

These findings show the endemic nature of *S. mansoni* infection in humans (and baboons: see Chapter 2) in Kibwezi Division. A control strategy would have to consider how to deal with the infection in baboons and probably other wildlife reservoirs.

Table 3.1 Prevalence of *S. mansoni* infection in Kibwezi Primary Schools

School	No. of pupils sampled	No. positive for *S. mansoni*	% positive
DPS	294	113	38.4
TPS	563	464	82.4
NPS	237	203	85.6
Total	1094	780	71.3

Table 3.2 Prevalence of *S. mansoni* infection in Kibwezi Primary Schools (percent infected according to sex)

Sex	DPS	TPS	NPS
Male	42.5	80.5	85.8
Female	33.6	84.3	85.5

Table 3.3 Prevalence of *S. mansoni* infection in Kibwezi Primary Schools (percent infected according to age)

Age group	DPS	TPS	NPS
1 (0-4 years)	0	20	0
2 (5-9 years)	25.7	68.9	70.4
3 (10-14 years)	59.7	88.3	92.8
4 (15-19 years)	65.1	91.7	94.3

Key: Age groups classified according to Ouma (1987)

Table 3.4   Arithmetic means of *S. mansoni* egg counts per gram in Kibwezi Primary Schools according to age

Age group	DPS	TPS	NPS
1 (0-4 years)	0	5.0	0
2 (5-9 years)	43.3	186.6	172.5
3 (10-14 years)	135.8	336.5	577.0
4 (15-19 years)	159.5	265.1	438.5

Table 3.5   Geometric means of *S. mansoni* egg counts ($\log_{10}$ [eggs/gm + 1]) according to age

Age group	DPS	TPS	NPS
1 (0-4 years)	1	1.8	1
2 (5-9 years)	2.8	24.6	34.1
3 (10-14 years)	17.0	79.0	238.7
4 (15-19 years)	23.3	92.3	187.1

Fig. 3.1   Map of Kibwezi showing the location of Dwa Primary School (DPS),
Thange Primary School (TPS) and Nzavoni Primary School (NPS)

Kibwezi R.

DPS

N

Dwa

Kibwezi

Plantations

Kikumbulyu station

Wanduli R.

Thange R.

NPS

TPS

Thange Market
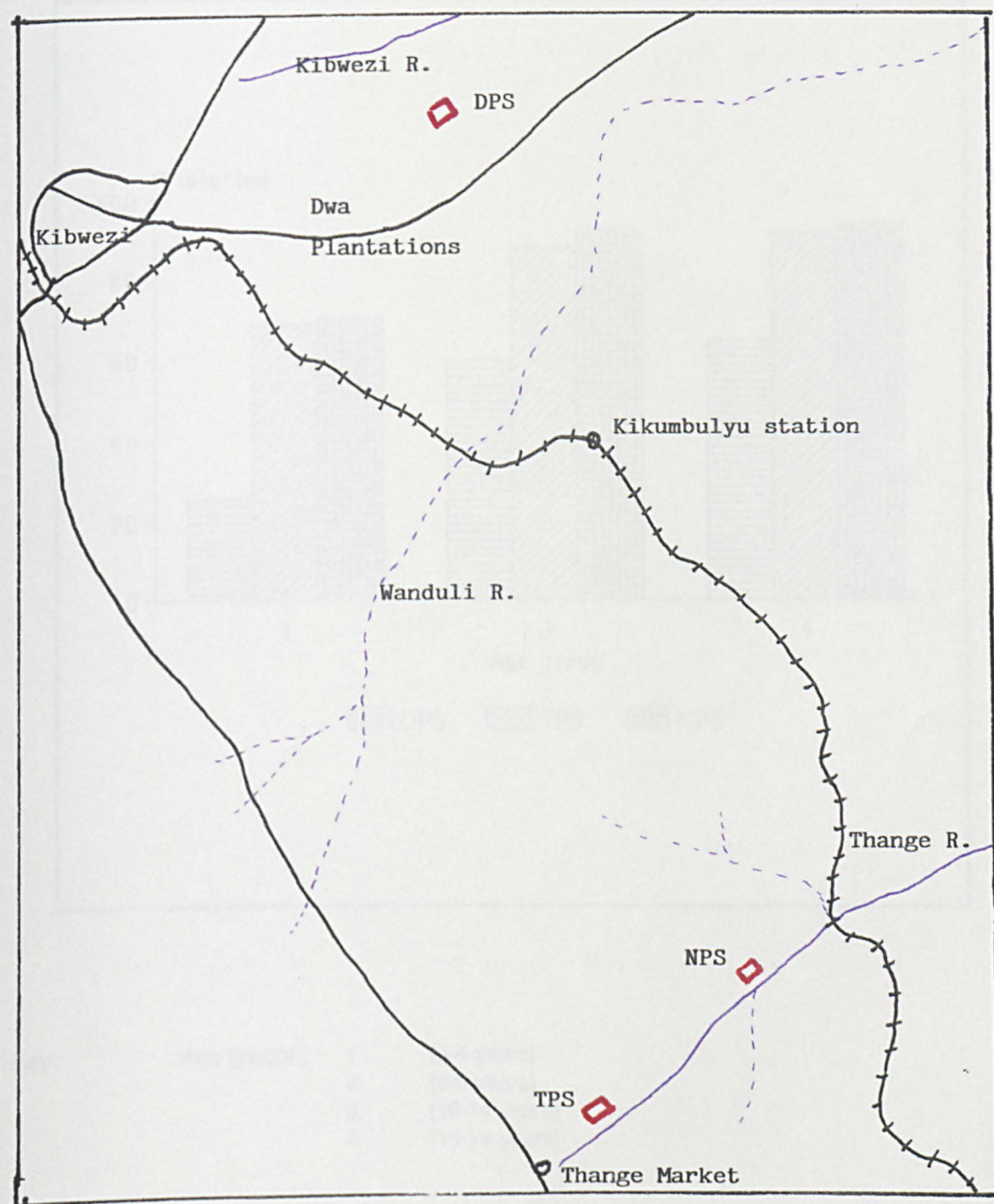
Key: School

River

Seasonal stream

Road

Railway

0   3 km

Scale

Fig. 3.2   Prevalence of *S. mansoni* infection according to age in 3 primary schools sampled in Kibwezi: Dwa Primary School (DPS), Thange Primary School (TPS) and Nzavoni Primary School (NPS)
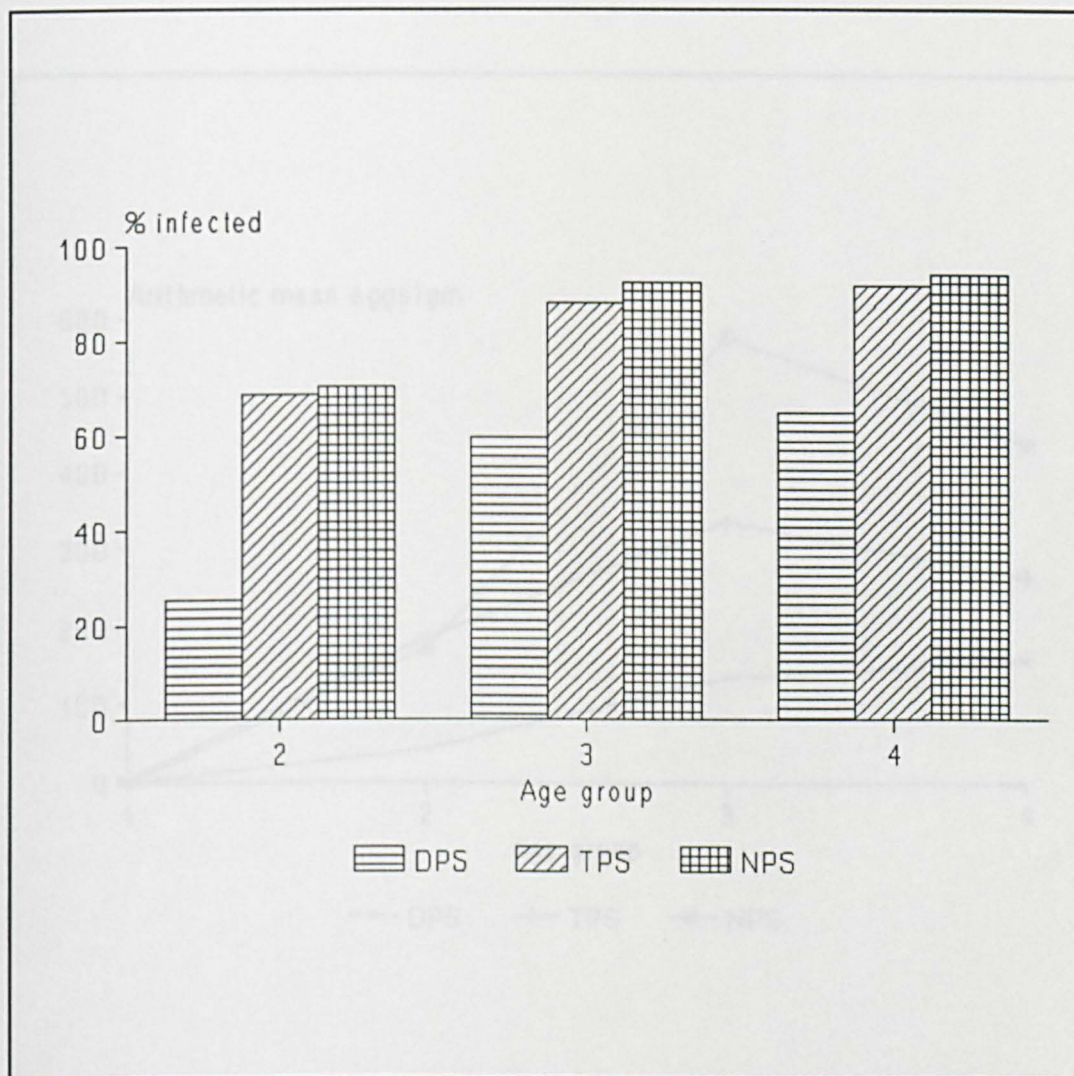
Key: Age groups: 1   (0-4 years)
                              2   (5-9 years)
                              3   (10-14 years)
                              4   (15-19 years)

Note: Age group 1 has been omitted from the figure because only one school was represented and hence no direct comparison can be made.

Fig. 3.3   Arithmetic means of *S. mansoni* egg counts from 3 primary schools in Kibwezi: Dwa Primary School (DPS), Thange Primary School (TPS) and Nzavoni Primary School (NPS)
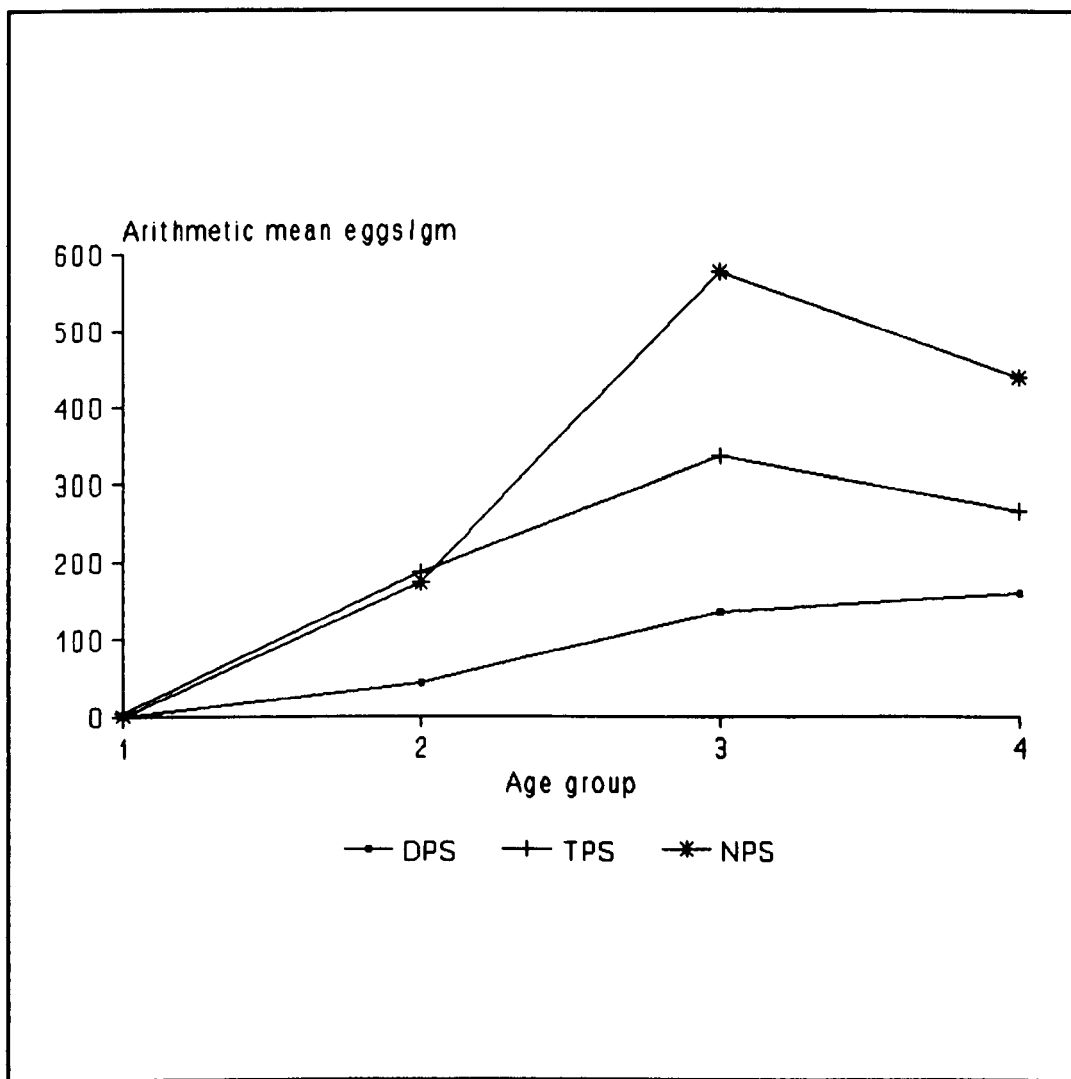
Fig. 3.4   **Geometric means of *S. mansoni* egg counts from 3 primary schools in Kibwezi: Dwa Primary School (DPS), Thange Primary School (TPS) and Nzavoni Primary School (NPS)**
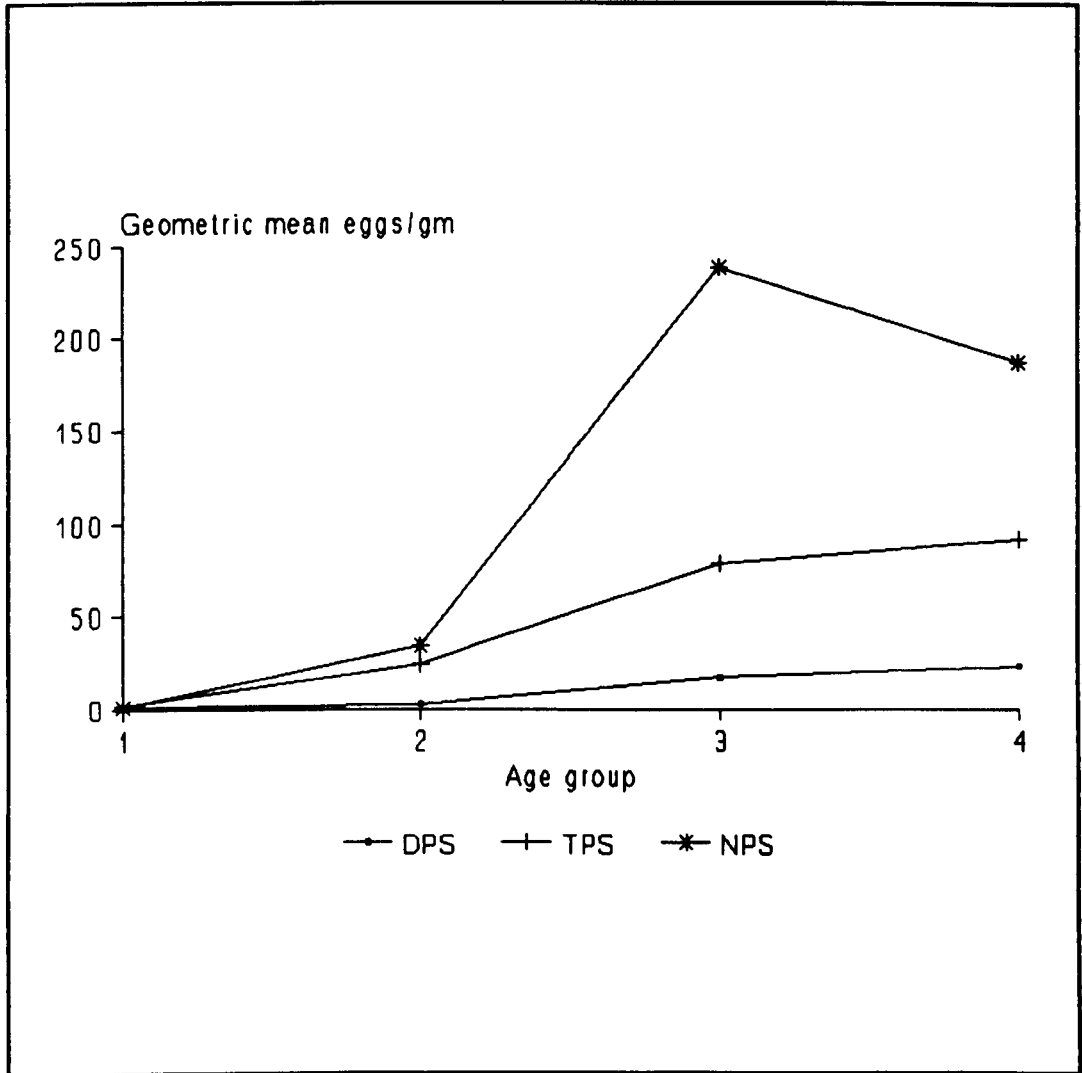
Fig. 3.5   Pupils from Thange Primary School

Fig. 3.6   Pupils drawing water with containers

Fig. 3.7   Pupils from Nzavoni Primary School

Fig. 3.8   Explaining sampling procedures to teachers and parents from Nzavoni Primary School

Fig. 3.9   Receiving Biltricide[R] tablets from Bayer[R] Company representative

Fig. 3.10   Treatment of infected school children
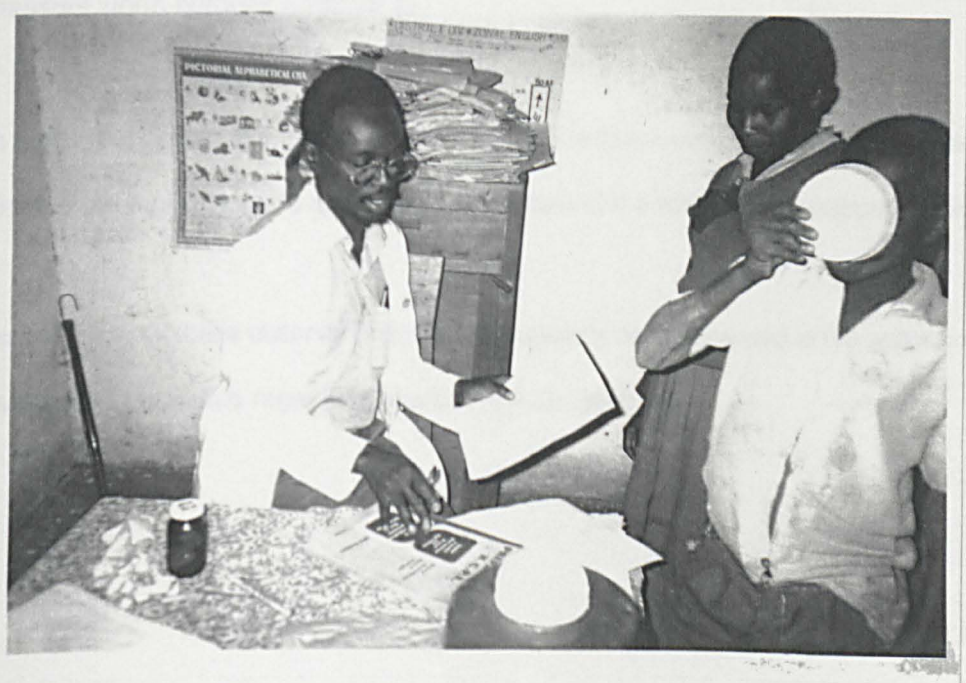
CHAPTER 4

THANGE RIVER WATER CONTACT AND SNAIL SAMPLING STUDIES

4.1   Introduction

Water is an important component necessary for the maintenance of the schistosome life cycle since it provides the habitat for the intermediate host snail as well as the two larval stages - miracidia (infective to the snail) and cercariae (infective to the definitive host).

Infection to man and other mammalian hosts depends on contact with water bodies infested with cercariae.

Thange River (Figure 4.1) is the main source of water for schoolchildren attending Thange and Nzavoni Primary schools who were found to have very high levels of S. mansoni infection (82.4% and 85.6% respectively) as reported in Chapter 3. Downstream from the human settlements, the river was also used by baboons in the surrounding bush. Of particular importance were the Thange and Kikumbulyu troops which were found to have heavy S. mansoni worm burdens (Chapter 2).

In order to establish the mode of transmission of schistosomiasis in the river, it was necessary to carry out a pilot study to assess contact pattern of the humans and baboons using the water.

To complement these observations, snail populations were assessed at the water contact points as well as their status regarding infection with S. mansoni.

4.2   Study sites

Three human and 2 baboon water contact sites (1 to 5) were chosen along the Thange River (Figure 4.1). Choice of sites depended on their regular usage and ease of access. The baboon sites were in the uninhabited bush downstream from the human sites.

As stated previously (Chapter 3), the Thange River is semi-permanent and flows continuously only in the wet months. This is governed by the rainfall pattern in the area which peaks in the months of March to May (long rains) and October to November (short rains). Figure 4.2 shows the rainfall pattern of DSE which borders Thange River. In the dry months, large sections of the river are reduced to scattered rock pools which become the only sources of water for humans and other animals. This is even more evident in the uninhabited bush where the sandy soil absorbs all the surface water leaving only that in the rock pools. The sites are described below.

Site 1: This is the main source of Thange River originating from rocky outcrops as a clear spring, dividing up into several streams which then converge on a wide sandy basin about 100 metres downstream. This basin forms an open water contact point which is used extensively by villagers from Thange Market (Figure 4.1) and children from Thange Primary school (Figure 4.3).

Site 2: Approximately 2km downstream (from site 1). This is an open, muddy pool at a meandering bend of the river, where the flow is markedly reduced. In addition to domestic water requirements, it is used as a watering point for livestock and as a pumping station for a small irrigation generator (Figure 4.4).

Site 3: About 1km downstream from site 2 and 500 metres from Nzavoni Primary school (NPS). It is used extensively by residents of Nzavoni Village and children from NPS for all their water

needs. There is also a brick maker who uses the site as a source of water for his trade (Figure 4.5).

Site 4: A collection of rock pools upstream from an old disused bridge for Masongaleni Estate, a part of DSE that was taken over by the Kenya Government in 1984 (DSE Records). This land has not yet been utilised and forms a large area of bush inhabited by various species of wildlife including baboons from the Kikumbulyu and Thange baboon troops (Chapter 2).

Site 5: A large rock pool overhang with acacia trees (Acacia tortilis) which is about 200 metres downstream from the disused bridge. It was frequently used by the Thange baboon troop (Chapter 2) as a drinking and sleeping site. Baboons slept in the trees above the water and could be seen depositing urine and faeces into the pool. When the river flow ceased in the dry season, the baboons defecated around the edges of the pool, forming a large "baboon toilet" (Figure 4.6).

4.2.1 Snail sampling sites

Snails were collected in and around the edges of all the water contact sites.

4.3 Materials and Methods

4.3.1 Human water contact observations

In order to make observations acceptable to the local people, a field observer from the area was recruited. Training was received from a Ministry of Health team based at Kambu and currently conducting some epidemiological research in schistosomiasis (Butterworth, 1990).

The methods used were adapted from those described by Ouma (1987). In order to estimate the age of subjects using the water without interfering with the activity, a demographic survey

was carried out in 32 selected households near the human water contact sites. The demographic baseline data obtained were similar to those reported by Ouma (1987). Details for each occupant included sex, age and occupation.

This information was used to assist in an estimate of age for those subjects who had not been registered or strangers who were just passing through.

Each site was observed for a total of 3 days, each day separated by a 2-week interval to reduce bias that could be introduced by changes in the weather, etc. Each site was observed over a Friday, Saturday and Sunday.

To facilitate easy recording, each activity was coded as shown in Table 4.1 and entered onto a water contact observation form (Appendix 4.1) for further analysis.

Each activity was recorded separately even it if was performed by the same individual. A person who crosses the river and stops to wash his/her face would have the two activities recorded separately.

The observer positioned herself at an appropriate vantage point in the shade (with an adequate number of recording forms, a pen and a watch) and recorded activity, time, duration and the degree of water contact for each individual from 0700 to 1700 hours inclusively (Figure 4.7).

The results were entered into an IBM personal computer with SPSS/PC statistical package and analysed.

4.3.2   **Baboon water contact studies**

As discussed earlier (Sections 1.7 and 2.2.4) baboons in this area are regarded as pests due to the damage they caused to agricultural and commercial crops. They are constantly harassed by the peasant farmers and DSE vermin control personnel whenever they are encountered. They were therefore very suspicious of humans and could not be approached for close observation.

The dense vegetation around the two baboon water contact sites made observations with binoculars difficult. In order to obtain basic information on baboon water contact, additional methods were used. These included field observations of a similar species of baboon in Amboseli National Park (Figure 2.1) and the preparation of a questionnaire on baboon water contact.

4.3.2.1   **Field observations of baboons in Amboseli National Park**

Amboseli National Park was selected for this study because IPR has a collaborative project on baboon behaviour (Amboseli Baboon Project) whose principal investigators are Drs S Altmann and J Altmann from the University of Chicago, USA. They have been studying baboons in the park for over 20 years (Altmann and Altmann, 1970).

Three troops of baboons were under long term behavioural study and accepted the presence of humans. Since they were in a protected park, they had not encountered hostile threats from humans and could easily be approached for close observation. The first 2 troops, code named "Lodge Group" and "Hooks Group" were within 2km of the base camp and were ranging in open savanna, easily accessible by motor vehicle.

Water contact observations were made by the author with the guidance of the principal investigators. Observations involved getting to the baboon sleeping site early (0600 hours) before they started moving away from the trees and then following them from a distance of 50 to 100 metres with a small four-wheel drive vehicle (Suzuki[R], Japan).

They were observed with a pair of binoculars (8 x 40 lens) until they came to the water contact site (mainly pools formed by underground springs). Their activities were recorded in a field notebook and photographs of various activities taken with the zoom lens of a 35mm standard camera.

4.3.2.2 Baboon water contact questionnaire

To complement the field observations, a questionnaire accompanied by an article (Appendices 4.2 and 4.3) were sent to the East African Wildlife Society magazine (Swara) for distribution to its members who might have observed baboons making water contact.

The article, entitled "Monkeys suffer from bilharzia too" (Muchemi, 1990), gave background information on schistosomiasis in non-human primates and the importance of water contact in the transmission. The readers were required to respond to the questionnaire (Appendix 4.3).

4.3.3 Snail sampling studies

Snail sampling was carried out at all the 5 water contact sites at monthly intervals from August 1989 to September 1990. The method used was based on the man-time approach developed by Klumpp and Chu (1977).

Equipment used included a snail scoop (Figure 4.8), a 20-litre plastic bucket and a pair of callipers.

A 50-metre section of the river, in and around the edges of the water contact site, was selected. Scooping for snails was done for 5 minutes by the same person and all the material placed in the plastic bucket (Figure 4.9). Collections were made between 1000 and 1200 hours. The contents of the bucket were transported as soon as possible to a temporary field laboratory where sorting for snails was carried out. Only *Biomphalaria pfeifferi* snails were selected and counted. They were sorted into 2 groups (Figure 4.10):

1.   small snails measuring 2-5mm shell diameter

2.   large snails measuring over 5mm shell diameter

Immediately after sorting, snails were selected for cercarial shedding. When numbers were less than 100, they were all used.

Where a large number of snails was collected, a minimum of 100 were randomly selected, both small and large and placed in individual 10ml plastic beakers or into single wells of recycled ELISA plates (Nunclon[R], Intermed, Denmark). Filtered pondwater was added and the snails were then placed under a 60 watt standard bench lamp (Anglepoise[R] Ltd., UK) for a period of 2 to 4 hours. This was usually in the afternoon between 1300 and 1600 hours. The cercariae were classified as schistosome or other trematode cercariae of bird or animal origin.

Information, including the state of flow in the river was recorded on a collection form (Appendix 4.4).

The snails were then removed from the wells and put into open one-litre plastic containers with pondwater, for holding them until they were returned to the river, at the site from where they were collected. This was done the same afternoon after collection or early the next morning.

4.4　　　　　Results

4.4.1　　　　Human water contact studies

In order to facilitate analysis, it was necessary to group water contact activities and age as shown in Table 4.2 and 4.3.

A total of 1560 contacts were observed at all the 3 sites. Of these 741 took place at site 1, 385 at site 2 and 434 at site 3. The activities performed were: domestic (1165 or 74.7%), crossing the river (261 or 16.7%), recreational (114 or 7.3%) and occupational - watering of vegetable nurseries (20 or 1.3%) as shown in Table 4.4.

More females than males made contact mainly to perform domestic activities (Table 4.5). Almost equal numbers of males and females crossed the river. Most water contact was made by subjects aged 30 to 49 years followed by those aged 15-19 and then 10-14 years (Table 4.6). Although all the activities took place throughout the day, there was a peak in the middle of the day (Table 4.17).

Most of the activities were performed within a short duration (Table 4.8).

4.4.2　　　　Baboon water contact observations

4.4.2.1　　　Observations in Amboseli National Park

It was observed that baboons in the 2 troops studied drank twice a day - early in the morning (0700 to 0900 hours) and in the middle of the afternoon (1500 to 1600 hours). The manner of drinking was the same in all animals regardless of age or sex (Figure 4.11). The animal would bend over, support itself on its hands and take a drink (Figure 4.12). This was either one long drink or several short sips, exposing only the mouth to the water. Short sips were common when the animal was unsure of its surroundings or close to a dominant individual. This pattern

of behaviour was seen in baboons from Kikumbulyu and Thange troops whenever they were observed drinking from the Thange River (Sites 4 and 5).

4.4.2.2   Baboon water contact questionnaire

A total of 2500 questionnaires were sent out with the Swara (East African Wildlife Society) Magazine but only 20 responses were received from the questionnaire particularly from people who had studied baboon behaviour from different parts of East Africa. Apart from those from behaviour experts, the rest had mistakes that made statistical analysis impossible.

A qualitative analysis of the responses showed that baboons have been observed making regular water contact. The main activity was drinking, with mainly the mouth being exposed to water. Other activities that have been observed were: playing/fighting by infants and juveniles; aggressive behaviour by adults such as males chasing females or dominant individuals chasing low ranking animals into the water; and the occasional feeding on aquatic plants.

4.4.3   Snail sampling on Thange River

A total of 11,196 B. pfeifferi snails were collected from the 5 sites. Site 3 had the highest count of 3712 while site 1 had the lowest count of 287 (Table 4.9 and Figure 4.13). The snail populations increased rapidly between August and September 1989 and then dropped drastically to a few or none by January 1990 in all sites. This was related to the rainfall which started in October 1989. Extensive flooding occurred on Thange River in November and December, thus flushing out most of the snails (Table 4.9 and Figure 4.14).

Of the snails collected a total of 3015 were examined for cercariae (Table 4.10). Only 10 (0.3%) were found to shed schistosome cercariae while 46 (1.5%) released cercariae of non-schistosome origin whose morphological features resembled those of Strigea cercariae and

4.5   Discussion

Results of water contact observation indicate that humans from the area along Thange River use it extensively. The finding that more females, particularly those in the middle age, made most of the contact can be explained by cultural trends common in most African ethnic groups. It is the females who perform domestic chores that require the use of water, ie. cooking, washing utensils and clothes. This finding differs slightly from that of Ouma (1987) who reported that females aged between 20-29 years made the most contact.

Children and young adults (ages 10-19 years) made the most contact with water (Table 4.6). This trend has been reported by other investigators (Ouma, 1987; Chandiwana and Christensen, 1988: Gryseels, 1991).

The main activities performed were either domestic or involved crossing the river. This pattern has been recently reported in Burundi by Gryseels (1991).

Most recreational activities, such as swimming and playing in the water, were performed by children aged 5-14 years (Table 4.6).

Although activities took place throughout the day, there was a peak during the middle of the day (Table 4.7). This unfortunately coincides with the peak of *S. mansoni* cercariae release by *B. pfeifferi* which has been reported to be between 1000 to 1400 hours (Jordan and Webbe, 1982). This means that the peak of water contact activities occurs when there is the greatest risk of infection.

Baboon observation in Amboseli National Park and responses to the questionnaire (Appendix 4.3) indicated that the animals make regular water contact. However, most of the contact is made when drinking and involves mainly the mouth. This implies that infection with

Baboon observation in Amboseli National Park and responses to the questionnaire (Appendix 4.3) indicated that the animals make regular water contact. However, most of the contact is made when drinking and involves mainly the mouth. This implies that infection with schistosomes in baboons would mainly be through the mouth since contact with water for other parts of the body is usually avoided.

Observations in DSE also indicated that baboons regularly drank water at least once a day, particularly if the source was within their range (author's unpublished observations).

The finding of infected *B. pfeifferi* snails in 4 out of the 5 water contact sites indicates the potential for a transmission cycle of *S. mansoni* along Thange River. Of more importance is the finding that 70% of the infected snails came from site 5 - the Thange baboon troop sleeping site. Laboratory investigations using mice identified the cercariae as *S. mansoni*. This shows that in cases where baboon sleeping sites are near the water, the animals may be more efficient in maintaining the schistosome life cycle than humans, particularly due to their direct contamination of water. This was suggested by Nelson (1960) from his observations in Kenya and also by Fenwick (1969) in Tanzania who noted that baboons were contaminating water in a rock pool below their sleeping site. In this way the baboon is better adapted as a maintenance host than man.

Table 4.1   Definition of water contact activities

Activities	Code
Crossing	01
Walking in water	02
Grazing cattle	03
Drawing water	04
Drinking water	05
Watering vegetables in nurseries	06
Bathing with soap	07
Bathing without soap	08
Bathing unspecified	09
Swimming	10
Playing in water	11
Washing parts of the body with soap	12
Washing parts of the body without soap	13
Washing parts of the body unspecified	14
Washing clothes/utensils with soap	15
Washing clothes/utensils without soap	16
Washing clothes/utensils unspecified	17

Table 4.2   Recoding of activities performed in water (Table 4.1)

Original code	New code	Name of activity
1	1	Crossing
3, 6	2	Occupational
4, 5, 7, 8, 9, 12 to 17	3	Domestic
2, 10, 11	4	Recreational

Table 4.3   Codes for age groups performing water contact activities

Age in years	Age group
0-4	1
5-9	2
10-14	3
15-19	4
20-29	5
30-49	6
50 and above	7

Table 4.4   **Water contact activities at 3 selected sites on Thange River**

Activity	Number of cases per site			Total
	1	2	3	
1 (crossing)	111	41	109	261
2 (occupational)	3	12	5	20
3 (domestic)	578	298	289	1165
4 (recreational)	49	34	31	114
Total	741	385	434	1560

Table 4.5   **Water contact activities on Thange River according to sex of subjects observed**

Activity	Number of contacts		Total
	Male	Female	
Crossing	137	124	261
Occupational	17	3	20
Domestic	459	706	1165
Recreational	69	45	114
Total	682	878	1560

Table 4.6 Age groups performing water contact activities on Thange River

Activity	Number of contacts according to age group							Total
	1	2	3	4	5	6	7	
Crossing	5	23	45	53	36	92	7	261
Occupational	0	4	9	2	4	1	0	20
Domestic	2	79	180	284	245	367	8	1165
Recreational	4	55	47	5	2	1	0	114
Total	11	161	281	344	287	461	15	1560

Key: Age groups: 1 = 0-4 years
                                2 = 5-9 years
                                3 = 10-14 years
                                4 = 15-19 years
                                5 = 20-29 years
                                6 = 30-49 years
                                7 = 50 (and above) years

Table 4.7 Time of day that water contact activities were carried out on Thange River

Activity	Contacts according to time			Total
	Early morning	Late morning	Afternoon	
Crossing	105	85	71	261
Occupational	7	6	7	20
Domestic	342	445	378	1165
Recreational	10	47	57	114
Total	464	583	513	1560

Key: Definition of time: Early morning = 0700-0959 hours
                                        Late morning   = 1000-1359 hours
                                        Afternoon   = 1400 + hours

Table 4.8   Duration of water contact activities performed on Thange River

Activity	Number of contacts by duration					Total
	1	2	3	4	5	
Crossing	260	1	0	0	0	261
Occupational	0	5	3	9	3	20
Domestic	972	85	51	35	22	1165
Recreational	32	35	22	21	4	114
Total	1264	126	76	65	29	1560
Percent of total contacts	81.0	8.1	4.9	4.2	1.9	100

Key: Duration: 1 = 1-5 minutes
                                 2 = 6-10 minutes
                                 3 = 11-15 minutes
                                 4 = 16-30 minutes
                                 5 = over 30 minutes

Table 4.9   Snail populations in Thange River water contact sites (August 1989 to September 1990)

Month	1989					1990									Total
	A	S	O	N	D	J	F	M	A	M	J	J	A	S	
Rainfall (mm)	0	0.8	79.5	207	139.6	12.5	38.6	232.1	188.5	9.2	0	0	0	0	907.8
Site 1	108	18	28	9	7	0	0	16	0	1	5	8	87	68	287
Site 2	144 (1)	200	66	150	41	3	0	0	0	0	0	0	0	0	604
Site 3	681 (1)	1183	1818	13	5	0	0	0	0	0	4	8	0	0	3712
Site 4	317 (1)	769	666	1481	133	0	1	0	4	0	0	0	2	8	3381
Site 5	1101 (2)	1102	392	508 (4)	71	9	0	1	1 (1)	0	0	3	0	24	3212

Key: ( ) Number with schistosome cercariae

Table 4.10 Recovery of schistosome cercariae from snails (*B. pfeifferi*) collected from Thange River water contact sites

Water contact site	No. with schistosomes	No. with other cercariae	Total screened
1	0	5	287
2	1	14	604
3	1	8	579
4	1	10	804
5	7	9	741
Total	10	46	3015

Fig. 4.1   Map of Kibwezi showing the Thange River water contact study sites
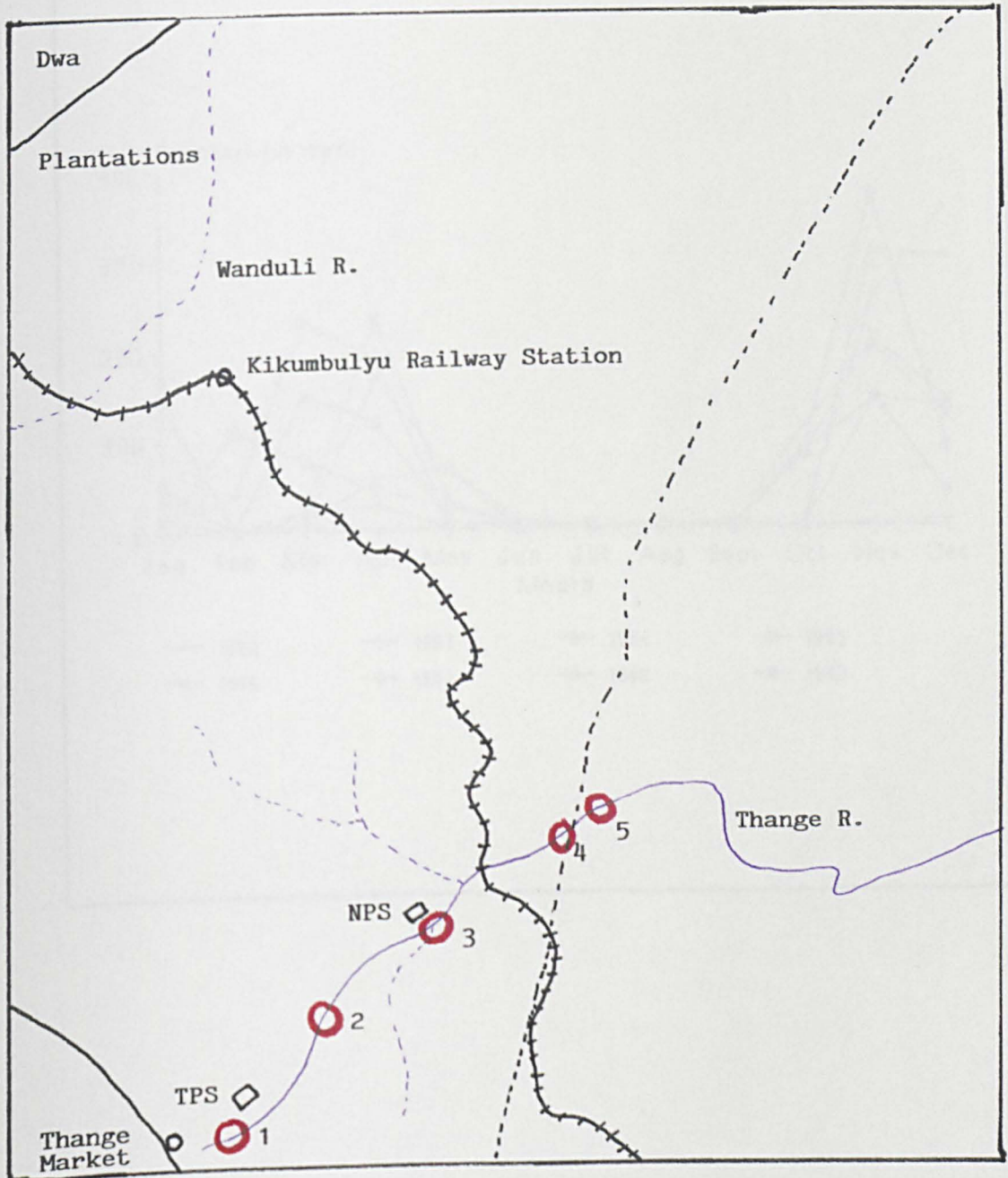
Key:

⭕	Water contact site
▬	River
- - -	Seasonal stream
────	All weather road
+++	Railway
----	Dry weather road
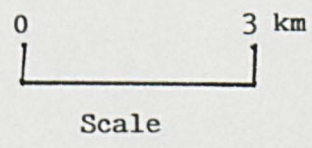
Fig. 4.2   **Monthly rainfall in Dwa Sisal Estate (1983-1990)**
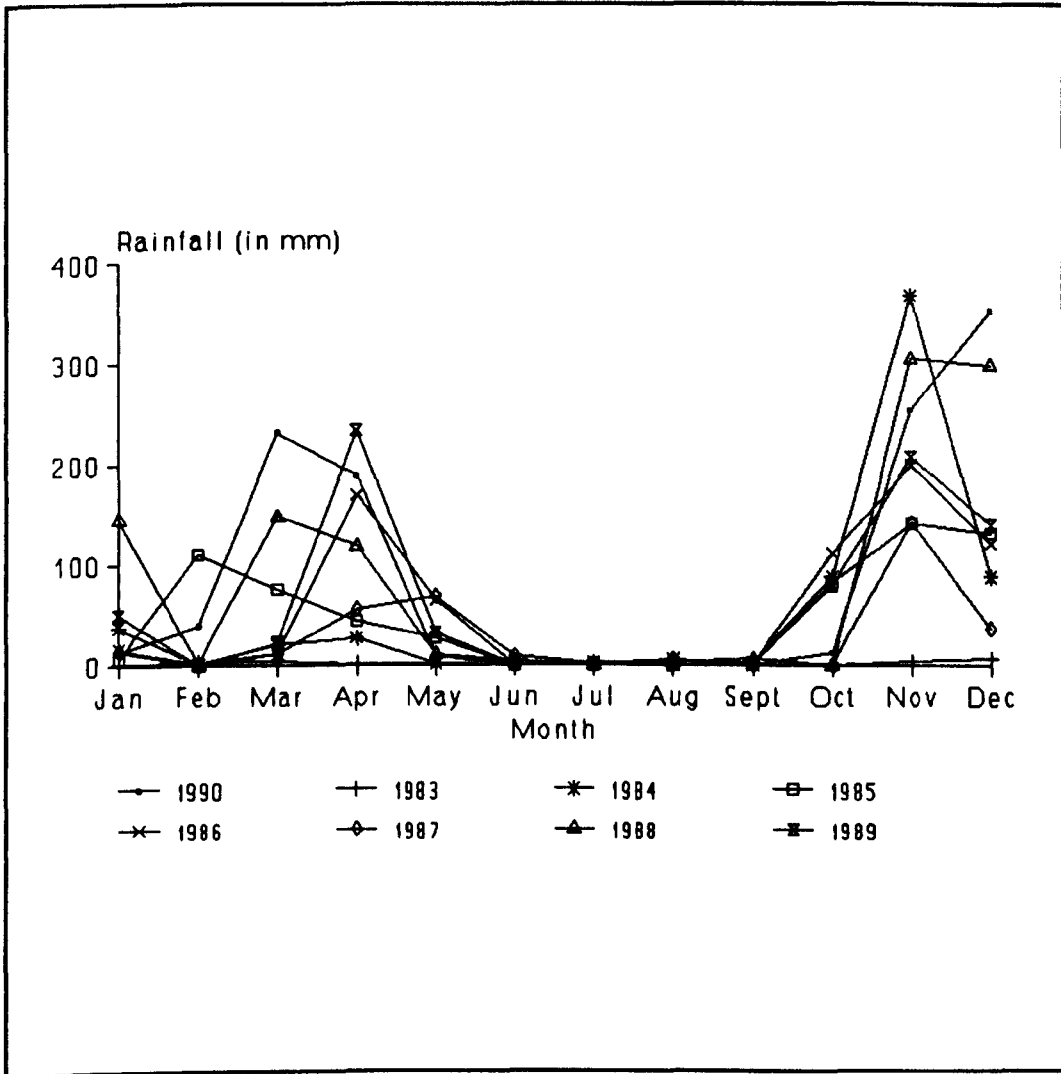
Fig. 4.3   **Thange Primary School children crossing the Thange River at Site 1**

Fig. 4.4   Water contact observation Site 2

Fig. 4.5   Drawing water at Site 3

Fig. 4.6a   Baboon water contact Site 5

Fig. 4.6b   Baboon faeces on the rocks at Site 5

Fig. 4.7   Water contact observation at Site 1

Fig. 4.8   IPR Snail Scoop

Fig. 4.8   IPR Snail Scoop
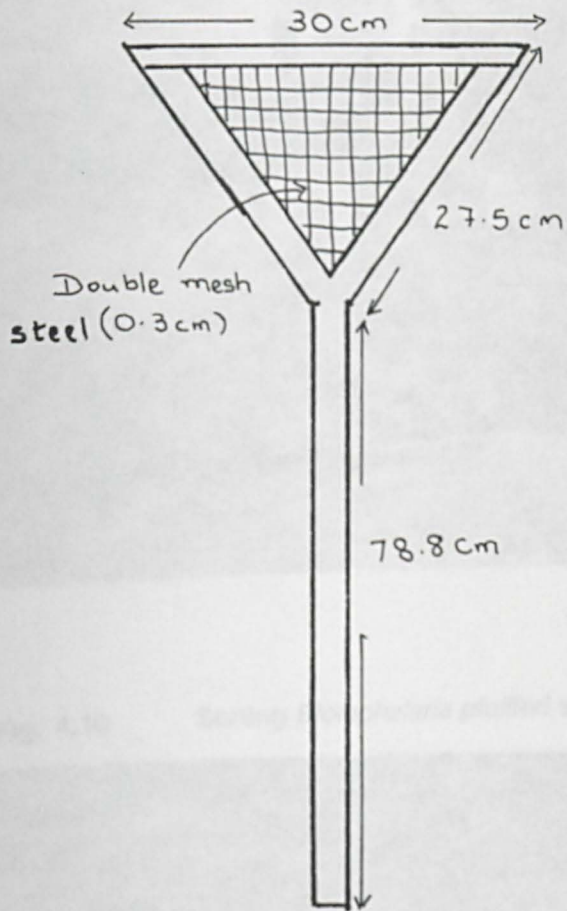
Fig. 4.9   Snail sampling at Site 5

Fig. 4.10   Sorting *Biomphalaria pfeifferi* snails for cercarial shedding
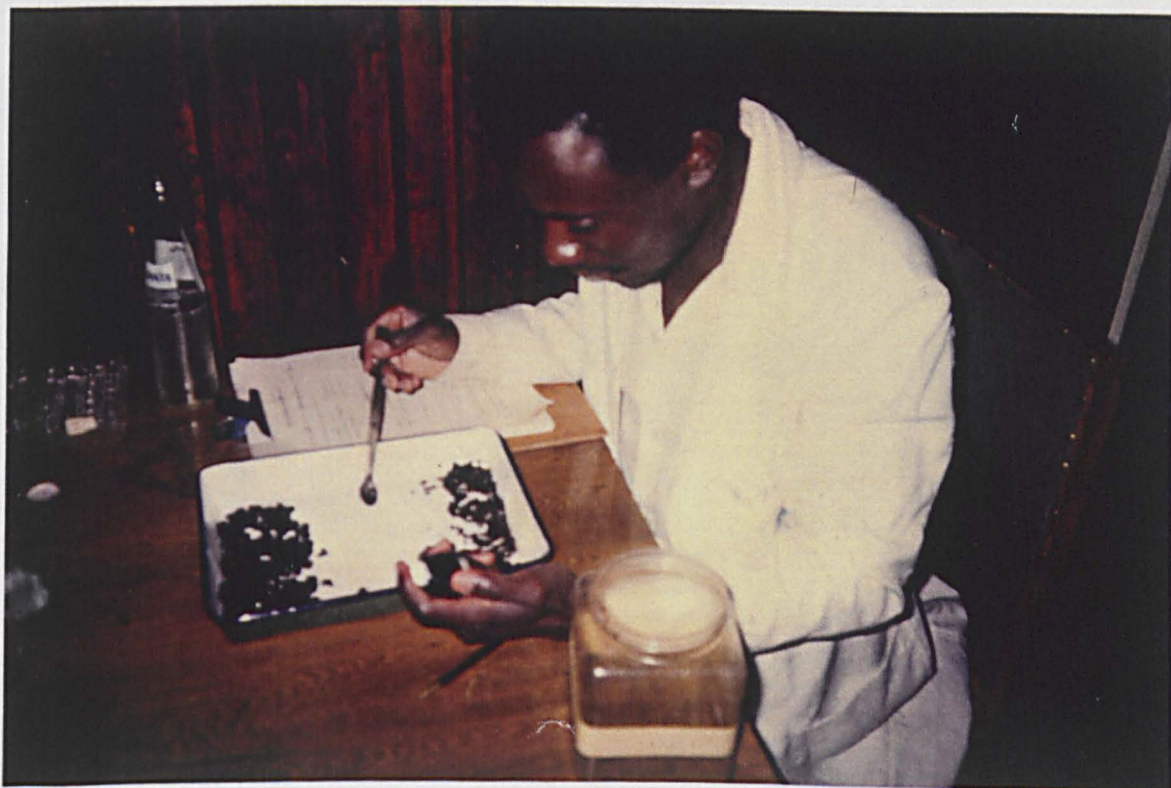
Fig. 4.11   Baboons drinking from springs in Amboseli National Park

Fig. 4.12   Characteristic baboon drinking posture

Fig. 4.13 **Monthly snail sampling from Thange River (August 1989 to September 1990)**

Total No. of snails collected

Month

Site 1   Site 2   Site 3   Site 4   Site 5
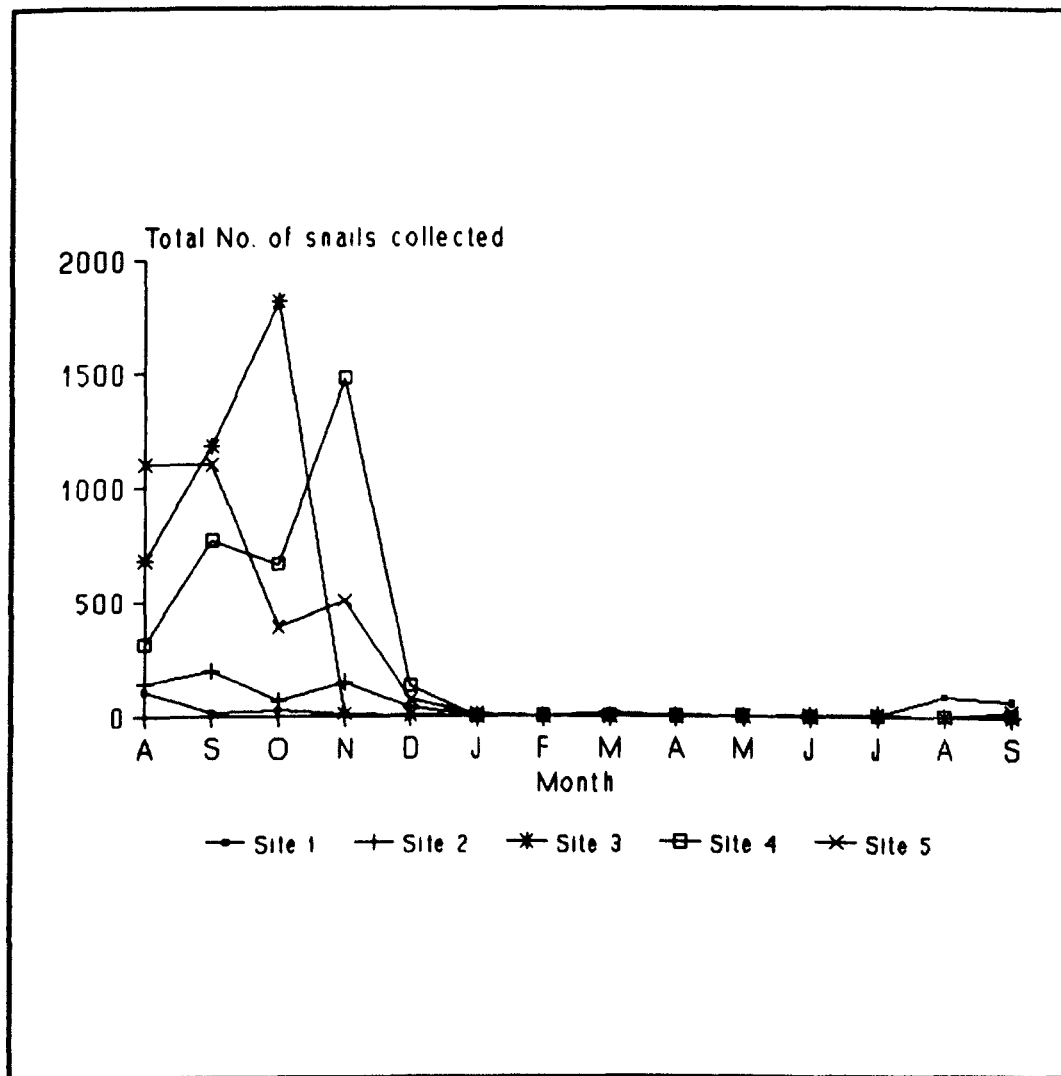
Fig. 4.14   **Monthly rainfall in Dwa Sisal Estate during the period of snail sampling (August 1989 to September 1990)**
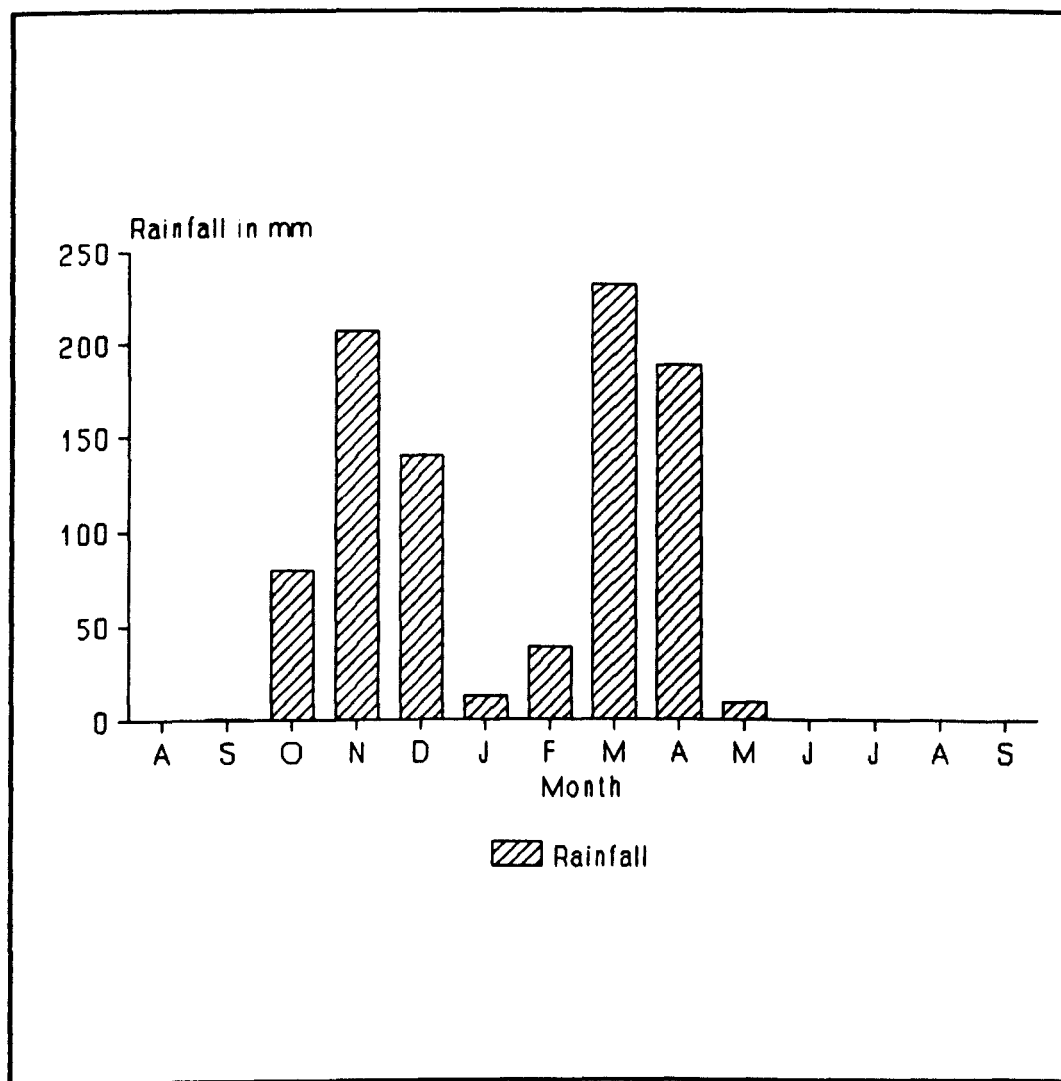
CHAPTER 5

LABORATORY INVESTIGATIONS OF S. MANSONI INFECTIONS IN BABOONS

5.1   **Introduction**

From the work already reported in Chapters 2 to 4, it became obvious that certain aspects of S. mansoni infection in baboons required investigation. From field observations it became clear that baboons visit water mainly to drink and even when they do so, mainly expose the mouth. On the other hand, natural S. mansoni infections were found in baboons from DSE, with some heavy worm burdens in the Thange troop. Therefore it was decided to investigate:

1.   The oral route of infection.

2.   The infectivity of natural S. mansoni baboon strain to captive baboons.

3.   The relationship between baboon and human S. mansoni strains from Thange River.

5.2   **Experimental design**

Five separate experiments were designed as below:

1.   An investigation on the relative efficiency of the percutaneous and peroral routes of infection with S. mansoni in captive baboons.

2.   Percutaneous exposure of captive baboons to a field strain of S. mansoni from Thange baboons.

3.   Peroral exposure of captive baboons to a field strain of S. mansoni from Thange baboons.

4.   Exposure of laboratory mice to schistosome cercariae recovered from naturally infected B. pfeifferi snails from Thange River baboon water contact site.

5.   A preliminary comparison of human and baboon S. mansoni strains from Thange using

isoenzyme electrophoresis.

5.3   An investigation on the efficiency of oral and percutaneous routes of S. mansoni infection

5.3.1   Introduction

Various studies on schistosomiasis in humans and other animals have led to a general consensus that the main route of infection with schistosomes in percutaneous (Jordan and Webbe, 1982; Kassuku et al, 1985). For most animals, particularly livestock and wildlife, including non-human primates, the main reason for making water contact is to drink. Herbivores, particularly, cattle, tend to stand in the water when drinking; other herbivores are reluctant to enter into water (Kassuku et al, 1985).

Reluctance of baboons to expose more than just the mouth when drinking suggests that the percutaneous route, though always used for experimental purposes (Smithers and Terry, 1965; Jordan et al, 1967; Sturrock et al, 1976), may not play a major role in natural infections. This requires investigation of the oral route as an alternative.

5.3.2   Materials and Methods

Eleven captive olive baboons (Papio anubis) were acquired from the IPR monkey colony. They were screened for S. mansoni infection at weekly intervals for 3 weeks using the miracidial hatching and modified Kato technique described in Appendices 2.1 and 2.2, and confirmed negative.

To condition them for the oral infection, the baboons were offered water and uninfected colony bred B. pfeifferi snails under observation for seven days as shown in Tables 5.3.1 and 5.3.2.

The snails were hidden in half portions of ripe bananas. The results influenced the group to which they were assigned. They were divided into 4 groups as follows:

Group I: 3 animals that rejected snails and were reluctant to take water under observation.

Group II: 3 animals that readily accepted water under observation.

Group III: 3 animals that were shy under observation and took water and snails with reluctance.

Group IV: 2 animals that readily ate snails whenever they were offered.

Cercariae were obtained from 30 *B. pfeifferi* snails that had been infected with a laboratory maintained *S. mansoni* using standard IPR procedures. A cercarial suspension was placed in a 50ml beaker, gently agitated with a pasteur pipette and 50µl aliquots taken with an Eppendorf pipette for staining with Lugols iodine and counting.

Positive snails were selected for feeding Group IV baboons. The protocol for infection is outlined below:

Group I: animals were sedated with a mixture of Ketamine and Rumpun [R] as described in Section 2.4.2.2. Using clothes pegs, a pouch was made in the groin of each animal and a suspension containing an estimated 836 cercariae placed in the pouch using the method of Smithers and Terry (1965) (Figure 5.1). After 30 minutes, the water was removed from the pouch using a pasteur pipette.

Group II: an estimated 811 cercariae were offered in 100ml of filtered pondwater into water containers from which the animals drank. A further 200ml of filtered pondwater was given after the cercarial suspension had been taken.

Group III: the animals were sedated as in Group I. They were then held in a sitting position while an estimated 830 cercariae in 100ml of filtered pondwater was given by stomach tube,

followed by 50ml cercariae-free water (Table 5.3.3).

Group IV: these animals were offered 4 half portions of ripe bananas each containing 2 *B. pfeifferi* snails picked from the positive batch that shed cercariae used to infect Groups I to III.

All the animals were housed in individual cages and regularly observed for clinical abnormalities.

From 6 weeks post infection, 24 hour faecal samples were collected weekly until week 12. They were examined for *S. mansoni* eggs using miracidial hatching and the modified Kato method (Appendices 2.1 and 2.2). They were then perfused at week 13 post-infection (Group I) and 16 weeks (Group II to IV) using the method of Smithers and Terry (1965) described in Section 2.4.2.5.

5.3.3   Results

The results of the weekly faecal *S. mansoni* egg counts are given in Table 5.3.4. Only Group I animals had detectable eggs in the faeces. Miracidial hatching tests were also positive only for Group I.

At perfusion, all groups were found with various numbers of *S. mansoni* worms (Table 5.3.3) except baboon number 1242 which had none. Large numbers of worms were recovered from Group I while Groups III and IV only harboured male worms.

5.3.4   Discussion

The results indicate that the percutaneous route is the most efficient method of *S. mansoni* infection. They also show that even in a single challenge the oral route can lead to infection. This is particularly evident in animals that drank the cercarial suspension as opposed to those

that were dosed by stomach tube. This is presumably due to the low pH in the gastric juice due to HCl which is likely to kill cercariae.

Eating infected snails also led to infection, probably because cercariae get released in the mouth as the tissues are macerated by chewing.

Although the single oral challenge did not lead to a viable infection, it would be interesting to find out if repeated challenge could change this situation. This was investigated using a field strain of *S. mansoni* from Thange baboon troop (Section 5.5).

5.4   Experimental infection of captive baboons with a field strain of *S. mansoni* from Thange baboon troop

5.4.1   Introduction

Natural *S. mansoni* infections have been reported in baboons by various authors (McQuay, 1952; Miller, 1959; Nelson, 1960; Fenwick, 1969; Fuller *et al*, 1979; Else *et al*, 1982; McGrew *et al*, 1989).

Although there have been suggestions that baboons may be potential reservoirs for *S. mansoni* infections in humans (Miller, 1960; Nelson, 1960; Fenwick, 1969), no investigation has yet been reported on how baboon to baboon transmission occurs in the wild.

The objective of this experiment was to investigate the susceptibility of captive baboons to a naturally acquired *S. mansoni* infection from baboons.

5.4.2   Materials and Methods

Three captive baboons (*P. cynocephalus*) weighing 4 to 7kg were acquired from the IPR baboon colony. They had been screened for *S. mansoni* using both miracidial hatching and Kato methods described earlier (Appendices 2.1 and 2.2) for 12 months and found negative.

A batch of 24 colony bred *B. pfeifferi* snails were selected from the IPR snail laboratory. They were packed in cottonwool dampened with filtered pondwater and put in a plastic container with an aerated lid (Figure 5.2) and transported to a field laboratory in Kibwezi.

Here the snails were maintained in an open plastic basin containing pondwater at 20-23°C and provided with soft lettuce *ad libitum*.

Fresh samples of baboon faeces were collected from the ground under the trees in Site 5 (Chapter 4) used by the Thange baboon troop (Chapter 2) as a sleeping and drinking site.

The samples were then transported to the field laboratory for processing. Each sample was thoroughly mixed and Kato slides prepared. The rest of the sample was processed for a miracidial hatching test.

Samples found positive for miracidia were pooled and used to infect the *B. pfeifferi* snails using the standard IPR procedure.

After an overnight conditioning in pondwater the snails were removed and packed in dampened cottonwool as described earlier. They were then transported to the IPR snail laboratory and maintained for 5 weeks post-infection before being examined for cercarial shedding.

Cercariae were collected after exposing the snails to light as described in Section 4.3.3, counted and used to infect the 3 baboons as described below.

The animals were sedated and exposed percutaneously to an estimated 909 cercariae each as shown in Table 5.4.1. They were then individually housed and faecal samples collected at weeks 8, 9, 25 and 49 post-infection as shown in Table 5.4.2.

They were perfused at week 49 post-infection.

5.4.3   Results

S. mansoni eggs were recovered from the 3 baboons as shown in Table 5.4.2. Miracidial hatching tests were positive for all animals from week 9 to 49. A large number of *S. mansoni* adults were recovered on perfusion (an average of 90%) from all the animals. The female worms were stained using the Fast Red B method (Appendix 2.3) and all found to be mature with a characteristic *S. mansoni* egg *in utero* (Figure 2.14).

5.4.4   Discussion

It is evident from the results that the natural baboon *S. mansoni* was highly infective to the captive baboons of the same species (*P. cynocephalus*). It would be assumed to be as infective to any other species of baboon since most have been found to be susceptible (McQuay, 1952; Miller, 1960; Else *et al*, 1982; McGrew *et al*, 1989). The percent recovery of adult worms from the estimated cercarial count was unusually high (90%). Experimental *S. mansoni* infections in baboons yield between 30% and 60% (Sturrock *et al*, 1976; Sturrock *et al*, 1988).

Although there might have been a technical error in the counting of cercariae used for infection, the recovery of such a high percentage of worms is still significant. It could be argued that

repeated passage of the parasite in baboons would make it more adaptive to members of the same species which might have been the case with the Thange baboon strain where *S. mansoni* was first reported in baboons over 30 years ago (Miller, 1959).

The important finding here is that baboons could maintain the natural infection for over 11 months and continue to release viable eggs in their faeces. The baboon, therefore, has the potential to become a suitable maintenance host for *S. mansoni* in Thange area.

5.5 Trickle oral challenge of captive baboons with a field strain of *S. mansoni* from the Thange baboon troop

5.5.1 Introduction

This was a follow-up from the previous experiment (Section 5.4) in which the field strain of *S. mansoni* was administered percutaneously. It was carried out with two main objectives:

a. To investigate the viability of the field strain of *S. mansoni* after the first passage.

b. To investigate the outcome of a repeated oral challenge of exposure to *S. mansoni* cercariae.

The oral challenge had already been shown to lead to an infection in a previous experiment (Section 5.3) but very few adult worms were recovered on perfusion after a single challenge. Since baboons drink on a regular basis in an environment where water is easily available (Altmann and Altmann, 1970) it would be likely for them to be exposed regularly if they drink from a contaminated source.

Sturrock *et al* (1976) showed that there was a build-up of infection after monthly percutaneous challenges with *S. mansoni*.

This experiment was designed to investigate the outcome of repeated oral challenges.

5.5.2   Materials and Methods

Three baboons (*P. cynocephalus*) were assigned to the experiment. They had been previously screened for *S. mansoni* using the modified Kato method as described in Section 5.4 and found to be negative. They were individually housed for the duration of the study.

A total of 288 *B. pfeifferi* snails, collected from Kibwezi Dwa Sisal Estate reservoir on Kibwezi River (described in Chapter 2), were screened for 4 weeks and found to be negative for cercariae. They were then each exposed to 6-8 miracidia of *S. mansoni* from a pooled sample of faeces collected and processed from baboons as described in Section 5.4. Only 39 snails survived up to week 6 post-exposure, when they started to release cercariae of *S. mansoni*.

The snails were shed together in a 500ml glass beaker containing pondwater and exposed to a 60 watt bench lamp as described in Section 4.3.3. After about 2 hours they were removed and returned to a glass tank containing pondwater and soft lettuce and kept in the dark for 2 days before they were removed and exposed to light as above for another cercarial shedding. This procedure was repeated 5 times over a two-week period by which time only 3 snails remained.

Cercariae recovered at each shedding were processed as follows:

They were counted as previously described (Section 5.3.2) and the suspension was offered to the baboons as described below.

The baboons were fasted for 24 hours before being offered a suspension containing an estimated 800 cercariae in 100ml of pondwater poured into clean aluminium drinking containers.

They were then observed from a distance for evidence of drinking from the containers.

For those animals drinking the cercarial suspension, another 100ml of cercariae-free pondwater was offered to wash down the suspension. For animals that rejected the suspension, the container was removed and thoroughly disinfected using 70% ethanol. All the containers were then washed.

A total of 5 challenges were made over a 2 week period.

Twenty-four hour faecal samples were collected from 7 weeks after the last challenge and processed using the modified Kato method and miracidial hatching test as described in Appendices 2.1 and 2.2. The baboons were then perfused for recovery of adult worms using the method modified from Smithers and Terry (1965) and described in Chapter 2, 9 weeks after the last challenge.

5.5.3 Results

The baboons were offered 800 cercariae in a 100ml suspension of pondwater on the first, second and fourth challenges. On the third challenge only 160 cercariae per animal were offered due to the reduced cercarial output from the snails. On the fifth challenge only 40 cercariae were recovered from the 3 snails alive. These were offered to baboon number 117 which had been very unwilling to drink the suspension in the first 3 challenges. Baboon number 114 rejected the suspension in the first 2 challenges and therefore drank only 960 cercariae on the third and fourth challenges. Baboon number 115 was very co-operative and took the suspension at all the times offered thus getting a total of 2560 cercariae. This information is summarised in Table 5.5.1.

Faecal egg counts per gram for weeks 7-9 post-last challenge are given in Table 5.5.2. Except animal number 117, the other 2 animals had positive miracidial hatching results in all the 3 weekly samples.

Due to unavoidable circumstances, the animals had to be perfused at week 9 after the last challenge. The number of worms recovered are given in Table 5.5.3. Animal number 115 which took the largest number of cercariae had a high worm load of 65. The other 2 animals which had only 2 challenges each had low worm burdens.

5.5.4   Discussion

The results in this experiment confirm two previous findings:

a.   That the oral route of exposure can lead to infection with S. mansoni. This was earlier shown in Section 5.3 in which the percutaneous and oral routes of exposure to S. mansoni were investigated.

b.   A repeated challenge of oral exposure to S. mansoni cercariae can lead to a build-up of infection and consequently lead to a viable infection which can be passed on to snails and hence other hosts. This is shown by the increased number of worms in baboon number 115 which took cercariae at all the challenges and also by the positive miracidial hatching test in the faecal samples collected from two of the animals.

Sturrock et al (1976) achieved similar results in baboons using the percutaneous route of exposure. They found that 5 monthly exposures of baboons to 200 S. mansoni cercariae per baboon gave similar results to a single exposure of 1000 cpb.

Kassuku et al (1985) obtained an infection with S. bovis using the oral route of exposure in goats.

The results in this experiment show that the oral route of exposure may be adequate to produce a high enough parasite load in baboons drinking regularly from a contaminated water source. They can also maintain the infection by passing out viable eggs in their faeces which would then infect susceptible snails to complete the parasite life cycle.

This is probably the method of infection in baboons sampled in DSE (Chapter 2). The proximity of Thange troop to their water contact site and their contaminative behaviour may account for the heavy worm burdens recovered from them.

5.6   **Exposure of laboratory mice to schistosome cercariae from naturally infected B. pfeifferi snails from Thange baboon troop sleeping site**

5.6.1   **Introduction**

During routine snail sampling on the Thange River, B. pfeifferi snails were screened and found to be shedding schistosome cercariae from 4 of the 5 water contact sites chosen (Chapter 4). Seven out of the 10 snails found positive for schistosome cercariae were collected from Site 5 (Chapter 4) which was the Thange baboon troop sleeping and drinking site.

Since schistosome cercariae, particularly those from human infections, have similar morphological features, it was necessary to infect mice in order to identify the parasites infecting the B. pfeifferi snails.

5.6.2   **Materials and Methods**

Ten laboratory bred BALB/c mice were acquired from the IPR animal colony. They were kept in two plastic mice cages, routinely used for laboratory mice (5 to a cage) and provided with formulated mice pellets and water ad libitum.

The mice were transported in their cages to a temporary field laboratory in Kibwezi which was also used for snail screening procedures and kept at room temperature (20-23°C).

Cercariae were collected from a single positive B. pfeifferi snail from Site 5 and used to infect 5 mice (approximately 40 cercariae per mouse) using the ring method of Smithers and Terry (1965).

The mice were transported back to the IPR animal colony and kept in a single cage. Pooled faecal samples were collected from week 6 post-challenge and processed using the Kato technique and miracidial hatching test (Appendices 2.1 and 2.2).

They were perfused after 16 weeks of infection using the method of Smithers and Terry (1965). Adult schistosomes thus obtained were stained using the Fast Red B method (Appendix 2.3) and females examined for reproductive status using the x 10 objective of the light microscope.

5.6.3   Results

Positive Kato and miracidical hatching tests were obtained 16 weeks after infection. The pooled sample contained 200 S. mansoni eggs per gram of faeces.

Three of the animals died due to unrelated causes in the same week and the remaining 2 were perfused. Only one was positive (6 males and 3 female adult worms). The worms were stained with Fast Red B and females found to have well-developed vitellaria with single lateral-spined ova in utero characteristic of S. mansoni.

5.6.4   **Discussion**

This experiment was carried out after the rains had started in Kibwezi and the only snail used was the last positive *B. pfeifferi* found. Extensive flooding of the Thange River occurred and snails were flushed and carried in the current.

The finding of lateral spined ova in the 3 adult females recovered is suggestive that the parasite was *S. mansoni* but no conclusive statement can be made because *S. mansoni* and *S. rodhaini*, both infective to mice, can hybridize to give *S. mansoni* type eggs. This experiment requires more mice and more positive snails to be conclusive.

5.7   **A preliminary comparison of human and baboon *S. mansoni* strains from Thange using isoenzyme electrophoresis**

5.7.1   **Introduction**

The finding of *S. mansoni* in humans and baboons using Thange River led to a need to establish the relationship between the two parasites.

In recent years, knowledge of genetic differentiation and diversity in a variety of organisms has been greatly enhanced by the study of protein variation. Enzyme electrophoresis has become an invaluable tool for the study of population genetics (Rollinson and Southgate, 1985).

This method provides a means for differentiating genetically distinct but often morphologically similar forms such as parasite strains. Fletcher *et al* (1981) used the method to assess genetic differences between *S. mansoni* strains from different geographic locations.   Enzyme polymorphisms in *S. mansoni* were studied in parasites from Africa, S.W. Asia, South America and the West Indies and results suggested that *S. mansoni* is as variable genetically as most

other organisms (Fletcher *et al*, 1981). The method has also been used to differentiate between *S. mansoni* and other closely related organisms such as *S. rodhaini* (Bremond *et al*, 1989).

In their study, Fletcher *et al* (1981) found that no differences in the mobility of schistosome electromorphs could be attributed to the differences in age of adult worms processed or their passage through different species of final or intermediate host.

This method was found most appropriate for the comparison of the human and baboon strains in Thange.

5.7.2 Materials and Methods

5.7.2.1 Propagation of human *S. mansoni* in mice

In order to obtain adult worms from infected human subjects it was necessary to infect laboratory mice with the cercarial stage.

Faecal samples were obtained from infected schoolchildren from Thange and Nzavoni Primary Schools (Chapter 3). These were pooled and miracidia hatched from them using filtered pondwater. Laboratory maintained *B. pfeifferi* snails from IPR were infected as in Section 5.4. After 5 weeks post-exposure, cercariae were obtained from the snails and used to infect BALB/c mice by the ring method of Smithers and Terry (1965). The mice were perfused 5 weeks post-infection and the worms, freshly obtained, used for enzyme electrophoresis.

5.7.2.2 Collection of baboon *S. mansoni*

S. mansoni adult worms were obtained at perfusion of Thange baboons (Chapter 2). They were placed in cryopreservation tubes (Nunclon[R], Intermed, Denmark) and kept at -20°C in a

portable freezer. Prior to electrophoresis, worms from each animal were thawed at room temperature (20°C) and placed in a petri dish containing distilled water.

5.7.2.3   Enzyme electrophoresis of *S. mansoni* worms

Starch gels were prepared according to the techniques detailed by Hillis and Moritz (1990). Individual male worms were crushed in 10µl of distilled water using a small mortar and pestle. The resulting homogenate was immediately absorbed on a 9.5 x 5mm tab of filter paper (Whatman No. 1) which was inserted into a cut made across the width of a cold starch gel as described by Fletcher *et al* (1981). Electrophoresis was then carried out using the techniques described by Fletcher *et al* (1981).

The gels were then stained for 4 enzymes: Phosphoglucomutase (PGM), Glucose phosphate isomerase (GPI), Malate dehydrogenase (MDH) and Lactate dehydrogenase (LDH).

5.7.3   Results

No reaction was obtained from LDH for unknown reasons. The other 3 enzymes gave clear and resolute staining as shown in Figure 5.3. The patterns of banding were similar for both the human and baboon derived worms in the homozygous and heterozygous electromorphs for the 3 enzymes.

In dimeric enzymes allelic frequencies were slightly different for both species as follows:

1.   GPI: Human   (a = 0.05, b = 0.95)

         Baboon   (a = 0.10, b = 0.90)

2.   <u>MDH</u>:   Human   (a = 0.10, b = 0.90)

                Baboon   (a = 0.30, b = 0.70)

PGM is a monomeric enzyme and showed the same pattern for both parasites.

These results are identical to those obtained by Fletcher *et al* (1981) for similar enzymes in *S. mansoni*.

Based on these results, no unique alleles could be identified in either human or baboon worms and there is no indication that the two parasite populations are different.

5.7.4   Discussion

Although the results highlight the similarity between the human and baboon parasites in Thange, this investigation is preliminary and requires a more detailed follow-up. It would have been more appropriate to compare a baboon parasite passaged through mice as for the human parasite. Storage temperatures were different for the baboon and human parasites, which seemed to affect the depth of staining for the various enzymes.

The fact that differences were not found in the electromorphs does not mean the parasites are the same. There is a need to use a wider range of enzymes. More sensitive techniques, such as DNA probes, may be needed to confirm the similarity of the two parasites.

Table 5.3.1   Amount of water (mls) taken by baboons while under observation per day for 7 days

Baboon number	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
1237	0	0	250	300	200	400	150
1238	0	0	200	200	250	250	150
1239	0	0	200	300	400	200	200
1240	0	0	250	350	400	250	250
1241	0	0	200	200	200	400	100
1242	0	0	150	300	150	400	0
1243	500	0	200	200	200	200	250
1244	0	0	0	250	150	150	150
1281	0	0	250	300	100	300	200
1282	0	0	250	300	400	200	50
1283	0	0	300	200	250	250	150

Table 5.3.2   Number of snails (inside banana) eaten by baboons per day for 7 days

Baboon number	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
1237	1	2	2	3	1	1	1
1238	1	0	2	2	0	1	1
1239	1	2	2	3	2	2	3
1240	1	0	1	2	2	0	1
1241	1	2	2	3	4	3	4
1242	1	1	2	4	3	4	0
1243	1	1	0	2	1	0	2
1244	1	1	0	1	2	2	1
1281	1	2	2	3	3	3	2
1282	1	2	1	1	1	2	3
1283	1	0	0	0	1	1	0

Note: Only one snail given to each animal on Day 1

Table 5.3.3   Recovery of adult *S.mansoni* from baboons infected by different routes

Baboon number	Route of exposure	Estimated No. of cercariae	No. of worms recovered		
			Male	Female	Total
1240	Percutaneous	836	231	216	447
1244	Percutaneous	836	231	198	429
1282	Percutaneous	836	141	149	290
1237	Voluntary drink	811	5	3	8
1243	of cercariae	811	1	2	3
1283		811	4	1	5
1238	Cercariae into	830	1	0	1
1242	the stomach	830	0	0	0
1281		830	1	0	1
1239	Eating infected	snails (2)	2	0	2
1241	snails	snails (4)	1	0	1

Table 5.3.4   Egg counts expressed as eggs per gram in baboon faeces collected 6-12 weeks post-infection with cercariae of *S. mansoni*

Baboon number	Week 6	Week 7	Week 8	Week 9	Week 10	Week 11	Week 12
1240	10	160	200	110	60	90	170
1244	50	320	540	200	90	280	240
1282	30	80	340	250	400	180	130
1237	0	0	0	0	0	0	0
1243	0	0	0	0	0	0	0
1283	0	0	0	0	0	0	0
1238	0	0	0	0	0	0	0
1242	0	0	0	0	0	0	0
1281	0	0	0	0	0	0	0
1239	0	0	0	0	0	0	0
1241	0	0	0	0	0	0	0

Table 5.4.1 Recovery of adult *S. mansoni* worms from captive baboons exposed to a field strain from Thange baboon troop

Baboon number	Route of exposure	Estimated No. of cercariae	No. of worms recovered		
			Male	Female	Total
112	Percutaneous	909	805	71	876
113	Percutaneous	909	675	111	786
116	Percutaneous	909	738	75	813

Table 5.4.2 Egg counts expressed as eggs per gram in baboon faeces 8-49 weeks post-infection with cercariae from a field strain of *S. mansoni*

Baboon number	Week 8	Week 9	Week 25	Week 49
112	50	30	50	70
113	0	200	50	190
116	10	50	40	70

Table 5.5.1   Oral challenge with *S. mansoni* cercariae recovered from a field strain from Thange baboon troop

Baboon number	Estimated number of cercariae at each challenge					
	First	Second	Third	Fourth	Fifth	Total
114	0	0	160	800	0	960
115	800	800 0	160	800	0	2560
117	0		0	800	40	840

Table 5.5.2   Egg counts expressed as eggs per gram (epg) in baboon faeces collected 7-9 weeks post-challenge with *S. mansoni* field strain

Baboon number	Weeks post-challenge (epg)		
	7	8	9
114	10	10	10
115	80	30	50
117	20	0	0

Table 5.5.3   Recovery of adult *S. mansoni* from baboons orally infected with a field strain

Baboon number	Estimated Total no. of cercariae	No. of *S. mansoni* worms		
		Male	Female	Total
114	960	7	6	13
115	2560	39	26	65
117	840	11	13	24

Fig. 5.1   Percutaneous infection of baboons with a schistosome cercarial suspension, by creating a pouch on the groin using clothing pegs

Fig. 5.2   **Diagram of snail transporting container**

Holes in lid for aeration
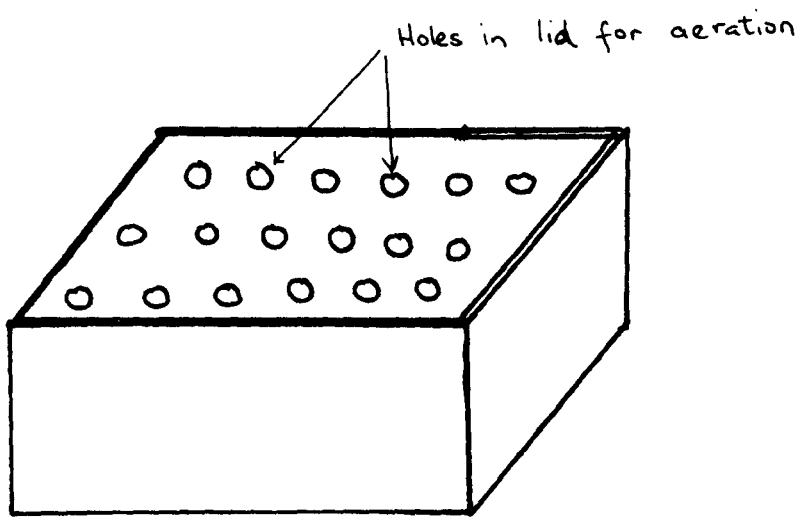
Fig. 5.3   Diagram of enzyme electrophoresis patterns in baboon and human
Schistosoma mansoni
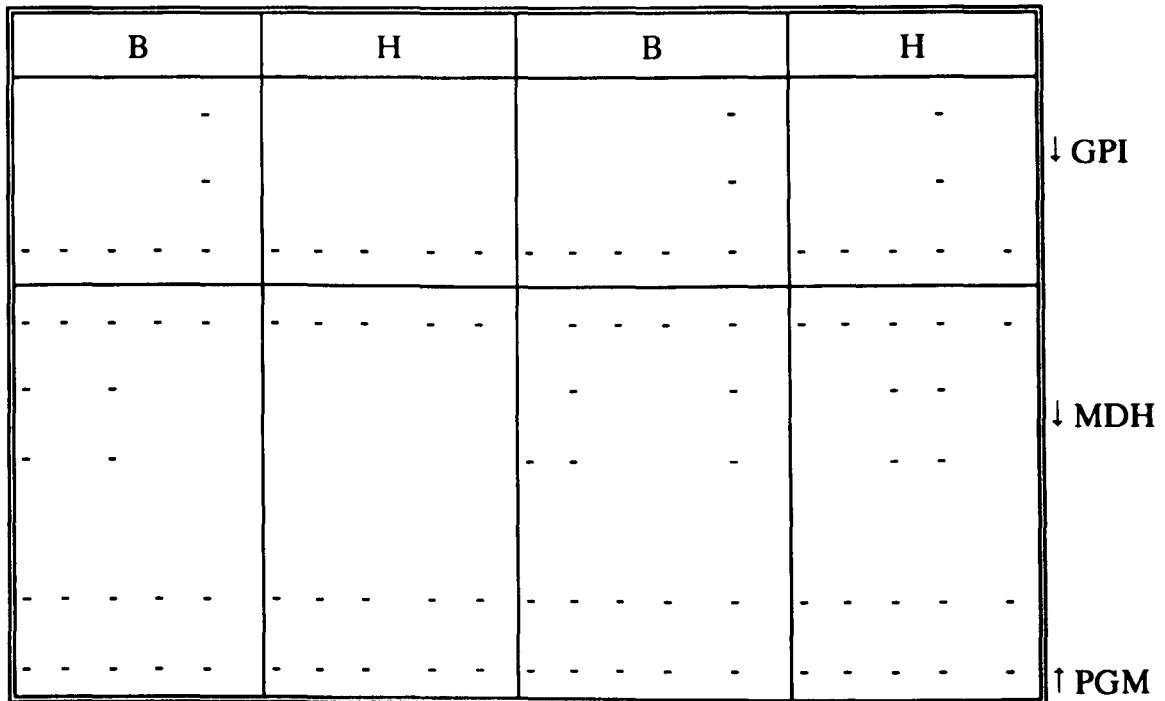
Key: B   = Baboon
       H   = Human

Note: Each sample slot represents one adult male worm. The arrows denote the direction of migration from the origin

CHAPTER 6

GENERAL DISCUSSION

Schistosomiasis is an important medical problem in Kenya as evidenced by hospital attendance records (Ouma, 1987) and the various control programmes going on in the country (Ouma *et al*, 1985; Butterworth *et al*, 1984; Butterworth, 1990).

Although several species of animals have been reported to have *S. mansoni* infections (Ouma and Fenwick, 1991), the baboon has for a long time been thought to be the ideal maintenance host of human *S. mansoni* (Miller, 1960; Nelson, 1960; Nelson *et al*, 1962; Fenwick, 1969).

Earlier investigations in Kibwezi (Miller, 1960; Nelson, 1960) found infections of *S. mansoni* in baboons (*Papio doguera*) but did not establish the sources of transmission for baboons and their relationship to those of humans using adjacent water sources.

The present study set out to investigate the potential of the baboon as a maintenance host of human schistosomiasis by:

a.   Sampling free-ranging baboons in Kibwezi to establish the prevalence and intensity of their infection with *S. mansoni*.

b.   Identifying potential transmission sources by observing them at their drinking sites.

c.   Sampling the human population sharing adjacent water sources to establish their relative prevalence and intensity of infection compared to that in baboons.

d.   Establishing the relative suitability of baboons and humans as maintenance hosts by observing their water contact patterns.

e.   Determination of the relationship between the baboon and human strains of the parasite.

Baboons from Dwa Sisal Estate (DSE) were sampled and found to have various levels of S. mansoni worm burdens which were related to their water contact sources. Those using the Kibwezi River were found to have low levels of infection, while animals using the Thange River had moderate to heavy worm burdens. The Thange troop was found to shed viable eggs in faeces which were infective to susceptible Biomphalaria pfeifferi snails. Cercariae obtained from these snails were highly infective to captive baboons of a similar species (P. cynocephalus). The strain of S. mansoni was still infective to another group of P. cynocephalus when they were orally inoculated.

Cercariae obtained from one naturally infected B. pfeifferi from the baboon drinking site gave a viable infection of S. mansoni in laboratory mice, thus suggesting that a transmission cycle was going on between the baboons and B. pfeifferi snails at the drinking site.

Snail sampling studies revealed large populations of B. pfeifferi at human and baboon water contact sites. The highest rate of schistosome infection in the snails was at the baboon sleeping/drinking site, thus suggesting that there was a higher infection pressure at the baboon site, obviously due to their more contaminative behaviour.

School children from 3 primary schools in Kibwezi were found to have varying levels of S. mansoni infection which was also related to the source of water. Children using the Thange River, upstream from the baboon site, had the highest prevalences (82.4% in Thange Primary School and 85.6% in Nzavoni Primary School). This was still lower than the 100% prevalence of infection in the 7 baboons from the Thange troop.

Baboons in Kibwezi and Amboseli were observed avoiding excessive contact with water except when drinking. However, observations by other investigators revealed that baboons engage in other activities that led to regular and extensive exposure to water, such as playing by juveniles and infants, and picking aquatic plants (and animals?). Behaviour experts in Tanzania observed

them swimming in Lake Tanganyika! (Nelson, personal communication).

Members of the Thange baboon troop used the trees above a rock pool from which they drank, for resting, dropping faeces and urine into it. Similar behaviour was observed by Nelson (1960). This makes baboons more suitable as maintenance hosts of S. mansoni in such areas.

Laboratory investigations revealed that although the percutaneous route of infection with schistosome cercariae is still the most efficient in baboons, the oral route can lead to viable infections particularly if drinking is repeated on a regular basis. This was also observed in goats by Kassuku et al (1985). Thus, even in cases where baboons avoid excessive exposure to water when drinking, they can still serve as maintenance hosts.

The preliminary finding of similar electromorphs in the human and baboon S. mansoni strains suggest that it may be the same parasite being maintained in either species. Unless more specific genetic characterisation is done, such as use of a wider range of protein enzymes and application of DNA technology, this finding cannot be confirmed.

The role of other animals in maintaining the Thange S. mansoni is not known, although the water contact sites are used by humans and other animals (Figure 6.1). Various rodent species, particularly mice, were observed in the reed-beds along the river.

These investigations clearly elucidate the potential of the baboon to act as a reservoir for human S. mansoni. There is evidence that it is capable of maintaining the infection in the snails and within a troop, particularly in areas where sleeping sites are near water. Most parts of Kenya which are endemic for schistosomiasis are in arid or semi-arid areas. Trees that are suitable as sleeping sites for baboons in such areas are found along the water courses thus making it possible for baboons to contaminate the water.

The finding of higher rates of infection in snails at the baboon sleeping and drinking site indicate that in certain cases the baboon may be more efficient in transmitting schistosomiasis than humans, who have not been observed deliberately defecating in the water (Ouma, 1987).

Further investigations are necessary to complement the findings of this investigation:

1. There is a need to examine humans living downstream from the Thange baboons for *S. mansoni* infection. The parasite recovered should be compared to that in the Thange baboons. This would help in establishing whether the baboon parasite infects humans. More detailed and advanced genetic differentiation should be carried out to establish how closely related the two parasites are.

2. *S. mansoni* from humans and baboons using Kibwezi River should be compared to that in Thange River to establish whether the low prevalence rates are the result of a less virulent strain. Morbidity studies should also be carried out in both areas as has been done by Butterworth (1990) with the Kangundo and Kambu *S. mansoni* strains, both areas of Machakos District.

3. The role of other species, particularly rodents, which are the other species reported to harbour natural infections, should be investigated in order to build a more complete epidemiological picture of *S. mansoni* in Kibwezi.

4. A control programme of *S. mansoni* in humans could be started in collaboration with other interested parties as this would establish the relative importance of the baboon reservoir once infection is reduced in humans.

Fig. 6.1   Humans and domestic livestock at a water contact site

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APPENDICES

Appendix 2.1   Miracidial hatching test

In the present study a technique modified from the methods of Weber (1973) and Upatham *et al* (1976) was used to test the viability of schistosome eggs.

One to two grams of fresh baboon faeces collected as described in section 2.4.2.1 was placed in a 50 ml plastic test tube. It was thoroughly mixed with normal saline and made up to the 45 ml mark. It was then kept in a dark chamber and allowed to settle for 20 minutes after which the supernatant was carefully decanted and the sediment resuspended in fresh saline. At least two further sedimentations were made in saline. The sediment was then resuspended using filtered pondwater and the contents poured into a transparent petri dish containing filtered pondwater.

The contents were then illuminated with a 60-watt bench lamp for two hours and then examined for miracidia under a dissecting microscope (x 40 magnification).

Appendix 2.2   Modified Kato technique

To screen baboon faeces for schistosome eggs a thick smear technique first developed by Kato and Miura (1954) and subsequently modified by Martin and Beaver (1968) and Katz *et al* (1972), was used.

A fresh faecal sample was thoroughly mixed and passed through a 105-mesh sieve made of stainless steel bolting cloth to remove coarse vegetation debris. Using a stainless steel template made to measure a volume equivalent to 50 mg (Siongok *et al*, 1976), the strained material was placed onto a microscope glass slide. It was then covered with a wettable cellophane coverslip (22 x 25 mm) pre-soaked in a solution prepared with 100 ml pure glycerine, 100 ml water and 1 ml of 3% aqueous malachite green.

The faecal material was evenly spread under the coverslip by inverting the glass slide and pressing gently on thick filter paper. Figure 2.12 shows the equipment used for this method.

Glycerin acts as a clearing agent and the malachite green stains the background surrounding the schistosome eggs.

Two slides were prepared from each sample and kept in slide boxes for later examination in the IPR laboratory using the x 10 objective of a light microscope. Schistosome eggs were counted and the results converted to eggs per gram.

Appendix 2.3   **Staining of adult schistosomes**

Worms collected using the perfusion method described in Section 2.4.2.5 and preserved in 70%

ethanol were stained for morphological assessment. The stain used was Fast Red B salt (G T

Gurr, London, UK). This stain was found most suitable for evaluating reproductive status in

trematodes and pseudophyllidean cestodes by Bell and Smyth (1958). It stains phenolic

substances in mature vitelline cells to give an orange or orange-red colour. This makes it

possible to assess development of the vitellaria in female worms.

The procedure for staining is as outlined below:

1.   Worms fixed at least overnight in 70% ethanol were rehydrated through 50% and 30%

       ethanol (15 minutes in each dilution).

2.   Transferred to distilled water (2 x 15 minute changes).

3.   Stained with freshly prepared 1% Fast Red B solution for exactly 3 minutes.

4.   Dehydrated through 30%, 50%, 70%, 90% and absolute ethanol (10 minutes in each

       dilution up to 90% and 2 x 15 minutes in absolute ethanol).

5.   Transferred to xylene for 15 minutes and then mounted in DPX onto a microscope slide

       with a glass coverslip.

The mounted worms were then examined for the reproductive status of the females using a light

microscope at x 100 magnification.

Appendix 2.4   **Tissue digestion for the recovery of schistosome eggs**

Schistosome eggs retained in tissues give an indication of the intensity of infection though the count is not necessarily directly proportional to the adult worm load (Sturrock *et al*, 1976). A convenient method of counting eggs in tissues by digesting the tissue with sodium or potassium hydroxide has been described by Cheever (1968). For this study KOH was used for digestion as described below.

Each organ sample collected after perfusion as described in Section 2.4.2.7 was thawed and chopped into small pieces which were put into a conical glass flask (250-500 ml).

A quantity of 10% KOH determined by the weight of the sample (10 ml for 1 gm) was poured into the flask and the contents vigorously shaken to form a suspension.

The suspension was kept at room temperature overnight (20-23°C) and incubated for up to 3 hours at 37°C the following morning to complete the digestion. The cloudy suspension that remained was thoroughly mixed and a 5 ml glass pipette used to take 1 ml which was put into a Sedgwick-Rafter slide.

The suspension in the Sedgwick-Rafter slide was searched for schistosome eggs using the low power (x 60 magnification) of a dissecting microscope. Four aliquots were similarly examined and the total egg count per organ calculated using a formula similar to that of Sturrock *et al* (1976) given below.

$$\text{Total tissue egg count} = \frac{W}{w} \times vol \times \frac{(R1 + R2 + R3 + R4)}{4}$$

where   W   = total weight of organ (gm)

        w   = weight of sample (gm)

        Vol   = volume of suspension (ml)

        R1 to R4   = readings from Sedgwick-Rafter slide

Appendix 2.5

Date

Animal Code _____   Sex _____   Age _____   Weight _____

Anaesthetic Notes

General Condition:

Excellent _____   Good _____   Fair _____   Poor _____

Respiratory _____   Circulatory _____

Lymph N _____   Spleen _____   Skin _____   Hair _____

Musc/Skel _____   Eyes _____   Other _____

Injuries

Mouth:

Gingiva _____   Calculi _____

Canine length _____   Teeth wear _____

Deciduous: L   R

Upper   PM2   PM1   C   I2   I1   I1   I2   C   PM1   PM2
Lower   PM2   PM1   C   I2   I1   I1   I2   C   PM1   PM2

Permanent:

Upper   M3 M2 M1 PM2 PM1 C I2 I1   I1 I2 C PM1 PM2 M1 M2 M3
Lower   M3 M2 M1 PM2 PM1 C I2 I1   I1 I2 C PM1 PM2 M1 M2 M3

Perfused _____   Serum sample _____   Faecal sample _____

Tissue samples:

Liver _____   Lung _____   S. Int. _____

L. Int. _____   Bladder _____

Comments

APPENDIX 4.1

WATER CONTACT OBSERVATION FORM

Site no: ☐☐   date: ☐☐☐☐☐☐   day of week: ☐   Observer: _____

Sheet no: ☐☐   Area: _____

Name/id. no.	Sex	Age/ yob	Duration (min) times	Activity		Degree

Monkeys suffer from bilharzia too

by Gerald Muchemi

A Kenyan research scientist needs information from Society members who have watched the behaviour of monkeys in and around water.

In the evolutionary tree, monkeys and apes are the closest relatives of man since they belong to the same taxonomical order. For this reason they share a variety of common 'human' ailments.

Researchers in Africa, Asia and South America have found natural infections of bilharzia (scientifically referred to as schistosomiasis) in a variety of monkey species. Bilharzia is a debilitating water-

borne disease of tremendous importance in tropical and sub-tropical countries throughout the world. According to recent World Health Organisation estimates, about 200 million people suffer from the disease and 600 million more are at risk as they perform their daily chores in and around water bodies.

In Kenya, bilharzia affects about two million people and the incidence is on the increase as more water areas are made available through man-made dams and irrigation schemes.

Bilharzia worms were first discovered from the intestinal veins of an Egyptian in Cairo in 1851 by Theodor Bilharz, a young German pathologist. Man and animals acquire the infection through contact with water containing the infective larvae (cercariae). These parasite stages are derived from freshwater snails hosts (specific to that particular disease) in which

they have undergone a developmental cycle. The cercariae penetrate through the wet skin of the animal or human host and are carried within the body via the blood circulation to the lungs, from where they migrate to the liver. In the liver the larvae mature into male and female adults. Once mated, the female worms produce numerous eggs. The presence of these eggs in the body elicits an immune response that leads to the main clinical disease, which is characterised by an enlarged liver and spleen. Most of the worm pairs localise in the veins of the intestines (intestinal bilharzia) or urinary bladder (urinary bilharzia). Some eggs are passed out through the faeces or urine and the damage to tissue that results during their passage leads to bloody faeces or urine. When the eggs come into contact with fresh water they hatch to release first stage larvae (miracidia), which seek and penetrate a vector snail in which they undergo further development to become cercariae and so continue the life cycle.

In many developing countries, including Kenya, agriculture and human settlement are rapidly encroaching on well-defined wildlife habitats. This has led to a growing conflict between human activity and wildlife conservation. In many cases wildlife has been exterminated or excluded by fencing, digging game moats, etc, but monkeys have adapted to the changing habitat. They raid crops from the farms and sleep in trees along watercourses where, in addition to drinking water that may be contaminated by humans, they risk contaminating the water if they themselves get infected.

At the Institute of Primate Research, our interest has been to study the current trend of bilharzia infection in monkeys, particularly in areas where the disease is common in people.

In a human population the prevalence of infection is directly related to the activities people perform in and around infected water bodies. The longer or more frequent the activity, the higher the chances of being infected. Similarly, with monkeys, infection will clearly depend on how much activity takes place at the water contact sites. Little knowledge is available on baboon water contact, and observations on such activities are clearly necessary to determine the most likely route(s) of infection (which may include drinking, wading across rivers, playing in water, etc).

In order to gain information on monkey water contact activity from as many people as possible, I have prepared a questionnaire addressed to anybody who may have encountered and observed monkeys by the water. Any response to this questionnaire would be greatly appreciated. **◊**

A loose copy of the questionnaire is included for all members living in East Africa. Any other members who think they have information that would help Dr Muchemi should write to him at the Schistosomiasis Project, Institute of Primate Research, P.O. Box 24481, Karen, Nairobi, Kenya.

INSTITUTE OF PRIMATE RESEARCH
NATIONAL MUSEUMS OF KENYA

<u>BEHAVIOUR OF BABOONS AND OTHER MONKEYS WHEN IN CONTACT WITH WATER BODIES</u>

Name of
Respondent:_____

Address:_____

Area monkeys seen: District _____ Division _____ Location _____

<u>Instructions for filling the form:</u>

Please answer each question by ticking (√) or circling (O) the appropriate answer in the list of response alternatives.

Q1.   Your answers to this questionnaire refer to:

1. Baboon
2. Vervet
3. Both
4. If any other please specify _____

Q2.   You observe monkeys as:

a). A hobby
b). Part of your work
c). In the course of other routine work

Q3.   Do you see monkeys on a:

1. Daily basis
2. Weekly basis
3. Monthly basis
4. On safari

Q4.   Is there a source of water near where you see the monkeys?

1. No
2. Yes

Q5.   Have you seen monkeys drinking from it?

1. No
2. Yes

Q6.   In a day how often do they drink from it?

1. Once
2. Twice
3. More than two times
4. Not known

Q7. When drinking do they?

1. Immerse the whole body
2. Only hands, feet and mouth
3. Only mouth
4. Not known

Q8.   Are there any activities you have observed them performing in or near water?

     1. No
     2. Yes

If response is "Yes" please answer Q9; if "No" skip to Q10.

Q9.   Which of the following activities have you seen them perform near water?

     a). Playing/fighting
     b). Swimming/wading/diving
     c). Other activities, please specify _____

Q10. a).   Have you observed any particular age group(s) that make more contact with water?

     1. No
     2. Yes

     b).   If "Yes" please indicate whether:

     (i).   Infants
     (ii). Juveniles
     (iii). Sub-adults and adults

Q11. Have you any ideas where these monkeys sleep or rest?

     1. No
     2. Less than one metre from the water
     3. About 50m away from the water
     4. More than 50m away from the water

Q12. Have you observed any defecation or urination near water?

     1. No
     2. Occasionally
     3. Frequently

-----------------------------------FIRST FOLD HERE ---

PLEASE POST TO:

                    Dr. Gerald Muchemi
                    Schistosomiasis Project
                    I.P.R.
                    P.O.Box 24481
                    Karen - Nairobi
                    Kenya.

-------------------------------------SECONDFOLDHERE------------------- ----------------------------

Appendix 4.4   Snail sampling form: Thange River

Date   ....   Collector   ....   Site No.   ....

Condition of River (tick appropriate):

Not flowing (pools)   = 1   ....

Flowing (slow to moderate)   = 2   ....

Flooded or flushed   = 3   ....

Number of *Biomphalaria pfeifferi* collected:

(Measurement = shell diameter)	2-5mm	> 5mm	Total
With other trematode cercariae			
With schistosome cercariae			
Total snails collected			

If not sampled give reason:

Other comments: