THE EFFECTS OF TEMPERATURE AND TYPE OF MOUSE BEDDING ON THE INFECTIVITY OF HUMAN SCHISTOSOME TYPE CERCARIAE IN MICE

BY

DAVID YETU ONYABE

A THESIS SUBMITTED TO THE POSTGRADUATE SCHOOL, AHMADU BELLO UNIVERSITY, IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF MASTER OF SCIENCE (M.Sc) IN ZOOLOGY

APRIL, 1993

DEDICATION

Dedicated to the millions in the tropics who suffer from the debilitating disease schistosomiasis.

DECLARATION

I hereby declare that this thesis is a record of my own research work. It has not been presented by me in any previous application for a higher degree.



David Yetu Onyabe

29/4/93

Date

CERTIFICATION

This thesis entitled "The effects of Temperature and Type of Mouse Bedding on the Infectivity of Human Schistosome Type Cercariae in Mice", meets the regulations governing the award of the degree of Master of Science of Ahmadu Bello University, and is approved for its contribution to scientific knowledge.

Professor C.G. Vajime
Internal Examiner

Dr. I. H. Nock
Internal Examiner

Head,
Department of Biological Sciences

Dean
Postgraduate School

ACKNOWLEDGEMENT

I am grateful to my supervisors, Professor Vajime and Dr. Nock for supervising this work, especially Prof. Vajime for his personal interest in me. A multitude of gratitude to Professor Okwuosa for putting so much energy into this work. I thank Professor Ham of University of Keele, U.K. for the very useful suggestions especially on the cold storage study. My thanks also go to the Onyabe family for the support and everything else.

I cannot express my gratitude enough to the entire technical staff of the Department of Zoology, University of Jos, where almost all of this work was carried out. Noteworthy are Mr. Ojobe, Kumbark, Moses, James, Peter, Chris, to mention only a few.

A million thanks also go to my friends, Agwale, Peter, Josephine, and Ayuk of the Postgraduate Entomology and Parasitology Laboratory, University of Jos, for their invaluable help and useful discussions.

I thank Mr. Ndams, Adamu and Nwanze for holding brief for me on my very frequent travels.

How can I forget to thank Mr. Alabi of University of Guelph, Canada, and Mr. Jib Odogba whose encouraging words spurred me on when the chips were down?

ABSTRACT

Five batches of mice each batch consisting of five replicates were exposed to cercariae at the rate of 200 cercariae per mouse at 5°C, 15°C, 25°c, 35°C and 45°C. It was observed that cercariae maintained at 25°C had the highest infectivity (15.2%) and those maintained at 45°C the lowest (1.8%). Infectivity of cercariae at 5°C was zero. Except for mice exposed to cercariae at 5°C, the Hb, g/lOOml and *PCV%*, of mice infected at other temperatures differed significantly ($P<0.05$) from those of the uninfected control. The tissue and faecal egg count, and wormload of mice infected at 15, 35 and 45°c also differed significantly ($P<0.05$) from those of mice infected at 25°C.

In another experiment, freshly emerged cercariae were stored at constant temperature (5°C) for 3,6,12 and 24 hour periods after which they were slowly warmed to 25 ± 1°C and used to infect four batches of mice, the batches corresponding with the four periods of storage. A fifth batch of mice was infected with freshly emerged cercariae and served as the infected control while a sixth batch served as an uninfected control. The results showed an inverse relationship between infectivity of cercariae and duration of storage. The wormload, tissue and faecal egg counts of infected mice differed significantly ($P<0.05$) from those of the infected

control regardless of duration of storage of cercariae while the Hb, g/lOOml and PCV% of infected mice except those exposed to cercariae cold stored for 24 hours differed significantly ($P<0.05$) from those of the uninfected control.

In yet another experiment one batch of five mice was reared on sawdust bedding and another batch of five mice was reared on shredded paper bedding for 7 days. It was found that infectivity of cercariae to mice maintained on shredded paper was higher than to mice on sawdust bedding. For all the indices of infectivity investigated, there was a significant difference ($P<0.05$) between shredded paper bred and sawdust bred mice.

TABLE OF CONTENTS

	PAGE
TITLE PAGE	i
DEDICATION	ii
DECLARATION	iii
CERTIFICATION	iv
ACKNOWLEDGEMENT	v
ABSTRACT	vi
TABLE OF CONTENTS	viii
LIST OF TABLES	xi
LIST OF APPENDICES	xii
CHAPTER ONE: INTRODUCTION	1
CHAPTER TWO: LITERATURE REVIEW	5
2.1 Cercarial Biology	5
2.2 Factors affecting the Penetration of the definitive host by cercariae	9
2.2.1 Reserved glycogen	9
2.2.2 Oxygen	10
2.2.3 pH	10
2.2.4 Age	11
2.2.5 Water Velocity	12
2.2.6 Salinity	12
2.2.7 Light	13
2.2.8 Temperature	14
2.2.9 Water turbidity and turbulence	15

2.3 The human factor in the epidemiology of schistosomiasis 16

2.3.1 Water contact pattern and prevalence of infection 16

2.3.2 Socio-economic factors in transmission of schistosomiasis 20

2.3.3 Developmental projects 22

2.4 Control of schistosomiasis 24

2.4.1 Chemotherapy 24

2.4.2 Sanitary Improvement and health education 26

2.4.3 Control of the snail intermediate hosts 27

2.4.4 The role of human behaviour in control of schistosomiasis 31

2.5 Economic importance of schistosomiasis 33

CHAPTER THREE: MATERIALS AND METHOD

3.1 Collection and observation of the snail intermediate hosts for infection. 36

3.2 Enumeration of cercarial number 36

3.3 Infection of mice with schistosome cercariae 37

3.4 Faecal egg count 42

3.5 Haemoglobin concentration 43

3.6 Packed cell volume 44

3.7 Recovery of adult worms from mice (wormload) 44

3.8 Tissue egg count 45

3.9 Hepatosplenomegaly 47

CHAPTER FOUR: RESULTS

4.1 Effects of temperature on infectivity 48

4.1.1 Wormload 48

4.1.2 Packed cell volume 48

4.1.3 Haemoglobin concentration 50

4.1.4 Faecal egg count 52

4.1.5 Tissue egg count 52

4.1.6 Hepatosplenomegaly 54

4.2 Cold storage experiment 54

4.2.1 Wormload 54

4.2.2 Packed cell volume 56

4.2.3 Haemoglobin concentration 58

4.2.4 Faecal egg count 58

4.2.5 Tissue egg count 60

4.2.6 Hepatosplenomegaly 61

4.3 Bedding experiment 61

4.3.1 Wormload 61

4.3.2 Packed cell volume 63

4.3.3 Haemoglobin concentration 65

4.3.4 Faecal egg count 65

4.3.5 Tissue egg count 66

CHAPTER FIVE: DISCUSSION AND CONCLUSIONS 69

REFERENCES 77

APPENDIX 90

LIST OF TABLES

Table 3.1:	Design of effect of temperature experiment	39
Table 3.2:	Design of the cold storage experiment	41
Table 3.3:	Design of the bedding experiment	42
Table 4.1:	Worm recovery in the temperature experiment	49
Table 4.2:	Mean packed cell volume and haemoglobin concentration in the temperature experiment.	51
Table 4.3:	Comparison of faecal and tissue egg counts in the temperature experiment	53
Table 4.4:	Mean worm recovery in the cold storage experiment	55
Table 4.5:	Packed cell volume and haemoglobin concentration in the cold storage experiment.	57
Table 4.6:	Comparison of faecal and tissue egg counts in the cold storage experiment.	59
Table 4.7:	Mean worm recovery in the bedding experiment	62
Table 4.8:	Mean packed cell volume and haemoglobin concentration in the bedding experiment.	64
Table 4.9:	Comparison of faecal and tissue egg counts in the bedding experiment	67

LIST OF APPENDICES

APPENDIX 1:	Detailed Results of the effects of temperature experiment	90
APPENDIX 2:	Detailed Results of the cold storage experiment	92
APPENDIX 3:	Detailed Results of the bedding experiment	94
APPENDIX 4:	Detailed Results of the two-tailed student's t-test	96

LIST OF FIGURES

FIGURE 1:	Summary of potential control measures in schistosomiasis control	25
FIGURE 2:	The tail immersion technique for infection of mice with schistosome cercariae	38
FIGURE 3:	Perfusion of a mouse for recovery of adult schistosomes.	46

CHAPTER ONE:

INTRODUCTION

Schistosomiasis or bilharziasis is the broad term for a group of parasitic infections caused by blood flukes of the genus Schistosoma. S. haematobium causes urinary Schistosomiasis while S. mansoni and S. japonicum are responsible for intestinal Schistosomiasis

Archaeological records in China and Egypt show that schistosomes have been parasitic companions of man for at least 3500 years and this relationship is believed to stretch much further back into prehistory (WHO 1989). Within the definitive host, the female produces large numbers of eggs, a small percentage of which gain access into the lumen of the gut or urinary bladder and are passed out while a greater percentage become trapped or embolized in various organs or tissues causing various degrees of morbidity or organ damage referred to as schistosomiasis. In the intestinal form of schistosomiasis, the colon or rectum, and liver are the major sites of egg embolization. A wide range of symptoms are associated with intestinal schistosomiasis such as liver and spleen enlargement (hepatosplenomegaly), weakness, diarrhoea, nausea, loss of weight, blood in stools etc. The disease has

low mortality but very high morbidity.

Several endemic foci of schistosomiasis have been identified in Nigeria, for example, Malumfashi (Pugh and Gilles, 1978), Katsina Ala (Okwuosa, Personal communication) among others. Transmission of schistosomiasis requires contact with snail infested freshwater. The disease is contracted by people as they go about the normal activities of their daily lives - washing, bathing, irrigating their fields, planting their rice etc (WHO, 1989). Infection is usually first contracted in childhood and exacerbated with each renewed contact with contaminated water. Transmission of schistosomiasis, however depends on the size of the snail host population, the rate of development of the schistosome within the snail host, the human water contact pattern, the level of infection in the population (Christensen and Furu, 1987) among others. These factors are in turn affected by conditions such as temperature and rainfall patterns (Christensen and Furu, 1987). Okwuosa (Personal Communication), suggested that the absence of infection from water contact in the early morning hours may be due to low water surface temperature and consequently low cercarial emergence and infectivity but that midday water contact was of much higher risk and would likely result in infection

since surface water temperature and, by implication, infectivity of cercariae, would have risen. This suggestion formed the basis for the study of the effect of temperature on the infectivity of schistosome cercariae in mice.

Often in the laboratories of developing countries, facilities for the proper cryopreservation of cercariae, for example liquid nitrogen, may not be available and Ham (Personal Communication), has suggested that cercariae that will not be used immediately may be stored in an incubator at 5°C and could be rewarmed gradually to produce infection. The object of the cold storage study was, therefore, to investigate the possible effect of cold storage of cercariae over variable periods of time on their subsequent infectivity to a mouse model.

The bedding experiment was mounted to investigate the suggestion by Wilson and Coulson (1984), that the type of mouse bedding could affect cercarial infectivity to mice. Therefore, mice bred on sawdust and shredded paper beddings were exposed to cercariae accordingly.

The three experiments were mounted with the following aims:

I. To determine the optimal surface water temperature of our freshwater systems at which

cercarial infectivity is at its peak.

II. To investigate the possible effect of duration of storage under refrigeration (5°C) on viability of the schistosome cercariae.

III To determine the differences between use of shredded paper and sawdust beddings vis-a-vis their effects on the infectivity of schistosome cercariae.

CHAPTER TWO

LITERATURE REVIEW

2.1 Cercarial Biology

The cercaria is the infective larval stage of Schistosoma spp. Cercariae of human Schistosomes emerge actively from the tissues of their snail intermediate host, Biomphalaria pfeifferi for S. mansoni, and Bulinus globosus for S. haematobium in Nigeria (Hira 1966, Asumu 1975, Okwuosa, 1979). Active emergence involves some physical effort on the part of the cercariae to emerge from the snail tissue as opposed to passive emergence in which the cercariae are voided from snail tissues without any part being played by the cercariae in the process. Generally, cercariae of trematodes show periodicity (based on a 24 hour cycle of activity) in emergence from their respective snail intermediate hosts such that their emergence coincides with the time of optimum availability of the definitive hosts for infection. Some cercariae emerge maximally during the daytime and others at night. Cercariae of S. mansoni, S. haematobium, S.bovis, S. mattheei, emerge maximally during the day time while those of S. japonicum and S. rhodaini show a preference for emergence during the early evening and night (Chappell, 1980). Since schistosome cercariae exhibit

a distinct diurnal periodicity related to light intensity, reversal of the environmental light pattern usually produces a reversal of the emergence pattern (Erasmus, 1972).

On emergence into the aquatic environment, the cercariae exist as free swimming larvae. This is only for a short while, about 36 hours (Chappell, 1980). The behaviour patterns of swimming cercariae are characterized by alternating periods of swimming and resting. The fork-tailed schistosome cercariae possess furcae at the end of the tail. During the rest period, they tend to sink but the furcae spread out so that the rate of descent is greatly reduced. On resumption of swimming, they rise again in the water. This is aided by their positive phototaxis mediated through eyespots (Chappell, 1980). In addition, schistosome cercariae are negatively geotatic. It is during the swimming period that they hopefully make contact with and penetrate the definitive host.

The mechanisms of host location employed by cercariae are not clear. It has been suggested that chemotaxis may play a part in host location by schistosome cercariae (Chappell, 1980). He added that though it is not clear whether schistosome cercariae

actually locate the host skin chemotactically, there is ample evidence that the specific site for cercarial penetration is selected by response to chemical components in the skin itself, perhaps, released by the sebaceous glands. He also observed that fatty acids may attract some schistosome cercariae to the definitive host since it has been observed experimentally that Schistosomattium douthitti cercariae fail to penetrate excised mouse ears if the skin is extracted with ether to remove fatty acids. Penetration resumes if ether extracted ears are rubbed with fatty acids. Fatty acids and skin lipids are, therefore, thought to be important stimulants only after the cercariae have actually attached to the host skin and are probably not chemical attractants themselves (Chappell, 1980).

The cercariae of Schistosoma mansoni are capable of penetrating a wide range of mammals but they do not necessarily develop to maturity in all of them. Thus, while the skin forms a distinct barrier, it is not the sole location in the host body at which host specificity may be determined (Chappell, 1980). Stirewalt (1966), has divided the penetration of mammalian skin by the cercariae of S mansoni into six stages as follows:

i. exploration of the skin

ii. secretion of mucus for attachment

iii. piercing and penetrating the stratum corneum through the muscular action of the cercarial body.

iv. Abrasion of host tissue

v. mucus secretion to soften the stratum corneum

vi. secretion of lytic enzymes.

Though cercariae are capable of entering at any site, they expend energy in locating the most suitable region such as wrinkles, ridges, areas where dead cells are flaking off, or even hair follicles (Chappell, 1980). According to Chappell (1980), exploration is accompanied by the secretion of mucus from the cercarial post-acetabular glands and this aids adhesion of the larva to the skin surface. The process of entry itself is part mechanical, brought about by the vigorous and abrasive actions of the cercariae, and part enzymic, due to the secretions of the acetabular glands, which are expelled by the body movements of the penetrating larva (Chappell, 1980). At the point of penetration, the cercaria sheds its tail outside the host and is then known as a schistosomulum.

2.2 Factors Affecting the Penetration of the Definitive Host by Cercariae

Generally, the survival and ability of digenean cercariae to actively penetrate their definitive host is affected by several factors. Some of these are:

2.2.1 Reserved Glycogen

Glycogen is known to be a common carbohydrate reserve in larval trematodes and it must provide a basic source of energy for these stages. Glycogen has been demonstrated in several cercariae and it occurs in muscular structures such as the suckers and pharynx, in the parenchymal cells, in caudal bodies in tail stems (Erasmus, 1972). On the basis of histochemical evidence, Cheng and Synder (1963), Cheng and Burton (1965), suggested that glucose derived from host tissues become absorbed by the sporocysts and immediately resynthesized as glycogen inside the cercariae.

Erasmus (1958), showed that the glycogen present in the caudal bodies of a forked tailed cercaria diminished during the free swimming period and that in cercariae lying on the bottom of the container, the caudal bodies had become reduced to a mass of granules free from glycogen. According to Wilson (1987), cercariae do not feed; consequently the size

of glycogen reserve has a significant effect on cercarial longevity. The act of penetration must utilize significant amounts of energy, estimated at 22-35% of the glycogen content of cercarial bodies (Wilson, 1987).

2.2.2 Oxygen

Vernberg (1963), has studied the respiratory characteristics of cercariae. She reported that cercariae are able to utilize oxygen and that oxygen is necessary for their survival and consequently, infectivity. Oliver et al (1953), showed that under anaerobic conditions, 85% of cercariae of Schistosoma mansoni were dead within 4 hours. The rates of oxygen consumption by cercariae are the highest within the life cycle of a trematode (Hunter and Verberg, 1955), indicating that oxygen availability (or the lack of it) is a very important factor in determining survival and infectivity especially of actively swimming cercariae.

2.2.3 pH (Hydrogen ion concentration)

The pH of water has been shown to play a role in the survival and infectivity of cercariae. Erasmus (1972), observed the survival of Schistosoma mansoni in several media monitored at 30°C. Fifty percent (described by the authors as the half life) of the cercariae were dead within 8-16 hours in spring water

pH 7.4. and no cercaria survived beyond 18 hours. In tyrode solutions containing 0.1% glucose, the half life was extended to about 20 hours with considerable numbers surviving 26 hours.

2.2.4 Age

It is obvious that for a particular cercaria, there is an optimum free swimming period which will enable it to reach its goal (e.g. leaf surface, definitive host, second intermediate host) and which will permit it to continue the complex behavioral patterns of entry and encystment (Erasmus, 1972). Should this swimming period be excessively prolonged, then the ability of the cercaria to enter into these later phases of activity is likely to be greatly reduced. According to Prah and James (1977), in the context of parasite transmission, the expected life span of an infective stage will not always be a direct reflection of the infective potential of the organism. They observed that infectivity is often a function of the age of the organism. Lawson and Wilson (1983), noted that the ability of Schistosoma mansoni to penetrate the tails of mice remains constant throughout their lives but that their capacity to establish themselves and then reach maturity decreased as they aged. Dutt and Srivastava (1962), reported that the cercariae of

Orientobilharzia dattai retained their infectivity to guinea pigs up to 45 hours after emergence but animals exposed to cercariae which had been in the water for 48-52 hours were negative.

It is, therefore, established that cercarial infectivity decreases with age.

2.2.5 Water Velocity

The infectivity of cercariae is affected by the velocity of the water. Radke et al (1961), demonstrated how the adult Schistosoma mansoni worm burden in mice diminished in relation to the velocity of the water and the distance from the origin of the cercariae. Christensen and Frandsen (1985), have observed that high water flow rates induce a reduced cercarial infectivity as a result of the time available for host recognition and host penetration being limited.

2.2.6 Salinity

Donnelly et al (1984), studying cercariae of Schistosoma mattheei, S. haematobium, and S. mansoni, observed no significant difference in cercarial longevity ($P>0.05$) in low salinities (0-0.525%) whereas further increases in salinity resulted in progressive decreases in survival. They reported that cercarial infectivity, as indicated by worm returns,

was reduced progressively with increased salinity upto a lethal limit of 1.05%.

By varying the concentration of sodium ions (Na +) and magnesium ions (Mg. ++) in the supporting media of S. mansoni cercariae, it has been shown that over a five-hour period, lowering of the Na+ concentration has no effect on the swimming activity of the cercariae, implying that an increase in the divalent - monovalent cation ratio should also have no significant effect (Becker and Lutz, 1976). However, the results showed that the duration of activity is lengthened in a high Mg ++ concentration. This effect is nullified by replacing Mg ++ with Ca++, showing that the proportion of the former is specifically responsible for the increased duration of swimming.

2.2.7 Light

Ghandour and Webbe (1975a), exposed cercariae of Schistosoma mansoni and S. haematobium to ultraviolet radiation wavelength 2537A° and found that cercariae were killed by exposure for 1-3 minutes but that exposure for 5-20 seconds appeard to have no visible effects. However, irradiation of cercariae for 5-20 seconds markedly increased their mortality during penetration of host skin and inhibited migration of schistosomula in the lungs. They noted that

cercariae were similarly affected when irradiated with ultraviolet light for 10 seconds. Ghandour and Webbe (1975 b), also studied the effects of gamma radiation from a cobalt - 60 source (6000r) on the cercariae of S mansoni. The results showed that penetration of irradiated cercariae proceeded almost normally in the skin but migration of schistosomula in the lungs was impeded and mortality appeared to be highest in the liver as all irradiated cercariae failed to develop into adult worms.

2.2.8 Temperature

Temperature has a marked effect on the infectivity of schistosome cercariae. Low temperatures appear to have an adverse effect on cercarial infectivity. Chen et al (1982), observed no infection in mice exposed to 1,500 schistosome cercariae at 5.5°C but recovered 0.4% adult worms from mice exposed to the same number of cercariae at 8.5°C. Stirewalt and Fregeau (1965), have reported infection of mice by schistosome cercariae at 7°C. On the other hand increased temperatures induce an increased rate of utilization of metabolic reserves and thereby a reduction in infectivity. Purnell (1966 a), suggested that high temperatures induced rapid movement in cercariae leading to a rapid depletion of

energy (glycogen) and consequently, reduced infectivity. He further suggested that the optimum temperature at which Schistosoma mansoni cercariae achieved highest infectivity was about 24°C. Similarly, stirewalt and Fregeau (1965), gave a range of 27-28°C as most suitable for infection. Purnell (1966 a), reported that at 39°C schistosome cercariae were uninfective. Conversely, Stirewalt and Fregeau (1965), reported that infection was possible at 45°C.

2.2.9 Water Turbidity and turbulence

Turbidity is the degree of cloudiness or suspended particles in the water. Turbulence describes the degree of "non-calmness" of the water body. Turbidity is closely related to turbulence. The higher the degree of turbulence the higher the turbidity of the water. Highly turbid water reduces cercarial infectivity by reducing the amount of light penetrating the water since schistosome cercariae show positive phototaxis (Christensen and Frandsen, 1985).

Cercarial infectivity is, however, affected by a complex of factors rather than one factor acting in isolation.

2.3 The Human Factor in the epidemiology of Schistosomiasis

Schistosomiasis is a disease of poor, rural (usually farming) communities. It is widely distributed in the tropical and subtropical areas of the world including parts of Africa, the middle east, Europe, South America, the Caribbeans and the far east (W.H.O, 1972). It is estimated that there are 200 million cases with a mortality rate of less than 200,000 per year, with 500-600 million people at risk of infection and 76 countries affected (WHO, 1990).

2.3.1 Water Contact Pattern and Prevalence of Infection

Transmission of schistosomiasis requires contact with water infested with the snail intermediate host of the disease. Water contact and exposure is associated with age, sex and other characteristics such as time spent in water, time of visit, activity in water and the level of submergence of the body in water.

Water contact activities may be broadly classified into the domestic, recreational, religious and occupational (Wilkins, 1987). Husting (1983), carried out some observations on water contact

activities in Zimbabwe. He observed that of female activities, the most important was washing dishes and this accounted for 47% of all female contact time, and exposure involved immersion of the extremities (hands and feet). The three most important male activities were washing the body, swimming and washing the extremities. Husting (1983), concluded that female water contact was more frequent and of longer duration than male. Wilkins et al (1984), also observed that women as a group spent over three times as much time at pools as did men. These observations suggest more exposure and presumably a greater risk of females contracting schistosomiasis than males. On the contrary, however, the prevalence of infection in most of the older age groups is greater in males than females e.g in northern Nigeria (Pugh and Gilles, 1978), in the Gambia (Wilkins et al, 1984). Sometimes the prevalence of infection have been found to be similar in the two sexes despite apparent differences in exposure to infection (Wilkins et al, 1984). Occasionally, the prevalence is higher in females, as in Mende communities in Sierra-Leone where fishing is a female occupation (Wilkins, 1987).

Certain age groups show higher water contact and therefore higher prevalence and intensity of infection than others. The constant features in

schistosomiasis surveys are a peak of infection between the age of 10 and 15 years and a gradual decline from this to a much lower level in those aged between 30 and 40 years (Polderman 1979, Wilkins, 1987). High egg counts are rare in subjects over the age of 30 (Wilkins, 1987). Although this pattern is common, its underlying causes are not clear (Warren, 1973). In particular, the epidemiological importance of reinfection in the previously infected or superinfection in the currently infected are poorly understood (Wilkins et al, 1984). These authors have identified two factors which could influence rates of acquisition and loss of infection. These are changes in the patterns of water contact with increasing age, and the development of acquired or concomitant immunity. Dalton and Pole (1978), studied a community on the Volta Lake, Ghana, and found that the younger subjects with a high mean intensity of infection had more water contact and exposure to infection than the lightly infected older subjects. They concluded that the pattern of infection in the community could be adequately explained in terms of the pattern of water contact. By contrast, Bradley and McCullough (1973), have applied the experimentally derived concept of concomitant immunity (Wilkins, 1987) to the epidemiology of schistosome infection. They suggested

that the relative stability of egg output which they had observed in a cohort of children for three years might represent the effect of concomitant immunity. They argued that if this were the case, the relationship of Schistosoma haematobium infection to age could reflect the acquisition of solid concomitant immunity by the age of ten years after which established worm burdens were subject to a constant mortality rate until the last worm was lost in the third or fourth decade of life and susceptibility regained. Wilkins and Scott (1978), in a study in the Gambia, observed the same relative stability mentioned by Bradley and McCullough (1973), but also found seasonal changes in egg counts, which suggested that superinfection was occurring. they argued that the relative stability reflected the persistence of worm burdens in a turnover state of dynamic equilibrium with acquisition and loss of worms occurring simultaneously. They suggested that if most subjects lost a constant proportion of their worm burden each year, the equilibrium level of infection in each subject would be determined by the rate of acquisition of new worms which might be influenced by individual patterns of water contact and or level of acquired/concomitant immunity. Wilkins (1987) has suggested that much further work

is needed to clarify the epidemiological role of protective immunity.

2.3.2 Socio-economic Factors in Transmission of Schistosomiasis

Certain human behavioral patterns facilitate the transmission of schistosomiasis. Infection of human populations occurs where such behavioural patterns result in exposure to cercariae. Contamination of water with faeces or urine containing schistosome eggs is necessary to maintain the infection in snail populations thus ensuring continuity of transmission (Anderson *et al*, 1982). This contamination is enhanced by the fact that inhabitants of those areas where schistosomiasis is endemic are often poor people, usually subsistence farmers, freshwater fishermen, plantation labourers etc, with low hygienic standards. Such poor communities almost always lack good toilet facilities and safe water for domestic use in the absence of which the inhabitants defecate and urinate indiscriminately. Lacking a safe water source, they obtain water for domestic purposes from the local ponds, streams or lakes which are often infested by the snail intermediate host (Nelson, 1972). Continuous contamination of the fresh water system ensures continuous transmission of

schistosomiasis between man and snail.

Santos (1988), has identified some socioeconomic factors relevant to the transmission of schistosomiasis.

Some of these are:

i. Religious beliefs: some religious practices favour the transmission of schistosomiasis. The Seventh Day Adventist for example, perform their baptism in streams without considering the danger of acquiring the disease. Conversely, the knowledge of the existence of the disease may have to be disregarded in favour of religious practices.

ii. Failure to use foot bridges: Probably some individuals do not feel it is necessary to waste time looking for footbridges in order to cross an infected stream. Indifference is a contributory factor to such attitude.

iii. Failure to provide for protective devices to avoid infection. Much as people might wish to provide themselves with protective devices such as boots to avoid infection, financial problems come into the picture. Thus, economic status in life affects transmission.

iv. Laundering in snail-infected streams inspite of prohibitions: People are not alarmed by the knowledge that the disease or infection is existing in their locality. In addition to this, many do not have water facilities near their homes and others enjoy socializing with friends and neighbours while doing their work.

2.3.3 Developmental Projects

According to Nelson (1972), the paradox of schistosomiasis is that while endemic areas are usually poor, rural communities, any attempt at increasing the economic development of such communities is likely to lead to increase in the prevalence of the disease. Such economic developments include road construction, mining activities, building of bridges and culverts and water resources development such as dam construction for agriculture and other purposes (Nelson 1972, WHO 1991). These developments increase the contact between man and the snail intermediate hosts of schistosomiasis (Nelson, 1972). perhaps of all the economic developments, the most important which facilitates the transmission of the disease is water resources development.

Nelson (1972), suggested that the building of

dams on rivers or lakes for irrigation and hydroelectric power purposes greatly reduces the velocity of such bodies of water and therefore makes the habitat conducive for the snail intermediate hosts which do not colonize fast flowing water. Such dams do not dry up during the dry season, therefore, facilitating year round transmission of the disease (Nelson, 1972). Damming for irrigation can also change an otherwise arid area into a conducive habitat for snails. The Aswan Dam in Egypt is an example of an economic development which has enhanced year round transmission of schistosomiasis (Nelson, 1972). Before the dam was built, there was a restricted breeding season for the snail intermediate hosts, and Biomphalaria, the intermediate hosts of Schistosoma mansoni, was confined to the Delta region. With the building of the dam, water became available for perennial irrigation in areas where before there was only a seasonal irrigation from the annual Nile floods, therefore, prolonging the transmission season. Nelson (1972), also noted that these snails had extended their distribution up the Nile Valley for several hundred miles.

Other human activities like mining, production of mud blocks, road construction etc, involve large excavations of the earth leaving large ditches, holes

etc, which gradually get colonized by snails. The water which has collected in such ditches and holes are used by the nearby villagers thus exposing them to the risk of infection through water contact.

2.4 Control of Schistosomiasis

The aim of the control of schistosomiasis is to attack the parasite at several different stages of its life cycle in order to interrupt transmission. According to Madsen (1986), the reductions in the probability of the successful passage of the parasite through the various stages are not just summed up but multiplied. A summary of methods or combination of methods that may be used to control schistosomiasis is shown in Figure 1.

2.4.1 Chemotherapy

Chemotherapy of schistosomiasis in man is achieved by treating infected individuals with such drugs as Praziquantel (Biltricide®), oxamniquine (Vansil®), and Metrifornate (Bilarcil®). A fourth drug Niridazole (Ambilhar) is no longer recommended for use (Simonsen, 1985), Oxamniquine is used for treatment of intestinal schistosomiasis but is ineffective for treatment of the urinary form of the disease while Metrifornate is useful in treating urinary but not intestinal schistosomiasis.

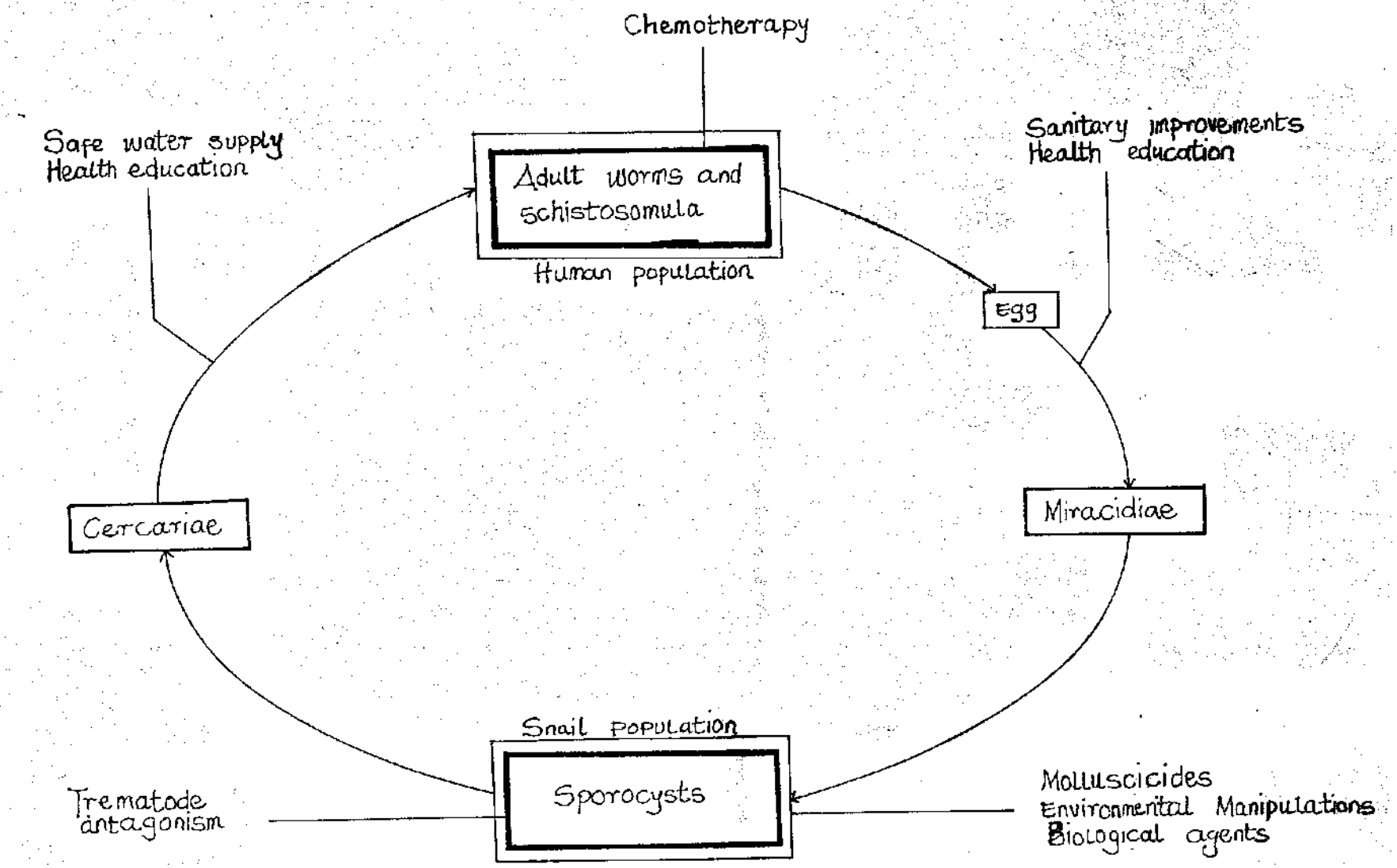


Fig 1: Summary of potential control measures in schistosomiasis control (from Madsen, 1986)

Praziquantel has the advantage of being useful in treating all forms of schistosomiasis and other helminth infections (Simonsen, 1985).

Mass chemotherapy in endemic areas is usually unsuccessful, for example, as recorded in Egypt (Encyclopaedia Britanica, 1972).

2.4.2 Sanitary Improvement and Health Education

According to Madsen (1986), sanitary improvement involves the provision of good housing which should have good toilet facilities, the provision of public toilets and the improvement of solid waste disposal so that such waste as faecal matter are properly treated and disposed of without contaminating the freshwater system. It also includes the provision of safe water for domestic use, for example, deep well.

The aim of health education is to raise the awareness of the people on the disease, the causative agent, mode of transmission, its prevention and control (Madsen, 1986). Usually the people are educated on the use of toilet facilities even if it is pit latrine provided by the sanitary improvement measures. They are also educated against indiscriminate defecation and coming in contact with freshwater. Health education is achieved by embarking

on mass literacy campaigns especially in endemic areas.

2.4.3 Control of the Snail Intermediate Host

Madsen (1986), has extensively reviewed control of freshwater snails. He suggested three major approaches for use in snail control and they include molluscicididing, environmental manipulations, and biological control.

Molluscicides used include niclosamide (bayluscide), triphenmorph (Frescon), sodiumpentachlorophenate (NaPCP), Copper sulphate, organotin compounds (such as tributyltin oxide, triphenyltin acetate etc) and plant molluscicides (e.g. Phytolacca dodecandra).

Niclosamide is the most commonly used of all molluscicides. Molluscicididing is the most important snail control method because it interrupts transmission rapidly, does not require the active cooperation of the local population, the cost-efficiency can be satisfactory etc. Madsen (1986), also recommends that a thorough knowledge of snail population and transmission dynamics is required before embarking on a snail control programme.

Environmental manipulations aim at rendering habitats unsuitable for the snails. Some methods are:

i. Clearing the weed by mechanical removal, use of herbicides etc.

ii. Increasing the depth of water margin since snails, with only a few exceptions, can inhabit only shallow margins.

iii. Periodic drying out of distribution and drainage systems when water is not needed for irrigation of crops. This will ensure that there is no transmission when the canals are dry. This method can only be used where an alternative source of water is available for the use of the local population.

iv. concrete lining of canals allow high flow rates thus reducing the need for deweeding and desilting.

v. Burying snails by ditch reconstruction

vi. Fluctuation of water level in storage ponds in order to strand snails.

Snail control may also be achieved by biological control methods which involves introducing the natural enemies (i.e predators or parasites) or competitors of the snails. Predators usually kill the

snails while parasites cause sterilization in snails. A competitor either reduces the amount of one or more resources available for another species (i.e exploitative competition) or the competitor will harm the other species during the process of obtaining the resources which both species are trying to gain (i.e interference competition). Examples of snail predators are the waterbug, Limnogeton fieberi, and flies of the family Sciomyzidae. Some echinostome rediae are reported to inhibit the development of schistosome sporocysts if they both infect the same snail. This method called trematode antagonism, is not yet popular. The most promising biological control method is interspecific competition among snail species. The most common competitor snails are Marisa cornuarietis, Helisoma duryi and, Thiara granifera.

Marisa cornuarietis destroys egg masses and juveniles of intermediate host snails and it preys on adult snails as well. It is also a voracious consumer of aquatic plants and in this way modifies the environment to the disadvantage of the intermediate host species. Madsen (1986), reported that Marisa reduced Biomphalaria glabrata populations almost to extinction over an 81 week observation in a study in Puerto Rico.

Helisoma duryi competes for food and interferes with both juvenile and adult snails, also consuming their egg masses. It causes reduced somatic growth and reproduction of various species of the snail intermediate hosts. Madsen (1983), reported that H. duryi was released into some drains in Tanzania in 1975 and by January 1981 when a snail survey was carried out in the area, the distribution of H. duryi and Biomphalaria pfeifferi showed almost no overlap. He explained that this could indicate competition but could also be due to their habitat preferences though no differences between the two sites dominated by either of the two snails could be observed.

Thiara granifera is an ovoviviparous, parthenogenetic snail. Madsen (1986), described field trials in St. Lucia which showed that Biomphalaria glabrata was apparently eliminated from marshes and streams 6-22 months after the introduction of T. granifera. Similarly, WHO (1981), reported the elimination of B. glabrata, the intermediate hosts of S. mansoni, after the introduction of T. granifera in the southern parts of U.S.A. T. granifera can, however, act as the snail intermediate host of the lung fluke, Paragonimus westermani, thereby discouraging its use.

2.4.4 The Role of Human Behaviour In the control of Schistosomiasis

So much of the success of schistosomiasis control programmes depends on human behaviour in every day life since control efforts will be frustrated by the repeated contamination of freshwater with faeces and urine. The importance of human behaviour in this respect is noted by Nelson (1972), who observed that "it is quite clear that there is very little hope of eliminating vast amount of helminth diseases from the world without drastic changes in human behaviour".

Human behaviour is influenced by some sociocultural factors as identified by Santos (1988). These include:

i. Low submission of stools: this is brought about by the fact that the people are not medically oriented and do not feel it a necessity to submit stools for parasitological tests unless bothered by symptoms of the disease.

ii. Poor compliance to treatment. People do not take their medicines as prescribed unless morbidity is already severe. Reasons are related to their religious beliefs, superstitious beliefs, customs and habits.

iii. Superstitious beliefs regarding cause of the disease: The superstitious beliefs of the people favour transmission of the disease. Where people believe the symptoms of the disease are caused by evil spirits, efforts towards treating them would amount to waste as such people might disregard taking the drugs, refuse to stay away from infested water and contaminate the freshwater with their urine and faeces.

iv. Prioritization of needs: Due to economic hardship, economic needs, rather than health needs, are given a higher priority.

A combination of several factors, such as low awareness of the disease, economic factors and the environment, make change in human behaviour even more difficult to achieve. For example, Tiaglo (1982), in a survey of inhabitants of different professions of a province in the Philippines found that 75% were aware of the disease but 25% did not seek medical advice for a year or more after contracting it owing mainly to financial problems and the distance from clinics. Most knew that water was involved in transmission but 40% had no alternative but to use natural water-river, streams, rice fields etc - for

their daily needs.

The ideal method of influencing a change in human behaviour to help in the control of schistosomiasis is to keep people away from infected water. But in the words of Nelson (1972), "who can be so heartless as to prevent children from swimming and washing in the only available water in countries where the midday temperature is usually over 90°F, and how can you keep a rice farmer out of his paddy fields?" A consideration of this complex of problems associated with control of schistosomiasis by altering human behaviour patterns led Nelson (1972), to declare rather frustratingly that "we are likely to have even less success in preventing children in the tropics from bathing in infected water or preventing indiscriminate defecation than the health educationalist in the western world have had in preventing people from committing suicide by smoking".

The key to change in human behaviour to enhance schistosomiasis control seems to lie in educating the people and providing a safe water source.

2.5 Economic Importance of Schistosomiasis

Farmers, freshwater fishermen, and labourers, for example in sugarcane or banana plantations, are the most susceptible to schistosomiasis since their

economic status in life and means of livelihood require contact with the often infected freshwater system (Santos 1988). According to Santos (1988), national economic loss is shown by the decrease in agricultural production due to affected farmers, increase in budgetary expenditure for the particular health problem, loss of livestock such as cows and pigs which are affected by the disease and deaths due to schistosomiasis.

In a study in the Philippines to assess the economic benefits of treatment of cases of schistosomiasis, Santos (1988), found that the average mandays lost by a worker with schistosomiasis before treatment was 45.5 per year. One year after treatment, the mandays lost dropped to 4, signifying a net gain of 41.4 mandays per year per infected person.

WHO (1967), reported that at least in areas of high endemicity, appreciable mortality was associated with schistosomiasis. Currently, it is estimated that schistosomiasis causes low direct mortality, but its chronic pathological effects might underlie mortality attributed to other causes (Andreano and Helminiak, 1988). It appears that the economic importance of schistosomiais is to a large extent due to morbidity caused by the disease rather than mortality.

Schistosomiasis is also economically important because drugs required for treatment could be quite expensive. This further depletes the already meagre income of such poor workers. Maiga (1988), observed a positive correlation between medical expenditures and the days per farmland missed due to bilharziasis in his study of peasant farmers in Mali, Molluscicides used in snail control are quite expensive. Madsen (1986), quoted a WHO document which put the price of bayluscide at 33.80 US dollars per kilogram of the 70% wettable powder formulation. He also noted that lining canals with concrete as a snail ecological control method would greatly add to the construction cost of such irrigation projects.

Apart from its economic importance schistosomiasis is known to have social consequences. The social importance of schistosomiasis revolves mainly around stigma of being infected. Herrin (1988), administered questionaires addressing various social consequences of suffering schistosomiasis on 176 infected and 921 non-infected people in the Philippines. Most respondents believed that most people look down on someone with schistosomiasis and that schistosomiasis infection of a household member will be a serious blow to the social status of the family.

CHAPTER 3

MATERIALS AND METHODS

3.1 Collection and Observation of the Snail Intermediate Hosts for Infection

The snail intermediate host of Schistosoma mansoni in Nigeria, Biomphalaria pfeifferi, were collected from small water bodies in Jos metropolis, specifically at the Dogon Karfe and Abattoir areas. The snails were taken to the laboratory and kept in total darkness for 24 hours to prevent them from shedding cercariae until desired.

The snails were observed for infection by putting them singly in specimen tubes and exposing them to sunlight in accordance with the method described by Christensen et al (1984).

Snails releasing pure populations of the human type brevifurcate cercariae were selected for use in the infection of mice. Since field collected snails may harbour unisexual infections, the cercarial suspension from at least five infected snails were mixed before use in infecting mice.

3.2 Enumeration of Cercarial Numbers

The enumeration of cercarial numbers was carried out as described by Christensen et al (1984). The

cercarial suspension (obtained from the snails) was gradually stirred and three representative drops were taken and transferred onto a glass slide. A drop of Lugols iodine was added to each drop to kill and stain the cercariae. The number of cercariae in each cercarial drop was taken by counting under the microscope. Where the counts obtained in the three samples differed from one another by more than 10%, new samples were taken and counted. The mean number of cercariae in the three samples was calculated and the total number of drops required to infect the mouse with 200 cercariae was obtained by dividing 200 by the calculated mean number of cercariae per drop.

3.3 Infection of Mice with Schistosome Cercariae

Exposure of mice to cercariae was achieved using the tail immersion technique as described by Christensen *et al* (1984) as shown in figure 2. Before exposure, each mouse was bathed in water for about 5 minutes to allow them defecate and urinate in order to reduce the tendency of defecating and urinating into the cercarial suspension. Each mouse was restrained in a restraining chamber and clamped vertically with its tail hanging downwards. The tail was immersed for 30 minutes in the specimen bottle

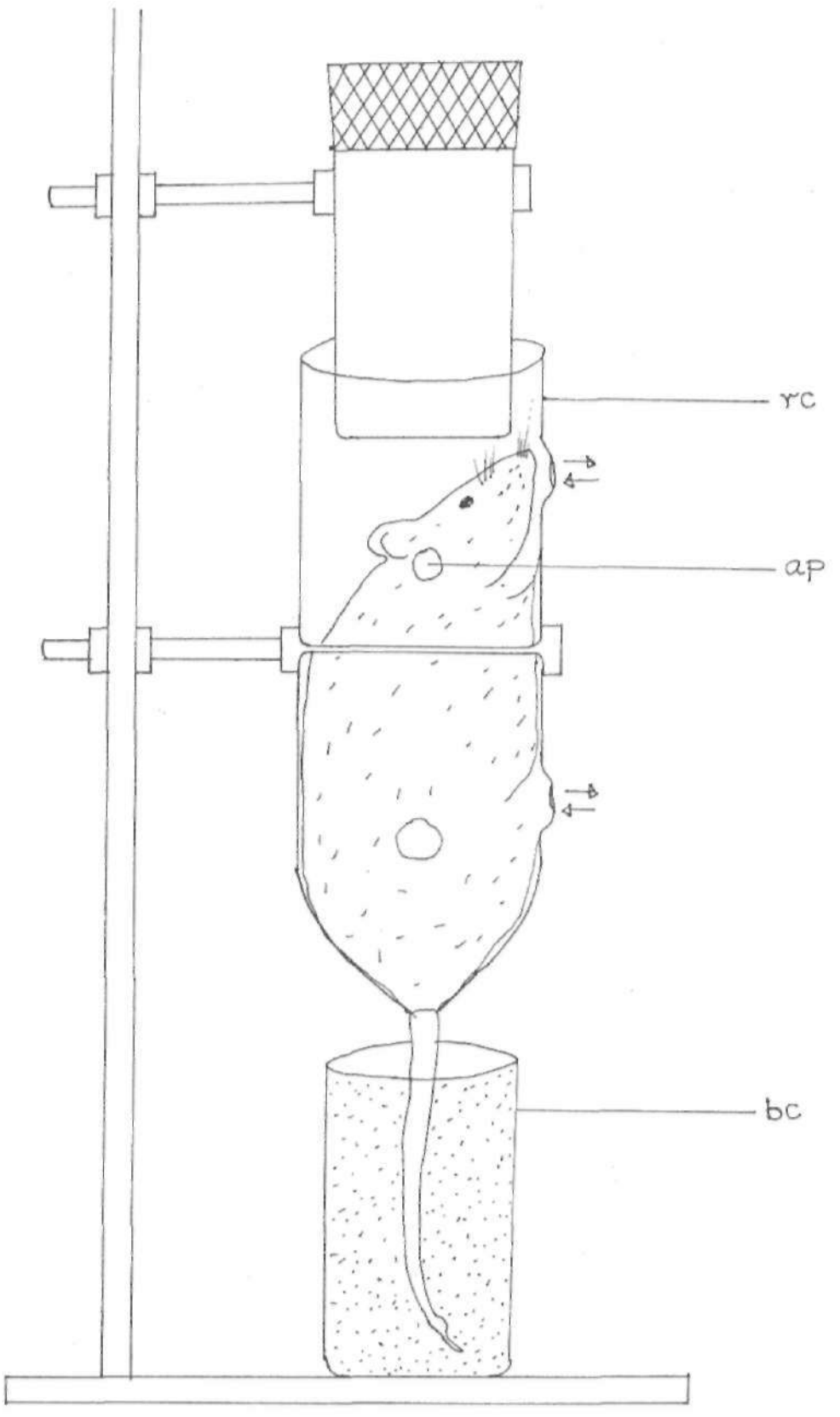


Fig 2: The tail immersion technique for infection of mice with schistosome cercariae (after Christensen et al 1984)
rc - restraining chamber containing mouse, ap - air pore,
bc - bottle of cercarial suspension.

containing water in which 200 cercariae had been introduced. Using a thermometer, the temperature of the water containing the cercariae was monitored and when noticed to fall or rise slightly beyond the desired temperature, warm or cold water was carefully added down the side of the bottle to increase or decrease the temperature respectively to the desired degree. The mice were exposed to cercariae suspended in water medium at the following temperatures: 5, 15, 25, 35, 45°C. There was an uninfected batch of five mice which served as the control. The design of the effect of temperature experiment is shown in Table 3.1. After exposure, the mice were put in labelled cages.

Table 3.1. Design of the effect of temperature experiment

Cercarial Dose Per mouse	Temperature(°C) of Exposure	Replicate Number of Mice
200	5	5
200	15	5
200	25	5
200	35	5
200	45	5
	Uninfected Control	5

The method described by Ham (personal Communication), and Krakower (1940), was used in conducting the cold storage experiment. The cercarial suspension obtained from snails was divided into specimen tubes and put in an incubator preset at 5°C. Each specimen tube containing cercariae was removed at the storage intervals of 3, 6, 12 and 24 hours and used for infecting a batch of 5 mice replicates at 200 cercariae per mouse. A batch of 5 mice was also infected with 200 cercariae which had not been subjected to cold storage per mouse. This served as the control. There was also a second control of 5 uninfected mice. On removal from the incubator, the cercariae were activated by gradual warming. The specimen tube was placed in a waterbath preset at 25°c. The temperature of the cercarial suspension was monitored with a thermometer and when it reached 25°c, it was removed and used for infecting mice as in the temperature experiment. Table 3.2 shows the design of the cold storage experiment.

Table 3.2 Design of the Cold Storage Experiment

Cercarial Dose Per mouse	Temperature(°C) of Exposure	Replicate Number of Mice
200	3	5
200	6	5
200	12	5
200	24	5
200	0 (Infected control)	5
-	Uninfected Control	5

The procedure used in the bedding experiment followed that described by Wilson and Coulson (1984). The same procedure for infection of mice as in previous experiments was adopted. However, the mice replicates to be reared on shredded paper bedding were transferred from sawdust to shredded paper 7 days prior to infection while the others remained on sawdust bedding. Infections were carried out at 25°C.

Table 3.3 Design of bedding experiment

Cercarial Dose Per mouse	Temperature(°C) of Exposure	Replicate Number of Mice	
		Shredded paper	Sawdust
200	25	5	5
-	Uninfected Control	5	

In all three experiments, cercarial infectivity was assessed by the following indices faecal egg count, haemoglobin concentration, packed cell volume, wormload, tissue egg count and a qualitative assessment of hepatosplenomegaly.

3.4. Faecal egg count

Each mouse was induced to defecate in a clean cage and each faecal pellet was weighed and assayed using the formol-ether technique. This technique has been documented by King (1973). The faeces was placed in five cubic centimetres of normal saline, emulsified using a glassrod, filtered and centrifuged for 2 minutes. The supernatant was discarded and the tube filled halfway with formal saline before adding $3CM^3$ (three cubic centimetres) of ether and shaking vigorously. The sample was again centrifuged for 2 minutes. The supernatant was discarded and a drop of

Lugols iodine was added to the sediment before observation and counting under the microscope. Observation of the faeces for eggs started five weeks after infection of mice and continued until the eggs were discovered in the faeces.

3.5 Haemoglobin concentration (Hb g/100 ml)

Haemoglobin concentration is a measure for evaluating the degree of anaemia in infected experimental animals. This was evaluated using the haemometer technique as documented by Blaxhall and Daisley (1973). The mice were anaesthetized just before perfusion with 0.2ml per 15g body weight of Nembutal (sodiumpentabarbitone) before collecting blood from them. The blood was sucked up to the 20cmm mark of the capillary pipette. 0.1N HC1 was added up to the "2" mark of the provided measuring tube. The blood in the capillary pipette was blown into the measuring tube containing 0.1N HC1 and allowed to stand for five minutes so as to form acid haematin. Distilled water was added drop by drop to the measuring tube until the colour of the solution matched that of the standard test rods. The value at which the colour of the solution matched that of the test rods was read off as Hb g/100ml. This procedure was repeated for each mouse.

3.6. Packed cell volume (PCV %)

The packed cell volume (PCV %) of each mouse was determined also to assess the degree of anaemia. The packed cell volume is the ratio of the red blood cells to the plasma volume of the blood. The lower the packed cell volume of the blood, the more the degree of anaemia that has occurred (King, 1973).

The procedure used in determining percentage packed cell volume was obtained from the description by Blaxhall and Daisley (1973). Blood from each mouse was drawn up to two thirds mark of two heparinised capillary tubes. This was done by simply holding the capillary tubes upright in the small specimen tube containing the blood sample. The blood ascended the tube by capillary action. One end of each tube was sealed with plasticine and centrifuged for 5 minutes in a heamatocrit centrifuge. The tubes were placed in a haematocrit reader and the value of PCV (x) was read off. The mean reading of the two tubes was taken as packed cell volume of the mouse.

3.7. Recovery of adult worms from mice (wormload)

The adult worms were recovered from the hepatic portal vein of the mice by the perfusion method as in Christensen et al (1984). Each mouse was killed with nembutal at a dose of 0.2ml per 15g body weight. The

mouse was dissected and pegged unto the perfusion apparatus (Figure 3) mounted with a flask before being perfused with 50ml of perfusion solution (15gm sodium citrate and 8.6 gm sodium chloride in 1 litre of water). Perfusion was achieved by severing the hepatic portal vein, then inserting the needle mounted on a 50ml syringe containing the perfusion solution into the left ventricle of the heart and gradually pumping this solution through the heart to the hepatic portal vein. Perfusion was complete when the mesenterial veins contained no more blood. If this was not achieved after using 50ml of perfusion solution, the procedure was repeated using another 50 ml of perfusion solution and using the same hole in the left ventricle. The adult worms were expelled into the flask mounted on the apparatus and were counted, under a dissecting microscope.

3.8 Tissue egg count

The procedure for determining tissue egg load was in accordance with the method described by Christensen et al (1984). The gastro-intestinal tract was wholly digested. The tissues were chopped into fine parts and 5% potassium hydroxide (KOH) was added in the ratio of 10 ml KOH per gramme of tissue. The tissues were then digested for three hours at

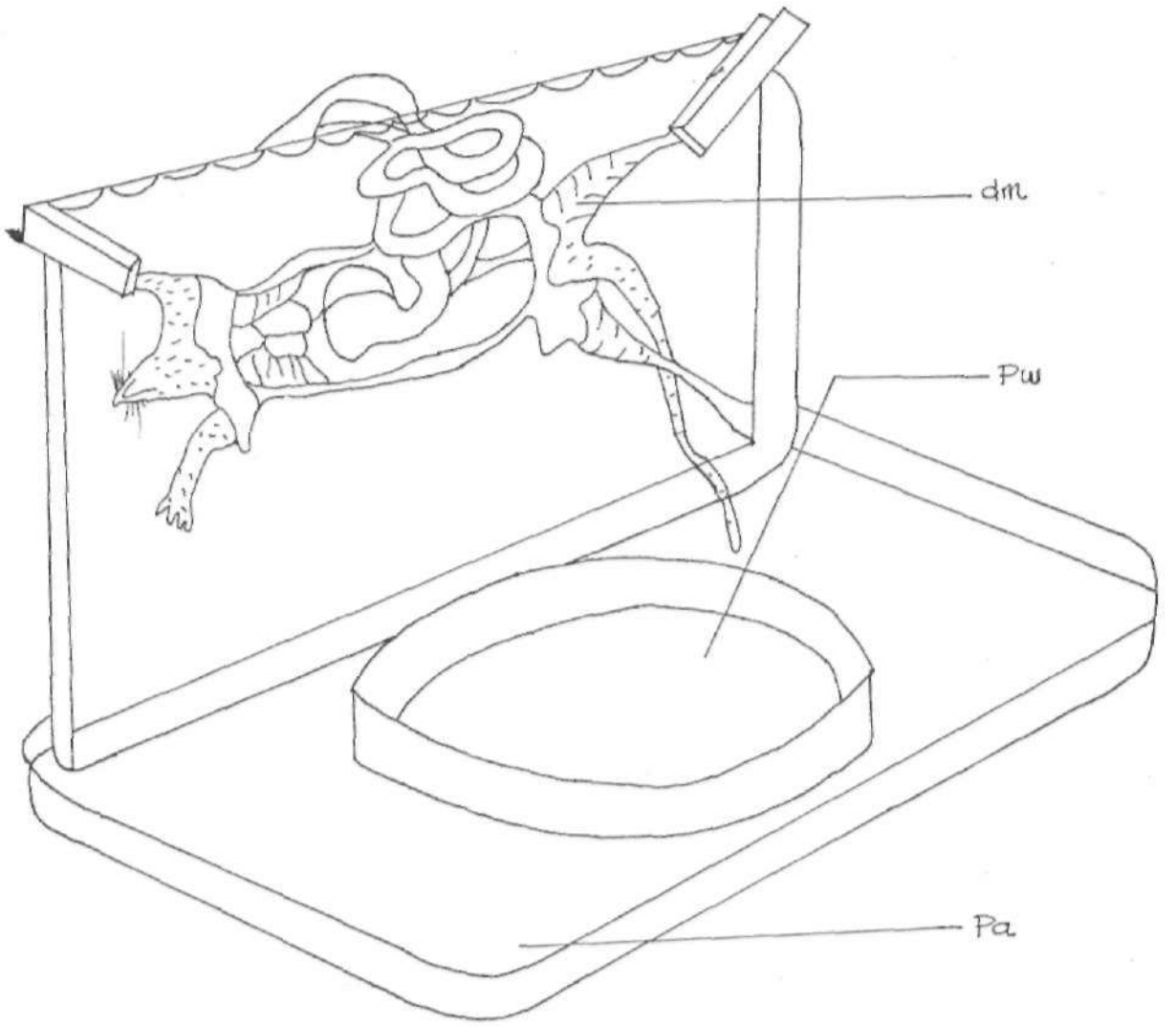


fig 3: Perfusion of a mouse for recovery of adult schistosomes (after Christensen et al 1984)
dm- dissected mouse, pw- perfused worms, pa- perfusion apparatus.

70°C in an oven. The eggs thus freed from the tissues were counted under the microscope. This procedure was repeated for each mouse and mean count for five replicates per batch determined.

3.9. Hepatosplenomegaly

Visual observation of the liver and spleen of all the mice including controls was made on perfusion and compared qualitatively to determine variation in degree of enlargement.

Except for hepatosplenomegaly, values recorded for all the indices used to assess cercarial infectivity were converted into mean values for all mice replicates receiving the same treatment. All mean values for parameters investigated were compared between experimental and control using the two-tailed student's t-test.

CHAPTER FOUR

RESULTS

4.1 Effect of temperature on infectivity

4.1.1 Wormload

Data on worm yield (Table 4.1) revealed that temperature played a vital role in cercarial infectivity. The optimum temperature was 25°C at which the worm yield ± SE (Standard error) was highest, 30.40 ± 2.83, representing 15.20% recovery while mean worm yield dropped to zero at lower temperatures down to 5°C. Also, a steady reduction in worm yield was recorded at temperatures higher than 25°C upto 45°C at which temperature only 3.60 ± 0.68 representing 1.80% of worms were recovered. The two-tailed students t-test showed that the mean wormload of the mice infected at 15,35, and 45°C differed significantly ($P<0.05$) from that of mice infected at 25°C.

4.1.2 Packed Cell Volume (PCV%)

The mean packed cell volume of infected mice showed varying degrees of anaemia when compared with that of the uninfected control. The highest mean value of PCV% was obtained in the uninfected control

Table 4.1: Worm recovery in the temperature experiment

Temperature (°C) of Infection	Mean Number of Worms ± S.E	Percentage Wormload	Two-Tailed Student's T-Test
5	0	0	-
15	4.60±0.40	2.30	P<0.05
25	30.40±2.83	15.20	
35	19.00±1.05	9.50	P<0.05
45	3.60±0.68	1.80	P<0.05

NOTE: Values for other temperatures were compared with value at 25°C.

being 56.90 ± 0.29 and the lowest value, 31.70 ± 0.46 was recorded in the mice infected at 25°C. Data on PCV% are presented in Table 4.2. The two-tailed student's t-test indicated no significant difference (P>0.05) between PCV% of mice exposed to cercariae at 5°C and the uninfected control. The same statistical test, however, indicated a significant difference (P<0.05) between PCV% of mice infected at 15, 25, 35, 45°C and the uninfected control.

4.1.3 Haemoglobin Concentration (Hb g/100 ml)

Haemoglobin concentration also indicated varying degrees of anaemia when Hb of infected mice were compared with that of the uninfected control. The lowest mean value of Hb was 7.74 ± 0.24 g/100ml for mice infected at 25°C while the highest was 11.40 ± 0.05 g/100 ml in the uninfected control (Table 4.2). Using a two-tailed student's t-test, no significant difference (P>0.05) was found between the Hb of mice exposed to cercariae at 5°C and the uninfected control. There was, however, a significant difference between Hb of the uninfected control and those of mice infected at 15, 25, 35, and 45°C.

Table 4.2: Mean Packed Cell Volume and Haemoglobin Concentration in the temperature experiment

Temperature (°C) of infection	Mean PCV (%)± S.E	Two-tailed Students T-Test	Mean Hb g/100ml ± S.E	Two-Tailed Students T-Test
5	56.70±0.20	NS	11.32±0.07	NS
15	44.80±0.58	P<0.05	10.60±0.19	P<0.05
25	31.70±0.46	P<0.05	7.74±0.24	P<0.05
35	34.40±0.91	P<0.05	9.06±0.08	P<0.05
45	49.10±1.70	P<0.05	10.88±0.16	P<0.05
Uninfected Control	56.90±0.29		11.40±0.05	

Note: Means for each treatment were compared with the control, NS=not significant.

4.1.4 Faecal Egg Count

Egg discharge in the faeces of infected mice was not noticed until fifty nine days post infection. No eggs were discharged with the faeces of mice exposed to cercariae at 5°C. Results of the faecal egg count shown in Table 4.3 indicated that the highest egg count ± SE was obtained from faeces of mice infected at 25°C being 4099.20±197.14 eggs/gm of faeces while the lowest count of 267.60±39.08 eggs/gm was observed in mice infected at 45°C. Using the two-tailed student's t-test, comparison of mean faecal egg count of mice infected at 15, 35, and 45°C with that of mice infected at 25°C showed a significant difference ($P<0.05$).

4.1.5 Tissue Egg Count

The number of eggs recovered from the tissues of infected mice was far greater than from the faeces as shown in Table 4.3. Tissue egg count ± SE was highest in mice infected at 25°C (23081.60 ± 1295.07 eggs). The number of eggs recovered from the tissues of mice infected above 25°C showed a decline upto 45°C from which mice the lowest egg count of 3314.80 ± 811.41 was obtained. Tissue egg count also decreased below 25°C reaching zero at 5°C. The two-tailed students t-test showed a significant difference ($P<0.05$) between the tissue egg count of mice infected at 25°C and those infected at 15, 35, and 45°C.

Table 4.3: Comparison of faecal and tissue egg counts in the temperature experiment.

Temperature (°C) of *infection*	Mean No. of eggs/ gram of faeces±SE	Two tailed Student's t-test	Mean No. of eggs *in* GIT± S.E	Two-Tailed Student's t-test
5	0	-	0	-
15	325.00±42.91	P<0.05	4831.00±578.74	P<0.05
25	4099.20±197.14	-	23081.60±1295.07	-
35	2737.20±257.24	P<0.05	16645.00±1381.92	P<0.05
45	267.60±39.08	P<0.05	3314.80±811.41	P<0.05

Note: Values for other temperatures were compared with values at 25°C.
GIT = Gastrointestinal tract.

4.1.6 Hepatosplenomegaly

Hepatosplenamegaly was observed in all infected mice but was not observed in mice exposed to cercariae at 5°C. It was most pronounced in those mice infected at 25 and 35°C which had very darkened livers and spleens with sandy patches. The livers bore scars. *Inflammation of the liver and spleen* was also observed in mice infected at 15 and 45°C but was comparatively less.

4.2 Cold Storage experiment

4.2.1 Wormload

The number of adult worms recovered from the mice decreased with increase in the duration of storage as shown in Table 4.4. The range of mean wormload ± S.E was 0.80±0.58 to 14.80±1.28 worms obtained from mice *infected with cercariae stored for 24 and 3 hours* respectively. These mean wormload values corresponded with 0.40 and 7.40 percentage worm yield for mice infected with cercariae stored for 24 and 3 hours respectively. Only four non-copula worms were obtain *from the hepatic portal veins of two of the five mice* infected after 24 hour storage of cercariae.

Table 4.4: Mean worm recovery in the Cold Storage experiment.

Duration (hours) of storage	Mean number of worms ± S.E	Percentage wormload	Two-tailed student's t-test
3	14.80±1.28	7.40	P<0.05
6	8.60±0.51	4.30	P<0.05
12	4.40±0.25	2.20	P<0.05
24	0.80±0.58	0.40	P<0.05
0 (INFECTED CONTROL)	30.40±2.83	15.20	

Note: Values for the various durations of storage were compared with value of infected control.

Statistical analysis using the two-tailed student's t-test showed a significant difference ($P<0.05$) between the mean number of worms recovered from mice infected with cercariae stored for zero hours (infected control) and from mice infected with cercariae stored for 3, 6, 12, and 24 hours. The same statistical test also showed a significant difference ($P<0.05$) between the mean number of worms recovered from mice infected with cercariae stored for various periods.

4.2.2 Packed Cell Volume (PCV%)

The degree of anaemia as assessed by percentage packed cell volume (PCV), was most severe, being 37.40±1.57%, in those mice infected with cercariae stored in the cold for 3 hours. The degree of anaemia became less severe as duration of storage increased since the mean values of PCV increased. Mice exposed to cercariae stored for 24 hours had a mean PCV of 56.60±0.29% which was comparable to that of the uninfected control, 56.90±0.29%. The mean PCV values of mice in the cold storage experiment are shown in Table 4.5. The two-tailed student's t-test showed a significant difference ($P<0.05$) between mean PCV of the uninfected control and those of mice infected with cercariae stored for 3,6, and 12 hours.

Table 4.5: Mean Packed Cell volume and Haemoglobin Concentration in the Cold Storage experiment.

Duration (hours)of Storage	Mean PCV (%) ± S.E	Two tailed Student's t-test	Mean Hb. (g/100ml) ± S.E	Two-Tailed Student's t-test
3	37.40±1.57	P<0.05	8.38±0.13	P<0.05
6	43.10±0.43	P<0.05	9.16±0.35	P<0.05
12	49.30±0.34	P<0.05	10.50±0.16	P<0.05
24	56.60±0.29	NS	11.64±0.08	NS
0 (INFECTED CONTROL)	31.70±0.46	P<0.05	7.74±0.24	P<0.05
UNINFECTED CONTROL	56.90±0.29		11.32±0.07	

Note: Values for the various durations of storage were compared with values of the uninfected control

NS = Not significant

4.2.3 Haemoglobin concentration (Hb g/100ml)

The haemoglobin concentration test showed that the degree of anaemia was most severe in mice infected with cercariae stored for 3 hours since such mice had the lowest mean Hb±SE of 8.38±0.13 g/100ml. With increase in the duration of storage, the Hb of mice appreciated (Table 4.5) an indication that the severity of anaemia lessened. The Hb ± SE of mice exposed to cercariae after 24 hours of cold storage was 11.64 ± 0.08 g/100ml, a figure comparable to 11.32 ± 0.07 g/100ml observed in the uninfected control.

The two-tailed student's t-test showed a significant difference ($P<0.05$) between the mean Hb of the uninfected control and those of mice infected with cercariae stored for 3,6, and 12 hours.

4.2.4 Faecal egg count

The number of eggs excreted by mice decreased as duration of cercarial storage increased as shown in Table 4.6. The highest mean count ± SE of 1973.20 ± 445.90 eggs/gm of faeces was obtained from mice infected with cercariae stored for 3 hours while the lowest mean ± SE of 387.40 ± 79.75 eggs/gm of faeces was obtained from mice infected with cercariae stored

Table 4.6: Comparison of faecal and tissue egg counts in the cold storage experiment

Duration (hours) of Storage	Mean No. of Eggs Gram of Faeces± S.E	Two tailed Student's t-test	Mean No of Eggs in GIT±SE	Two-Tailed Student's t-test
3	1973.20±445.90	P<0.05	13514.40±607.34	P<0.05
6	1049.00±72.88	P<0.05	4405.80±254.86	P<0.05
12	387.40±79.75	P<0.05	2637.80±358.01	P<0.05
24	0		0	
0(INFECTED CONTROL)	4099.20±197.14		23081.60±1295.07	

Note: Values of the various durations of storage were compared with values of the infected control

GIT = Gastrointestinal tract

for 12 hours. As in the uninfected control, mice exposed to cercariae after 24 hours cold storage excreted no eggs.

Using the two-tailed student's t-test, it was found that the mean faecal egg count of the infected control differed significantly ($P<0.05$) from the mean egg counts of mice infected with cercariae stored for 3, 6, and 12 hours.

4.2.5 Tissue egg Count

Mice infected with cercariae stored for 3 hours had the highest mean count ± SE of 13514.40±607.34 eggs, while those infected with cercariae after 12 hours cold storage had the lowest mean ± SE of 2637.80 ± 358.01 eggs. No eggs were recovered from the tissues of mice exposed to cercariae stored for 24 hours. The mean ± SE count of 23081.60 ± 1295.07 eggs recorded for the infected control greatly exceeded that of the experimentals. These results are reflected in Table 4.6.

The two-tailed student's t-test showed a significant difference ($P<0.05$) between tissue egg counts of the infected control and of mice infected with cercariae after 3, 6, and 12 hours.

4.2.6 Hepatosplenomegaly

The liver and spleen of mice exposed to cercariae stored for 24 hours showed no enlargement or sandy patches. They were normal in appearance and comparable to those of the uninfected controls. However, the livers and spleens of mice infected with cercariae stored for 3,6, and 12 hours did show enlargement as did the infected control. Of the experimentals, liver and spleen enlargement was observed to be most pronounced in mice infected with cercariae stored for 3 and 6 hours.

4.3 Bedding Experiment

4.3.1 Wormload

Mean worm recovery ± SE and, consequently, percentage wormload was higher in mice infected and reared on shredded paper (30.40 ± 2.83 worms and 15.20% respectively) than in mice infected but raised on sawdust bedding (21.40 ± 0.92 worms and 10.70% respectively). Infection was carried out at 25°C. A comparison of worm recoveries from mice raised on the two types of beddings is given in Table 4.7.

Table 4.7: Mean worm recovery in the Bedding Experiment.

Type of mouse bedding	Mean number or worms ± S.E	Percentage wormload	Two-tailed Student's t-test
Shredded paper	30.40±2.83	15.20	
Sawdust	21.40±0.92	10.70	P<0.05

Note: Value of Sawdust was compared with value for Shredded paper.

Statistical analysis using the two-tailed student's t-test showed that the wormload of mice infected and reared on shredded paper differed significantly ($P<0.05$) from that of mice reared on sawdust bedding.

4.3.2 Packed Cell volume (PCV%)

The percentage packed cell volume (PCV) showed that the degree of anaemia was more pronounced in mice infected and reared on shredded paper than those infected but reared on sawdust bedding since the mean PCV ± SE of mice raised on sawdust bedding was higher being 33.70 ± 0.34% compared with 31.70 ± 0.46% for mice on shredded paper bedding (Table 4.8).

The two-tailed t-test showed a significant difference ($P<0.05$) between the mean PCV of mice infected and reared on shredded paper and mice infected but raised on sawdust bedding. There was also a significant difference ($P<0.05$) between PCV of the uninfected control and those of mice raised on shredded paper and sawdust beddings.

Table 4.8: Mean Packed Cell Volume and Haemoglobin Concentration in the Bedding experiment

Type of mouse bedding	Mean PCV (%) ± S.E	Two tailed Student's t-test	Mean Hb. (g/100ml) ± S.E	Two-Tailed Student's t-test
Shredded Paper	31.70±0.46		7.74±0.24	
Sawdust	33.70±0.34	P<0.05	8.52±.0.16	P<0.05
Uninfected Control	56.90±0.29	P<0.05	11.40±.0.05	P<0.05

Note: Values of Sawdust and the control were compared with values for shredded paper

4.3.3 Haemoglobin Concentration (Hb g/100 ml)

Comparison of the haemoglobin concentration of the infected mice showed lower values hence more pronounced anaemia in mice raised on shredded paper than on sawdust bedding. A mean ± SE of 7.74± 0.24 g/100 ml was recorded for mice infected and reared on shredded paper compared with 8.52 ± 0.16 g/100ml for mice infected but reared on sawdust bedding. Hb results are reflected in Table 4.8.

The two-tailed student's t-test confirmed a significant difference ($P<0.05$) between the Hb of mice infected and reared on shredded paper and on sawdust bedding. The same statistical test also showed a significant difference ($P<0.05$) when the mean Hb values of infected mice raised on either sawdust or shredded paper bedding was compared with that of the uninfected control.

4.3.4 Faecal egg count

More eggs were recovered from the faeces of mice infected and raised on shredded paper bedding than from the faeces of those infected but reared on sawdust bedding. The mean faecal egg count ± SE for mice on shredded paper was 4099.20 ± 197.14 eggs/gm

compared with 2888.80 ± 237.47 eggs/gm for mice on sawdust. Faecal egg count is shown in Table 4.9.

Using the two-tailed student's t-test, a significant difference ($P<0.05$) was found between the mean faecal egg count of infected mice raised on shredded paper and on sawdust beddings.

4.3.5 Tissue egg count

More eggs were recovered from the tissues than from the faeces of infected mice (Table 4.9), as was the case in the other experiments. Mean tissue egg count ± SE was higher for mice reared on shredded paper (23081.60 ± 1295.07 eggs) than for mice raised on sawdust bedding (17521.80 ± 632.92 eggs). No eggs were recovered from the tissues of the uninfected control.

Table 4.9: Comparison of faecal and tissue egg counts in the Bedding experiment

Type of mouse bedding	Mean No. of Eggs Gram of Faeces± S.E	Two tailed Student's t-test	Mean No of Eggs in GIT±SE	Two-Tailed Student's t-test
Shredded Paper	4099.20±197.14		23081.60±1295.07	
Sawdust	2888.80±237.47	P<0.05	17521.80±632.92	P<0.05

Note: Values of sawdust were compared with values of shredded paper
GIT = Gastrointestinal tract

The two-tailed students' t-test showed that the mean tissue egg count of mice infected and reared on shredded paper differed significantly ($P<0.05$) from that of mice infected but reared on sawdust bedding.

CHAPTER FIVE

DISCUSSION AND CONCLUSIONS

In the temperature experiment, percentage worm recoveries rose from zero at 5°C upto a maximum at 25°C, and above this, decreased upto 45°C. It has been observed that schistosome cercariae would survive 45°C but not 50°C (Purnell, 1966b). The zero infectivity observed for cercariae at 5°C is consistent with the findings of Chen et al (1982), who investigated the adhesion precipitation technique for the recovery of schistosome cercariae from natural waters. These authors reported no infection in mice exposed to cercariae at 5.5°C because majority of the mice froze to death while others were numbed.

The findings of this study disagree with those of Purnell (1966a), who reported that at 39°C, Schistosoma mansoni cercariae were uninfective. However, he observed that infectivity reached a peak about 24°C, a value comparable to the peak infectivity observed at 25°C in this study. The presence of infection at 45°C in the present study, tallies with the observations of Stirewalt and

Fregeau (1965), who investigated cercarial infectivity at extreme temperatures and reported that infection was possible at 45°C.

Anaemia, in the temperature experiment, though observed in all infected mice, was most pronounced in those exposed to cercariae at 25°C. Generally, the lower the value of PCV% and Hb g/100ml, the more is the severity of anaemia that has occurred. The absence of anaemia in mice exposed to cercariae at 5°C is due to the zero infectivity of cercariae at this temperature.

Anaemia in schistosomiasis is usually significantly correlated with number of infecting cercariae so that the higher the number of infecting cercariae, the higher would be the degree of anaemia (Okwuosa, in press).

The observations on faecal and tissue egg counts in the temperature experiment suggest cercarial infectivity was highest at 25°C and lowest at 45°C and zero at 5°C. The relatively low faecal egg count compared to the tissue egg count observed in this study has also been reported by Okwuosa (in press). He indicated that it is a factor of the immuno-competence of the experimental host as influenced by host strain and age. The immuno-competence of the host determines the degree of tissue response and the

extent of egg embolization and so determines how many eggs successfully reach the lumen of the gastrointestinal tract and the time required for the passage (Okwuosa, in press).

The qualitative assessment of hepatosplenomegaly in infected mice revealed that liver and spleen enlargement was most pronounced in those infected at 25°C and least in those mice infected at 45°C. The degree of liver and splenic enlargement appears to depend on the number of cercariae that successfully penetrate the definitive host. According to von Lichtenberg (1987), there is a clear correlation between schistosome eggs in the stools and the degree of liver or splenic enlargement; the higher the mean count, the higher the prevalence of these organomegalies and the risk of progression to severe disease.

From the observations on the effects of temperature on cercarial infectivity, it would be seen that infectivity of cercariae was highest at 25°C, lowest at 45°C and zero at 5°C. Enzymes usually require a narrow range of temperatures in order to function optimally. Ham (personal communication), suggested that the zero infectivity at 5°C and low infectivity at 45°C is probably because the histolytic enzyme system of the cercariae are

inactivated at 5°C and lysed at 45°C. Furthermore, at high temperatures, cercariae move very rapidly but age quickly and soon die and even if some cercariae do manage to penetrate, they may not mature since they are likely to have used up energy (glycogen) reserves by vigorous movement (Purnell, 1966a). This suggestion may also explain the low cercarial infectivity at 45°C. It could be inferred that at 25°C, the cercariae move with just adequate speed required during host finding and penetration rather than dissipating energy through very rapid swimming. The results suggest that 25°C, or thereabouts, represents the optimum temperature which favours the action of cercarial histolytic enzymes, rate of energy consumption, and the process of infection in general.

The above observations on the relationship between cercarial infectivity and temperature suggest that under field conditions, infection with schistosome cercariae probably occur over a wide range of temperatures. From the results, it could be inferred that temperature influences cercarial infectivity, infectivity being low in the early mornings, rising to a peak about mid-day and showing a decline thence into the night. However, caution should be exercised in applying the findings of this

study to field conditions since penetration of the definitive host by cercariae is influenced by a complex of factors such as water contact pattern, duration of contact, activity in water, water salinity, turbulence, velocity, turbidity, among others, all of which were eliminated from this study.

In the cold storage study, an inverse relationship was observed between worm recovery and duration of cold storage. Percentage worm recovery decreased as duration of cold storage increased. Infection could barely be produced with cercariae that had been 24 hours in the cold as a percentage worm yield of only 0.40 was obtained from mice infected with such cercariae. This disagrees with the suggestion by Krakower (1940) that infection may still be produced after cercariae had been 4 days in the cold at 5°C. Cercariae used in this study may have been less resistant to thermal stress than those used by Krakower (1940).

Records of all the indices used to assess cercarial infectivity in the cold storage study suggest that cercarial infectivity decreased as the duration of cold storage increased. This may be partly due to the fact that infectivity decreases with age (Purnell, 1966a Prah and James, 1977). The results also suggest that cold storage of cercariae

causes a decrease in infectivity since cercariae not subjected to cold storage consistently showed a higher infectivity than those cold stored. Temperature has a marked effect on the metabolic rates of all organisms. At such low temperatures as 5°C, metabolic rates decrease deleteriously and may lead to death. For those cercariae that survived the low temperature, their infectivity decreased with age.

In the bedding experiment, the indices of cercarial infectivity suggest that cercariae were more infective to mice bred on shredded paper than to those on sawdust and that sawdust adversely affects cercarial infectivity. Why this is so is not known. The results agree with the findings of Wilson and Coulson (1984), who compared the effects of type of bedding on the routes (tail and abdomen) of infection of mice. They suggested that sawdust may contain substances which "contaminate" the skin and hamper either the penetration of the skin by the cercariae or the onward migration in the definitive host by schistosomula, or the maturation of the schistosomula into adult worms in the definitive location in the definitive host.

All the indices of infectivity used, and therefore, the results of the three experiments may

have been affected by the following:

i) The 200 cercariae used in infecting mice were estimates subject to experimental error. There is no guarantee that each mouse received exactly 200 cercariae.

ii) The ratio of male: female cercariae was not known at the time of infection of mice since there is no method of telling the sex of a cercaria. For this reason, the number of in copula worms varied in mice that were given the same treatment. This is important since only paired worms lay eggs which are responsible for the disease.

iii) A large proportion of schistosome cercariae die while penetrating the skin of experimental hosts and large numbers of schistosomula die within 10 minutes of penetration (Clegg and Smithers, 1968).

Conclusion

i) Infection with schistosome cercariae can occur over a wide range of temperatures. Infectivity, however, is highest at about 25°C, lowest at 45°C and non-existent at 5°C.

ii) The infectivity of cercariae decreases with increase in the duration of cold storage. Cold storage of cercariae for use in experimental schistosomiasis appears to be only of limited use and it is recommended that if this method of cercarial preservation must be used, storage should be for as short a period as possible. Alternatively, cercarial dose of infection could be increased, for example 400 cercariae, if cold stored.

iii) Sawdust bedding appears to inhibit cercarial infectivity compared to shredded paper bedding. It is therefore recommended, that use of sawdust bedding for mice in experimental schistosomiasis be avoided.

REFERENCES

Anderson, R.M., Mercer, J.G., Wilson, R.A. Carter, N.P. (1982). Transmission of Schistosoma mansoni from man to snail. Experimental studies of miracidial survival and infectivity in relation to larval age, water temperature, host size and host age. Parasitology (pt2) 1.339-360.

Andreano, R. and Helminiak, T. (1988). Economics, health and tropical diseases: A review. In: Herrin, A.N. and Rosefield, P.L. (Eds) Economics, Health and Tropical Diseases pp19-72.

Asumu, D.I. (1975). Studies on the biology of snails and their larval trematodes in Ibadan freshwater systems. Ph.D Thesis. University of Ibadan, pp 1-440.

Becker, W. and Lutz, W. (1976). The effect of salt solutions of differing ionic composition on the swimming activity of the cercariae of Schistosoma mansoni Zeitschrift fur Parasitenkunde 50(1): 99-101.

Blaxhall, P.C. and Daisley, K.W (1973).

Routine haematological methods for use in fish blood. Journal of Animal Biology 5:771-781.

Bradley, D.J. and McCullough, F.S. (1973).

Egg output stability and the epidemiology of Schistosoma haematobium infection. Part II. An analysis of the epidemiology of endemic Schistosoma haematobium. Transactions of the Royal Society of Tropical Medicine and Hygiene,, 67: 491-500.

Chappell, L.H. (1980).

Physiology of Parasites. Blackie, Glasgow and London. pp.117-118.

Chen, G.Y. Yi., D.S., Li, W.S., Chen, S.Z. and Wu, G. (1982). Adhesion-precipitation technique for schistosome cercariae recovery from natural waters. Chinese Medical Journal, 95(10): 762-764.

Cheng, T.C. and Burton, R.W (1965).

Relationships between Bucephalus sp and Crassotrea virginica: Histopathology and sites of infection. Chesapeake Science. 6:3-16.

Cheng, T.C. and Snyder, R.W. (1963).

Studies on host-parasite relationships between larval trematodes and their hosts IV. A histochemical determination of glucose and its role in the metabolism of molluscan host and parasite. Transcations of the American Microscopic Society, 82: 343-346.

Christensen, N.O. and Frandsen, F. (1985).

An introduction to the taxonomy, morphology, biology, and transmission ecology of species of the genus Schistosoma causing human African schistosomiasis. Danish Bilharziasis Laboratory Handbook, pp.1-33.

Christensen, N.O. and Furu, P. (1987).

Basic aspects of the epidemiology of human African Schistosomiasis. Danish Bilharziasis Laboratory Handbook pp.1-20.

Christensen, N.O., Gotsche, G. anmd Frandsen F. (1984).

Parasitological techniques for use in routine laboratory maintenance of schistosomes and for use in studies on the epidemiology of human and bovine schistosomiasis. Danish Bilharziasis Laboratory Handbook, pp.1-40.

Clegg, J.A. and Smithers, S.R. (1968).

Death of schistosome cercariae during penetration of the skin II. Penetration of mammalian skin by Schistosoma mansoni. Parasitology, 58: 111-128.

Dalton, P.R. and Pole, D. (1978).

Water contact patterns in relation to Schistosoma haematobium infection. Bulletin of the World Health Organization 56: 417-426.

Donnelly, F.A.,Appleton, C.C. and Schutte, C.H.J. (1984).

The influence of salinity on the cercariae of three species of Schistosoma. International Journal for Parasitology., 14(1): 13-21.

Dutt, S.C and Srivastava, H.D. (1962)

Biological studies on Orientabilharzia dattai (Dutt and Srivastava 1952). Dutt and Srivastava, 1955 - a blood fluke of ruminants. Indian Journal of Veterinary Science, 32: 216-228.

Encylopedia Britainica (1972).

Schistosomiasis. 19:1156-1157.

Erasmus, D.A. (1958).

Studies on the morphology, biology and development of a strigeid cercaria (Cercaria X Baylis, 1930). Parasitology 48: 312-335.

Erasmus, D.A. (1972).

The Biology of Trematodes. Edward Arnold (Publishers) Limited, London, pp.98-110.

Ghandour, A.M. and Webbe, G. (1975 a).

The effect of ultraviolet radiation on cercariae of Schistosoma mansoni and Schistosoma haematobium. Journal of Helminthology. 49(3): 153-159.

Ghandour, A.M. and Webbe, G. (1975 b).

The effects of gamma radiation on cercariae of Schistosoma mansoni. Journal of Helminthology. 49(3): 161-165.

Herrin, A.N. (1988).

Perspectives of disease impacts: What can they tell us? In: Harrin. A.N. and Rosenfield, P.L. (Eds) Economics, Health and Tropical Diseases. pp.225-232.

Hira, P.R. (1966).

Studies on the ecology of the intermediate snail host of Schistosoma haematobium in Ibadan, Western

Nigeria, and on some aspects of the biology of the parasite. Ph.D Thesis, University of Ibadan. pp. 1-310.

Hunter, W.S. and Vernberg, W.B. (1955).
Studies on oxygen consumption in digenetic trematodes I. Experimental Parasitology. 4: 54-61.

Husting E.L. (1983):
Human water contact activities related to the transmission of bilharziasis (Schistosomiasis). Journal of Tropical medicine and Hygiene. 86: 23-35.

King, M. (1973).
Medical Laboratory for Developing Countries. Oxford University Press, England pp.7.1-7.2.

Krakower, C.A (1940).
Some observations of the effects of physical and chemical agents on the cercariae of Schistosoma mansoni. Puerto Rican Journal of Public Health and Tropical Medicine, 16:26.

Lawson, J.R. and Wilson, R.A. (1983).
The relationship between the age of Schistosoma mansoni cercariae and their ability to penetrate and

infect the mammalian host. Parasitology. 87 (3): 481-492.

Madsen, H. (1986).

Control of freshwater snails, Danish Bilharziasis Laboratory handbook P1-50.

Madsen, H. (1983).

Distribution of Helisoma duryi, an introduced competitor of intermediate hosts of schistosomiasis in an irrigation scheme in northern Tanzania. Acta Tropica 40:297-306.

Maiga, A.M. (1988).

Epidemiology and the socioeconomic consequences of schistosomiasis among peasant farmers of Niger. In: Herrin, A.N. and Rosenfield, P.L. (Eds). Economics, Health and Tropical Diseases, pp 233-239.

Nelson, G.S. (1972).

Human behaviour in the transmission of parasitic diseases. In: Canning, E.U. and Wright, C.A. (Eds) Behavioural Aspects of Parasite Transmission. Academic Press, London, pp.109-122.

Okwuosa, V.N. (in the press).

An evaluation of the morbidity of graded doses of brevifurcate cercariae of Schistosoma mansoni in well nourished mice. Journal of Agriculture, Science and Technology

Okwuosa, V.N. (1979).

The ecological studies of freshwater snails with particular reference to schistosome vectors in parts of Lagos, Oyo and Ogun states of Nigeria. Ph.D. Thesis, University of Ibadan, pp.1-284.

Oliver, L.,Von Bran, T. and Mehlman, B. (1953).

The influence of lack of oxygen on Schistosoma mansoni cercariae and on infected Australorbis glabratus. Experimental Parasitology, 2: 258-270.

Polderman, A.M. (1979).

Transmission dynamics of endemic schistosomiasis. Tropical and Geographical Medicine. 31(4): 465-475.

Prah, S.K. and James, C. (1977).

The influence of physical factors on the survival and infectivity of miracidia of Schistosoma mansoni and Schistosoma haematobium I. Effects of temperature and ultraviolet light. Journal of Helminthology, 51. 73-85.

Pugh, R.N.H. and Gilles, H.M. (1978).
Malumfashi Endemic Diseases Research project III. Urinary Schistosomiasis: a longitudinal study. Annals of Tropical Medicine and Parasitology, 72: 421-482.

Purnell, R.E. (1966 a).
Host-parasite relationships in schistosomiasis I. The effect of temperature on the infection of Biomphalaria sudanica tanganyicensis with Schistosoma mansoni miracidia and of laboratory mice with Schistosoma mansoni cercariae. Annals of Tropical Medicine and Parasitology, 60: 90-96.

Purnell, R.E. (1966 b).
Host-parasite relationships in schistosomiasis III. The effect of temperature on the survival of Schistosoma mansoni miracidia and on the survival and infectivity of Schistosoma mansoni cercariae. Annals of Tropical Medicine and Parasitology, 61: 182-186.

Radke, M.G., Ritchie, L.S. and Rowan, W.B. (1961).
Effects of water velocities on worm burdens of animals exposed to Schistosoma mansoni cercariae released under laboratory and field conditions. Experimental parasitology, 11: 323-331.

Santos, A.T. (1988).

Economic aspects related to schistosomiasis transmission and control. In Herrin, A.N. and Rosenfield, P.L. (Eds). Economics, Health and Tropical Diseases, pp.449-452.

Simonsen, P.E. (1985).

Chemotherapy of schistosomiasis. Danish Bilharziasis Laboratory handbook

Stirewalt, M.A. (1966).

Skin penetration mechanisms in helminths In: Soulsby, E.J.L. (Ed.), Biology of Parasites, Academic press Limited, London. pp.41-60.

Stirewalt, M.A. and Fregeau, W.A. (1965).

Effects of selected experimental conditions on penetration and maturation of Schistosoma mansoni in mice I. Environmental. Experimental Parasitology, 17: 168-179.

Tiaglo, T.V. (1982).

Health knowledge, attitudes and practices related to schistosomiasis in Leyte. Tropical medicine, 24(2): 103-114.

Vernberg, W.B. (1963).

Respiration of digenetic trematodes. Annals of New York Academy of Science, 113: 261-271.

Von Lichtenberg, F. (1987).

Consequences of infections with schistosomes. In: Rollinson, D. and Simpson, A.J.G. (Eds), The Biology of Schistosomes, from genes to latrines, Academic Press Limited, London, pp 185-232.

Warren, K.S. (1973).

Regulation of the prevalence and intensity of schistosomiasis in man: immunology or ecology? Journal of Infectious Diseases, 127: 595-609.

WHO (1972).

A consideration of the economic impact of Schistosomiasis. WHO BULLETIN, 47: 559-566.

WHO (1981).

Data sheet on the biological control agent Thiara granifera WHO/VBC/81.833.

WHO (1967).

Measurement of the public health importance of bilharziasis. Report of a WHO Scientific group. WHO Technical Report Series No. 372, p.12.

WHO (1989).

Tropical Diseases, Progress in International Research 1987-1988. Ninth programme Report, pp.55-62.

WHO (1991).

Tropical diseases, Progress in Research, 1989-1990. Tenth programme Report pp.41-47.

WHO (1990).

Tropical Diseases 1990 TDR-CTD/HH 90.1 pp.6-7.

Wilkins H.A. (1987).

The epidemiology of Schistosome infections in man In: Rollinson, D. end Simpson, A.J.G. (Eds) The Biology of Schistosomes, from genes to latrines Academic Press Limited London, pp. 379-398.

Wilkins, H.A., Goll, P.H., Marshall, T.F. Dec. and Moore, P.J. (1984). Dynamics of Schistosoma haematobium infection in a Gambian community I, the pattern of human infection in the study area. Transactions of the Royal Society for Tropical Medicine and Hygiene, 78: 216-221.

Wilkins, H.A. and Scott, A. (1978).

Variation and stability of Schistosoma haematobium egg counts: a four year study of Gambian children. Transactions of the Royal Society of Tropical Medicine and Hygiene, 72: 397-404.

Wilson, R.A. (1987).

Cercariae to liver worms: Development and migration in the mammalian host In: Rollinson, D. and Simpson, A.J.G. (Eds), The Biology of Schistosomes, from genes to latrines, Academic Press Limited, London, pp.115-146.

Wilson, R.A. and Coulson, P.S. (1984).

The effects of soft wood sawdust bedding on the maturation of an infection of Schistosoma mansoni in mice exposed to cercariae via the tail or abdominal skin. Transactions of the Royal Society of Tropical Medicine and Hygiene, 78(3): 411-412.

APPENDIX I

DETAILED RESULTS OF THE EFFECT OF TEMPERATURE EXPERIMENT

WORMLOAD

TEMPERATURE (°C) OF EXPOSURE	1	2	3	4	5	MEAN ± SE	PERCENTAGE WORMLOAD
5	0	0	0	0	0	0	0
15	6	4	4	5	4	4.6 ± 0.4	2.30
25	29	33	40	26	24	30.40 ± 2.83	15.20
35	20	19	20	15	21	19.00 ± 1.05	9.50
45	3	6	3	4	2	3.60 ± 0.68	1.80
CONTROL (UNINFECTED)	-	-	-	-	-	-	-

PACKED CELL VOLUME (%)

TEMPERATURE (°C) OF EXPOSURE	1	2	3	4	5	MEAN ± SE
5	56	57	57	56.50	57	56.70 ± 0.20
15	43	45	46	44	46	44.80 ± 0.58
25	32.50	32	30	32.50	31.50	31.70 ± 0.46
35	33.50	33.50	33	38	34	34.40 ± 0.91
45	50.50	44	51	46.50	53.50	49.10 ± 1.70
CONTROL (UNINFECTED)	57.50	57	56.50	56	57.50	56.90 ± 0.29

HAEMOGLOBIN CONCENTRATION

TEMPERATURE (°C) OF EXPOSURE	REPLICATES 1	2	3	4	5	MEAN ± SE
5	11.20	11.50	11.50	11.20	11.20	11.32 ± 0.07
15	10	11	10.50	10.50	11	10.60 ± 0.19
25	7.90	7.40	7.00	8.20	8.20	7.74 ± 0.24
35	9	9	8.90	9.40	9	9.06 ± 0.08
45	11	10	11.20	10.50	11.20	10.88 ± 0.16
CONTROL (UNINFECTED)	11.50	11.40	11.20	11.40	11.50	11.40 ± 0.05

FAECAL EGG COUNT PER GRAMME

TEMPERATURE (°C) OF EXPOSURE	REPLICATES 1	2	3	4	5	MEAN ± SE
5	0	0	0	0	0	0
15	241	425	322	219	418	325±42.91
25	4329	3381	4464	4349	3973	4099.20±197.14
35	2920	2480	3176	1859	3251	2737.20±257.24
45	394	245	167	221	311	267.60 ± 39.08
CONTROL (UNINFECTED)	-	-	-	-	-	-

TISSUE EGG COUNT

TEMPERATURE (oC) OF EXPOSURE	REPLICATES 1	2	3	4	5	MEAN ± SE
5	0	0	0	0	0	0
15	6017	5418	5122	2625	4973	4831 ± 578.74
25	24672	24868	25327	22154	18387	23081.60 ± 1295.07
35	18416	13621	17844	13228	20116	16645 ± 1381.92
45	3602	6286	2017	1744	2925	3314.80 ± 811.41
CONTROL (UNINFECTED)	-	-	-	-	-	-

APPENDIX 2

DETAIL RESULTS OF THE COLD STORAGE EXPERIMENT

WORMLOAD

DURATION (HOURS) OF STORAGE	REPLICATES 1	2	3	4	5	MEAN ± SE	PERCENTAGE WORMLOAD
3	17	13	18	11	15	14.80±1.28	7.40
6	9	8	9	7	10	8.60±0.51	4.30
12	5	4	4	5	4	4.40±0.25	2.20
24	0	3	0	0	1	0.80±0.58	0.40
0 (INFECTED CONTROL)	29	33	40	26	24	30.40±2.83	15.20
UNINFECTED CONTROL	-	-	-	-	-	-	-

PACKED CELL VOLUME (PCV, %)

DURATION (HOURS) OF STORAGE	REPLICATES 1	2	3	4	5	MEAN ± SE
3	33.50	40	35	42	36.50	37.40±1.57
6	43	42.50	43.50	44.50	42	43.10±0.43
12	49.50	48	49.50	50	49.50	49.30±0.34
24	56	56.50	57.50	56	57	56.60±0.29
0 (INFECTED CONTROL)	32.50	32	30	32.50	31.50	31.70±0.46
UNINFECTED CONTROL	57.50	57	56.50	56	57.50	56.90±0.29

HAEMOGLOBIN CONCENTRATION (Hb, g/100 ml)

DURATION (HOURS) OF STORAGE	REPLICATES 1	2	3	4	5	MEAN ± SE
3	8.20	8.80	8.50	8.40	8	8.38±0.13
6	8	9.50	8.80	10	9.50	9.16±0.35
12	10	10.50	10.50	11	10.50	10.50±0.16
24	11.80	11.60	11.40	11.80	11.60	11.64±0.08
0 (INFECTED CONTROL)	7.90	7.40	7.00	8.20	8.20	7.74±0.24
UNINFECTED CONTROL	11.20	11.50	11.50	11.20	11.20	11.32±0.07

FAECAL EGG COUNT PER GRAMME

DURATION (HOURS) OF STORAGE	REPLICATES 1	2	3	4	5	MEAN ± SE
3	2891	1173	3218	1415	1169	1973.20 ± 445.90
6	1091	852	1076	944	1282	1049.00 ± 72.88
12	630	322	240	518	227	387.40 ± 79.75
24	0	0	0	0	0	0
0 (INFECTED CONTROL)	4329	3381	4464	4349	3973	4099.20 ± 197.14
UNINFECTED CONTROL	-	-	-	-	-	-

TISSUE EGG COUNT

DURATION (HOURS) OF STORAGE	REPLICATES 1	2	3	4	5	MEAN ± SE
3	14872	13557	13838	11244	14061	13514.40±607.34
6	5276	4518	4344	3715	4176	4405.80±254.86
12	2171	2092	3124	1984	3818	2637.80±358.01
24	0	0	0	0	0	0
0 (INFECTED CONTROL)	24672	24868	25327	22154	18387	23081.60±1295.07
UNINFECTED CONTROL	-	-	-	-	-	-

APPENDIX 3

DETAILED RESULTS OF THE BEDDING EXPERIMENT

WORMLOAD

TYPES OF MOUSE BEDDING	REPLICATES 1	2	3	4	5	MEAN ± SE	PERCENTAGE WORMLOAD
SHREDDED PAPER	29	33	40	26	24	30.40 ± 2.83	15.20
SAWDUST	23	20	24	19	21	21.40 ± 0.92	10.70
UNINFECTED CONTROL	-	-	-	-	-	-	-

PACKED CELL VOLUME (PCV, %)

TYPES OF MOUSE BEDDING	REPLICATES 1	2	3	4	5	MEAN ± SE
SHREDDED PAPER	32.50	32	30	32.50	31.50	31.70 ± 0.46
SAWDUST	33	33.50	34.50	33	34.50	33.70 ± 0.34
UNINFECTED CONTROL	57.50	57	56.50	56	57.50	56.90 ± 0.29

HAEMOGLOBIN CONCENTRATION (Hb, g/100ml)

TYPES OF MOUSE BEDDING	REPLICATES 1	2	3	4	5	MEAN ± SE
SHREDDED PAPER	7.90	7.00	8.20	7.40	8.20	7.74 ± 0.24
SAWDUST	8.40	8.80	8.20	8.20	9	8.52 ± 0.16
UNINFECTED CONTROL	11.50	11.40	11.20	11.40	11.50	11.40 ± 0.05

FAECAL EGG COUNTPER GRAMME

TYPES OF MOUSE BEDDING	REPLICATES 1	2	3	4	5	MEAN ± SE
SHREDDED PAPER	4329	3381	4464	4349	3973	4099.20 ± 197.14
SAWDUST	3322	2280	3308	2338	3196	2888.80 ± 237.47
UNINFECTED CONTROL	-	-	-	-	-	-

TISSUE EGG COUNT

TYPES OF MOUSE BEDDING	REPLICATES 1	2	3	4	5	MEAN ± SE
SHREDDED PAPER	24672	24868	25327	22154	18387	23081.60 ± 1295.07
SAWDUST	19567	18218	16472	17324	16028	17521.80 ± 632.92
UNINFECTED CONTROL	-	-	-	-	-	-

APPENDIX 4

DETAILS OF THE TWO-TAILED STUDENTS T-TEST

EFFECTS OF TEMPERATURE ON INFECTIVITY

WORMLOAD

TREATMENT (°C)	MEAN ± SE	CALCULATED t (t calc)	TABULATED t (t tab(0.05)	
15	4.60 ± 0.40	8.99	2.31	P<0.05
25	30.40 ± 2.83	-	-	-
35	19.00 ± 1.05	3.77	2.31	P<0.05
45	3.60 ± 0.68	9.18	2.31	P<0.05

NOTE: Mean for each treatment was compared with the mean for the 25oC treatment.
df = (n - 1) x 2
= (5 - 1) x 2
df = 8

PACKED CELL VOLUME

TREATMENT (°C)	MEAN ± SE	CALCULATED t (t calc)	TABULATED t (t tab(0.05)	
5	56.70 ± 0.20	0.56	2.31	P>0.05
15	44.80 ± 0.58	18.56	2.31	P<0.05
25	31.70 ± 0.46	46.00	2.31	P<0.05
35	34.40 ± 0.91	23.46	2.31	P<0.05
45	49.10 ± 1.70	4.53	2.31	P<0.05
Control	56.90 ± 0.29	-	-	-

NOTE: Mean for each treatment was compared with the mean for the control
df = 8

HAEMOGLOBIN CONCENTRATION

Treatment ^{0}C	Mean ±SE	Calculated t (tcalc)	Tabulated t (ttab(0.05)	
5	11.32±0.07	0.67	2.31	P)0.05
15	10.60±0.19	4.10	2.31	P(0.05
25	7.74±0.24	15.18	2.31	P(0.05
35	9.06±0.08	22.72	2.31	P(0.05
45	10.68±0.16	3.08	2.31	P(0.05
Control	11.40±0.05	-	-	-

Note: Mean for each treatment was compared with the mean for the control. df = 8

TISSUE EGG COUNT

Treatment ^{0}C	Mean ±SE	Calculated t (t calc)	Tabulated t (t tab (0.05)	
15	4831±5678.74	12.84	2.31	P(0.05
25	23081.60±1295.07	-	-	-
35	16645.00±1381.92	3.41	2.31	P(0.05
45	3314.60±811.41	12.91	2.31	P(0.05

Note: Mean for each treatment was compared with the mean for $25^{0}C$ treatment. df=8

FAECAL EGG COUNT

TREATMENT (°C)	MEAN + SE	CALCULATED t (t calc)	TABULATED t (t TAB (0.05)	
15	325.00 + 42.91	18.67	2.31	P<0.05
25	4099.20 + 197.14	-	-	-
35	2737.20 + 257.24	4.19	2.31	P<0.05
45	267.60 + 39.08	19.03	2.31	P<0.05

NOTE: The Mean for each treatment was compared with the mean for the 25 °C treatment.

: df = 8

COLD STORAGE EXPERIMENT

WORMLOAD

DURATION (HOURS) OF STORAGE	MEAN ± SE	CALCULATED t (t calc)	TABULATED t (t TAB(0.05)	
3	14.80 ± 1.28	5.01	2.31	P<0.05
6	8.60 ± 0.51	7.56	2.31	P<0.05
12	4.40 ± 0.25	9.12	2.31	P<0.05
24	0.80 ± 0.58	10.21	2.31	P<0.05
0	30.40 ± 2.83	-		

NOTE: Mean for each treatment was compared with the mean for the 0 hours (infected control)

: df = 8

PACKED CELL VOLUME

DURATION (Hours) OF STORAGE	MEAN ± SE	CALCULATED t (t calc)	TABULATED t (t tab(0.05)	
3	37.40 ± 1.57	12.16	2.31	P<0.05
6	43.10 ± 0.43	26.55	2.31	P<0.05
12	49.30 ± 0.34	16.99	2.31	P<0.05
24	56.60 ± 0.29	0.73	2.31	P>0.05
UNINFECTED CONTROL	56.90 ± 0.29			

NOTE: The Mean for each treatment was compared with mean for uninfected control.
: df = 8

HAEMOGLOBIN CONCENTRATION

DURATION (Hours) OF STORAGE	MEAN ± SE	CALCULATED t (t calc)	TABULATED t (t tab(0.05)	
3	8.38 ± 0.13	19.06	2.31	P<0.05
6	9.16 ± 0.35	6.09	2.31	P<0.05
12	10.50 ± 0.16	4.70	2.31	P<0.05
24	11.64 ± 0.08	2.05	2.31	P>0.05
UNINFECTED CONTROL	11.32 ± 0.07	-	-	-

NOTE: Mean for each treatment was compared with the mean for the (uninfected control)
: df = 8

FAECAL EGG COUNT

DURATION (Hours) OF STORAGE	MEAN ± SE	CALCULATED t (t calc)	TABULATED t (t tab(0.05)	
3	1973.20 ± 445.90	4.35	2.31	P<0.05
6	1049.00 ± 72.88	14.49	2.31	P<0.05
12	387.40 ± 79.75	17.42	2.31	P<0.05
24	-	-		-
0	4099.20 ± 197.14	-		-

NOTE: The Mean for each treatment was compared with mean for uninfected control.
df = 8

TISSUE EGG COUNT

DURATION (Hours) OF STORAGE	MEAN ± SE	CALCULATED T (TCALC)	TABULATED T (T TAB(0.05)	
3	13514.40 ± 607.3	6.68	2.31	P<0.05
6	4405.80 ± 254.86	14.12	2.31	P<0.05
12	2637.80 ± 358.01	15.19	2.31	P<0.05
24	-	-	-	-
0	23081.60±1295.07	-	-	-

NOTE: Mean for each treatment was compared with the mean for the infected control (Zero hours)
df = 8

BEDDING EXPERIMENT

WORMLOAD

BEDDING	MEAN ± SE	CALCULATED t (t calc)	TABULATED t (t tab(0.05)	
SHREDDED PAPER	30.40 ± 2.83	3.01	2.31	P<0.05
SAWDUST	21.40 ± 0.92	-	-	

NOTE: Mean for Shredded paper was compared with mean for sawdust
df = 8

PACKED CELL VOLUME

BEDDING	MEAN ± SE	CALCULATED t (t calc)	TABULATED t (t tab(0.05)	
SHREDDED PAPER	31.70 ± 0.46	3.48	2.31	P<0.05
SAWDUST	33.70 ± 0.34	-	-	

NOTE: Mean for Shredded paper was compared with mean for sawdust
df = 8

HAEMOGLOBIN CONCENTRATION

BEDDING	MEAN ± SE	CALCULATED t (t calc)	TABULATED t (t tab(0.05)	
SHREDDED PAPER	7.74 ± 0.24	2.72	2.31	P<0.05
SAWDUST	8.52 ± 0.16	-	-	-

NOTE: Mean for Shredded paper was compared with mean for sawdust
df = 8

FAECAL EGG COUNT

Bedding	Mean ± SE	Calculated t (t calc	Tabulated t t tab(0.05)	
Shredded paper	4099.20 ± 197.14	3.51	2.31	P<0.05
Saw dust	2000.60 ±237.47	-	-	-

Note: Mean for shredded paper was compared with mean for sawdust. df = 8

TISSUE EGG COUNT

Bedding	Mean ± SE	Calculated t (t calc)	Tabulated t t tab(0.05)	
Shredded paper	17501.00 ±632.92	3.85	3.21	P<0.05
Sawdust	23041.60 ± 1295.07	-	-	-

Note: Mean for shredded paper was compared with mean for sawdust. df = 8.