

**A SURVEY OF THE PREVALENCE OF *SCHISTOSOMA BOVIS*  
AND ITS POSSIBLE PUBLIC HEALTH IMPORTANCE IN KWALE DISTRICT  
KENYA**



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## DECLARATION

I hereby declare that this thesis is my original work and has not been submitted for the award of a degree in any other University.

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## DEDICATION

This work is dedicated to my family, first to my dear husband John Kamanja, who was both a father and a mother to our children while I was away, our daughter Prudence Njeri and son Kelvin Ngure who allowed me to be absent from home to undertake this programme and to their aunts Grace Wanjiru and Faith Wakarima for being so kind and understanding throughout the period I was away.

To God be all the glory for the great things He has done

## ABSTRACT

Schistosomiasis is caused by trematodes of the genus *Schistosoma* which are widespread in Africa and affect both man and livestock. *Schistosoma bovis* is usually a livestock parasite but may occasionally parasitise humans. Studies were carried out to determine the prevalence of *S. bovis* in cattle and its possible public health importance in Kwale District between the months of March and September 2005.

The study was done by carrying out abattoir surveys where the mesenteric veins of the carcasses were visually examined for the presence of adult *S. bovis* worms. Three abattoirs were visited which included Ngombeni and Kwale slaughter houses in Matuga division and Mwambungo slaughter house in Msabweni division. Rectal faecal sample analysis for identification of *S. bovis* eggs was carried out using the sedimentation technique. A total of 492 cattle faecal samples from various divisions in the district were examined.

Snail sampling was carried out in various water bodies using the scooping method. In seasonal rivers, and during the dry season, snails were recovered by digging in riverbeds. The snails that were recovered were put in 24-well microtitre plates and put under the shade for at least two hours to induce shedding of cercariae from the snails.

Human stool samples from school children from Matuga Msabweni and Kinango divisions were analyzed for *S. bovis* eggs using the Kato Katz technique and urine samples were analyzed using the filtration technique. Records at Kwale KEMRI Laboratory were examined for any recorded cases of *S. bovis* eggs in the last two years. To confirm the identity of *S. bovis* adult worms, a polymerase chain reaction (PCR) technique was used on selected *S. bovis* adult worms from slaughtered cattle. A one way analysis of variance (ANOVA) was used to compare the difference in frequencies of the number of animals infected for the different sexes and age groups of the sampled animals and to check for any

significance of the differences in frequency of *S. bovis*. A graphical presentation was used to compare frequencies of *S. bovis* over the study period.

Rectal faecal sample analysis gave a point prevalence of *S. bovis* eggs as 16.9%. The age group of less than 3 years and the age group of above 6 years gave a prevalence of 18.1% and 21.1%, respectively and the difference in prevalence was not statistically significant at 95 % confidence interval. The point prevalence of *S. bovis* adult worms by abattoir survey was 25.1%. The incidence of *S. bovis* worms over the study period followed the rainfall pattern and was highest in May, but lowest in September 2005.

Snails of the genus *Bulinus*, the intermediate hosts of *S. bovis* were recovered from the various water bodies in Kwale district. The snail population followed the rainfall pattern being abundant during the wet months compared to the dry months. Cercarial shedding in the microtiter plates were only recorded in September.

No *S. bovis* eggs were recovered from the stool samples from school children but other helminth parasites recovered included *Trichuris trichuria* and *Ascaris lumbricoides* while, eggs of *S. haematobium* were recovered from three urine samples. Examination of hospital records did not reveal any record of *S. bovis* eggs over the previous two years but other parasites recorded over the same period were *Plasmodium falciparum*, *S. haematobium*, *T. trichuria* and *A. lumbricoides*.

*Schistosoma bovis* is prevalent in Kwale district as the polymerase chain reaction (PCR) confirmed that the adult worms recovered from slaughtered cattle were *S. bovis*.

The snail intermediate hosts for *S. bovis* were present in Kwale District and the population followed a rainfall pattern. Ova that resembled *S. haematobium* in shape but bigger than *S. bovis* and *S. haematobium* in size were recovered from some rectal faecal samples. Further investigations may be necessary to confirm their identity.

## CHAPTER ONE

### 1.0 Introduction

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

Ruminant *Schistosoma bovis* is widespread in Africa, the Mediterranean and the Middle East (Dinnik and Dinnik, 1965; Johansen, 1994). In some countries such as the Sudan, the prevalence of *S. bovis* infection in cattle in the enzootic areas may be high, ranging between 37.2% and 90.8% (Dinnik and Dinnik 1965), and being highest in 18 month old cattle (Majid, *et. al.*, 1980). Although cattle naturally infected with *S. bovis* do acquire immunity as manifested by a decline in prevalence and intensity of infection with increase in age (Majid *et. al.*, 1980), this resistance may be achieved at the expense of considerable morbidity and mortality in the young calves (Hussein, 1980). *Schistosoma bovis* infection may remain as a long standing chronic infection, with low morbidity but causing continuous ill health and low productivity in affected herd (Makundi *et. al.*, 1998).

Schistosomes are important parasites because of their medical and veterinary importance. Domestic livestock have been reported to harbour natural infections of medically important schistosomes including *S. bovis* (Rollinson and Southgate, 1987). Although *S. bovis* is primarily a ruminant schistosome it has been isolated from human stool (Raper, 1951; Kisner *et. al.*, 1953; Chungu *et. al.*, 1986). This however, may not necessarily suggest human infection with *S. bovis* but could be a case of spurious infection (Kinoti *et. al.*, 1988).

Schistosomes use fresh water snails as intermediate hosts and hence creation of large man made dams for hydro-electric power generation and establishment of irrigation schemes for farming will increase habitats for aquatic snails and risk of schistosomosis for both livestock and humans. An example is Mwea Rice Irrigation Scheme where high prevalence of *S. mansoni* has been reported since the initiation of the irrigation project (Doumenge *et. al.*, 1987). Kwale District in Coast Province has low lying areas and seasonal rivers which get flooded during heavy rains but stagnant water masses persist during the dry season. Damming activities in these areas create conditions favourable for habitation by fresh water snails of both veterinary and medical importance. These water systems are used by humans and animals making conditions conducive for occurrence of snail borne diseases like schistosomosis Anonymous (2000).

*Schistosoma haematobium* which is in the same species group as *S. bovis* is also common in Kwale district (Mkoji *et. al.*, 1999). Fresh water snails of the genus *Bulinus*, the intermediate hosts for schistosomes species in the *S. haematobium* group are present in this area (Highton, 1974). These predispose to the presence of *S. bovis*. Although extensive studies have been undertaken on *S. haematobium* in the human population in Kwale, very little is known about *S. bovis* in the area.

Schistosomosis is a major health problem in livestock and man due to production losses occasioned by ill health, mortality, stunted growth in livestock and reduced output in man (McCauley *et. al.*, 1984). There is need therefore to determine the prevalence of *S. bovis* in Kwale District in order to be able to institute control measures. Knowledge of the parasite species present, herd structure, grazing management and climatic conditions will assist in the implementation of sustainable control programmes (Nansen, 1991).

## 1.1 Hypotheses

This study was carried out under the following hypotheses

- 1) That *S. bovis* is prevalent in cattle in Kwale District.
- 2) That infected snail intermediate hosts are prevalent in Kwale district.
- 3) That *S. bovis* is of public health importance in Kwale District.

## 1.2 Objectives

These hypotheses were tested with the aim of achieving the following objectives.

- 1) To determine the prevalence of *S. bovis* in cattle in Kwale District, coastal Kenya.
- 2) To determine the presence and relative abundance of the intermediate snail hosts in selected watering points.
- 3) To determine the public health significance of *S. bovis* in the study area.

### 1.3 Significance of the study

Cattle play an important role in the agricultural sector contributing 20% of the total production. Demand for milk and meat is estimated to exceed production by approximately 32% and 23% respectively (Anonymous, 1981). With the rapid increase in human population it is becoming increasingly important to maximize agricultural production through improved management practices and control of production limiting diseases such as helminthosis in cattle.

The rapid increase in human population also puts a strain on social and sanitary facilities. This results in people and livestock gathering at water catchment areas providing an ideal environment for proliferation of intermediate hosts and maintenance of water borne zoonotic diseases like schistosomiasis caused by *S. bovis*. *Schistosoma bovis* is occasionally a parasite of man and *S. matthei* together with *S. incognitum* may be infective to man. Although the animal schistosomes are of relatively little importance as direct pathogens in man, they could possibly confer some degree of heterologous immunity against *S. mansoni* or *S. haematobium* when they penetrate human skin (Rollinson and Southgate 1987). *Schistosoma matthei* has been reported to hybridise with *S. haematobium* and there may be a possibility of *S. bovis* hybridising with *S. haematobium*.

The present study aimed at establishing the prevalence of bovine schistosomes in Kwale District and its possible presence in the human population in the area. This information may be used to formulate intervention measures with the aim of improving cattle productivity and improve poverty reduction measures in the area. This may also enable formulation of other additional intervention measures to reduce both livestock and human infestation with schistosomes.

## CHAPTER TWO

### 2.0 LITERATURE REVIEW

#### 2.1 *Schistosoma* infections in cattle and man

Flukes of the genus *Schistosoma*, the adult stages of which are found in the vesical plexus of the hepatic portal system, cause schistosomiasis which is one of the most important helminthic diseases of man (Soulsby, 1982). The parasites were first described by a German pathologist who found the adult worms in the mesenteric veins of an Egyptian man in Cairo during a post mortem. Several species have since been described affecting man and livestock (Rollinson and Southgate, 1987).

Five species of schistosomes are pathogenic to man. Of these, the most important are *S. mansoni*, *S. haematobium* and *S. japonicum* (Rollinson and Southgate, 1987). *Schistosoma mekongi* and *S. intercalatum* which also infect humans tend to have a more limited distribution. Animal schistosomes such as *S. matthei*, *S. incognitum* and *S. bovis*, do occasionally also infect humans (Rollinson and Southgate 1987). *Schistosoma bovis*, *S. haematobium* and *S. matthei* are closely related phylogenetically and belong to the haematobium group complex which utilizes snails in the genus *Bulinus* as intermediate hosts (Rollinson and Southgate 1987). *Schistosoma matthei* is primarily of veterinary importance, being found commonly in cattle, sheep and goats in Southern Africa; the definitive host range is not however restricted and this parasite can infect man and hybridisation with *S. haematobium* occurs (Rollinson and Southgate 1987). *Schistosoma bovis* was first described from cattle in Egypt and has been described in both domestic animals and man (Chunge, *et al.*, 1986) and has also been observed in human stool in Uganda (Raper, 1951), South Africa (Kisner *et al.*, 1953), Zimbabwe (Blair, 1966) Kenya (Chunge *et al.*, 1986) and the Republic of Niger (Mouchet *et al.*, 1988). Kinoti, *et al.*, (1988) have suggested that *S. bovis* infections



reported in humans may have been as a result of spurious infections acquired possibly through the eating of meat from *S. bovis* infected bovines.

Information about the snail hosts of *S. bovis* in East Africa exists (Brown, 1994), and although the parasite exclusively utilizes some *Bulinus* species in East Africa as intermediate hosts it shares several host species with the human pathogen *S. haematobium*. At a global level, the snail host of *S. bovis* include *Bulinus forskali*, *B. africanus*, *B. truncatus*, *B. globosus*, *B. nuxalus* and *Planorbharius metidjensis* (Southgate and Knowles 1975, Brown, 1994). Within the East African region *S. bovis* is transmitted by the *Bulinus forskalii* groups species, the *Bulinus africanus* group species, and the *Bulinus truncatus* group species (Brown, 1994).

## 2.2 Distribution of schistosomes

Human schistosomiasis is known to be endemic in 76 countries of the world and it is estimated that 200 million people are infected and up to 600 million exposed to infection because of poverty, ignorance, poor housing, sub standard hygiene practices and inadequate sanitary facilities (WHO, 1985). In Kenya it occurs in the coastal area, parts of central Kenya, within the Athi River basin and in western Kenya around Lake Victoria (Barber *et al.*, 2000).

Schistosomiasis is one of the most widespread parasitic diseases, ranking second only to malaria in terms of its socio-economic and public health importance in tropical and sub tropical areas (WHO, 1990). It is also the most prevalent of the water borne diseases and one of the greatest risks to health in rural areas of developing countries (WHO, 1990). Both *S. mansoni* and *S. haematobium* are endemic in Kenya. In the 1960s one million persons were estimated to be infected but with increasing human population and water resource development the figure has risen to 3 million (Dnumenge *et al.*, 1987).

*Schistosoma bovis* has been reported as an immunological analogue of *S. haematobium* (Agnew *et al.*, 1989, Pardo *et al.*, 2004) and *S. haematobium* has been reported in the South African buffalo (Bason and Kruger, 1970) therefore it is possible that *S. haematobium* could infect cattle which belong to the bovidae family as the buffalo.

### 2.3 Life cycle of *Schistosoma bovis*

The life cycle of all schistosomes is complex and involves a vertebrate host as the definitive host and a snail intermediate host (Figure 1). The snail intermediate hosts of *S. bovis* in East Africa include the *Bulinus africanus* group species primarily (*B. africanus*, *B. globosus*, *B. nasutus*), *B. joriskali*, *B. truncatus* and under some circumstances *B. tropicus*. The life cycle involves the egg, miracidium, first and second sporocysts, cercaria, schistosomulum and the adult worm. The ovigerous female usually in the gynaecophoric canal of the male where copulation takes place, leaves the canal and penetrates deeply into the small vessels of the mucosa or sub mucosa of the intestine, laying eggs in the capillaries. From here the eggs pass through the intestinal wall into the lumen and outside in the faeces. When laid the eggs are fully embryonated and they continue their development as they pass out in the faeces (Soulsby, 1982).

Eggs hatch to miracidia after contact and dilution of the faeces with water while agitation ensures optimal hatching. The miracidium swims ceaselessly during its short life of 5-6 hours. When they swim in the vicinity of a suitable host they are stimulated to swim more rapidly and change direction more frequently thus increasing the chance of encountering the snail host. Miracidium infect aquatic snails of the *Bulinus* species where it penetrates the tissues of the snail shedding its epithelium and begins to develop into a mother sporocyst near its point of entry. After about 2 weeks the mother sporocyst gives rise to the daughter

sporocysts which usually migrate to other organs of the snail and continues producing daughter sporocysts for up to 6 or 7 weeks.

Cercariae with forked tails are developed in the daughter sporocyst and they start to emerge from the snail host about 4 weeks after the initial penetration by the miracidium. The emerging cercariae swim to the surface of the water and slowly sink to the bottom and live for 1-3 days. Development of cercariae within the snail host is dependent on temperature and time of the year. The infection is common in man and animals where the rainfall is highest and there is a marked increase in incidence with increasing water conservation. If the cercariae come into contact with the skin or the rumen of a suitable host they attach to it and penetrate the epidermis assisted by the cephalic gland secretions. The tail of the cercaria is cast off and penetration can be accomplished in 10-30 seconds but generally in less than half an hour.

The cercaria transform into a small elongated body known as the schistosomulum, which within 24 hours enters the peripheral circulation or the lymphatic system and are carried via the heart to the lungs within 4 to 7 days. They are then carried to the liver via blood and from 8 days onwards, schistosomulac are found in the portal vessels of the liver. Pairing of the worms occur in the portal veins before they reach maturity in the mesenteric veins. Cattle and humans may become infected when standing in shallow waters through the skin or orally when drinking water that is infested with the snail intermediate hosts and contaminated with faecal material from infected animals and man.

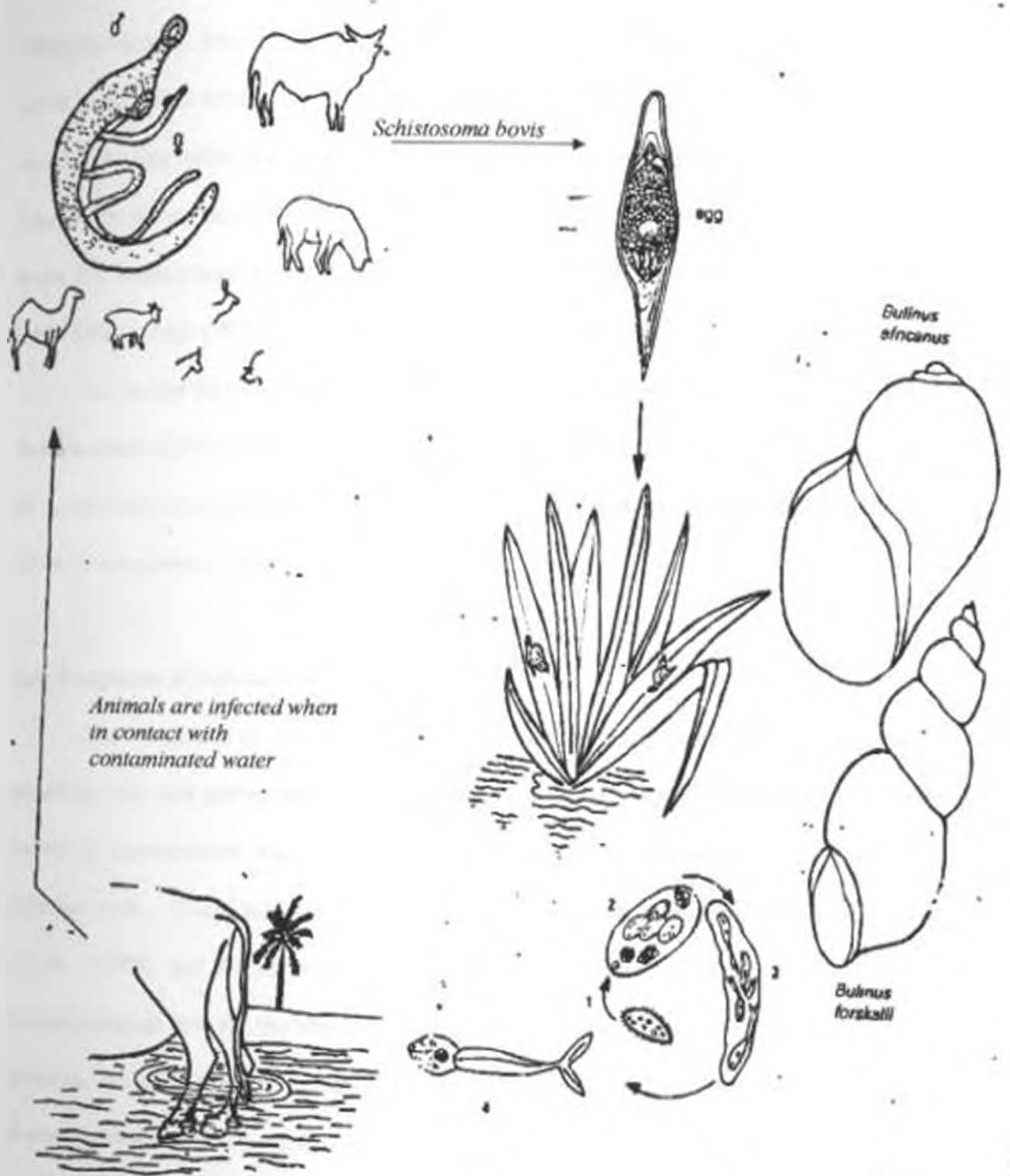


Figure 1. Life cycle of *S. bovis* adapted from Tropical Veterinary Parasitology p 88

## 2.4 Importance of *Schistosoma bovis* in cattle

A study carried out on *S. bovis* infections showed that animals that had died or were slaughtered after falling sick had high faecal egg counts, were emaciated, had diarrhoea and were weak. Sick animals were usually weak and could not cope with adverse conditions such as drought, starvation and trekking long distances to livestock markets (Makundi *et al.*, 1998). Studies in Sudan showed that cattle severely infected with *S. bovis* were so weak that they were left behind near homesteads with calves, as healthy animals left for grazing to distant areas (McCauley, 1983).

In studies based on farms/villages in Iringa in Tanzania, Makundi *et al.* (1998) found that ruminant schistosomiasis may be of great economic importance due to its association with ill health, loss of condition and sometimes death. This is especially so due to the focal nature of the transmission (Makundi *et al.*, 1998).

## 2.5 Diagnosis of *Schistosoma* species infections

The diagnosis of schistosome infections can be made from clinical, parasitological, physiological and histopathological findings (Saad *et al.*, 1984). Differentiation between *S. bovis*, *S. haematobium* and *S. mattheei* can be made using polymerase chain reaction (PCR) (Barber *et al.*, 2000) followed by digestion of the PCR product and electrophoresis. Agnew *et al.* (1989) and Pardo *et al.* (2004) also found out that *S. bovis* can be used as an immunological tool for the diagnosis of *S. haematobium* especially on imported cases of the disease, while Barber *et al.* (2000) showed the close association between *S. bovis* and *S. haematobium* and that these two species can be differentiated by using the PCR.

### 2.5.1 Clinical findings

The clinical signs depend on the number of infective cercariae, number of worms reaching maturity and the pathology caused. These signs include poor body condition, diarrhoea, inappetance and marked anaemia. However, these signs can be a manifestation of other parasitic infections or nutritional disorders and laboratory diagnosis is always encouraged (Saad *et al.*, 1984).

### 2.5.2 Faecal examination

Egg morphology for species identification has been emphasized in most situations except where hybridisation occurs as eggs can be obtained readily from faecal samples. The size and shape of the egg, the egg-laying site and the geographical area of the host are useful clues to the identity of the parasite. Difficulties in identification arise when distinctive morphological characters are lacking as for example *S. haematobium* and *S. curassoni* which share similar egg morphologies or when species overlap in their distribution, as in many parts of Africa; it is necessary to employ further reliable methods of characterisation (Rollinson and Southgate 1987).

Bovine faeces can be screened for schistosome eggs by a thick smear technique as described by Kato *et al.* (1972). This technique has been evaluated by Idris and Jabril, (2001). The filtration technique (Kassuku *et al.*, 1985) and the sedimentation technique (Hansen and Perry 1994) can also be used.

#### 2.5.2.1 The Kato-Katz technique

In this technique a small amount of faecal material is placed on a newspaper or scrap paper and a piece of nylon screen is pressed on top so that some of the faeces sieve on top and

accumulate on the nylon cloth. A flat sided spatula is used to scrap across the upper surface on the screen to collect the sieved faeces. A template is placed on the slide and the sieved faeces are added with a spatula so that the hole in the template is completely filled with about 49 mg of faeces. The spatula is passed over the filled template to remove excess faeces from the edge of the hole and the template is removed carefully so that a cylinder of faeces is left on the slide. The faecal material is covered with a glycerol pre-soaked cellophane strip with Malachite green stain used to clear the background surrounding the schistosome eggs.

The slide is inverted and the faecal sample pressed firmly against the hydrophilic cellophane strip to spread evenly. The slide is placed on the bench with cellophane upwards to enable evaporation of water while glycerol clears the faeces. The slide is then kept for one or two hours at room temperature to clear the faecal material prior to microscopic examination (Idris and Jabril; 2001).

#### **2.5.2.2 Sedimentation technique**

Trematode eggs have high specific gravities and therefore the eggs do not float in common floatation fluids, but they may be concentrated by sedimentation. The technique is a combination of washing and sieving of faeces to remove the smallest and the largest faecal particles. This technique utilises the high gravity of the eggs, which facilitates their sedimentation in beakers with steeply sloping sides and the method is qualitative not quantitative. Faecal samples are preserved by adding 1% formalin while being transported from the field (Hansen and Perry, 1994).

#### **2.5.3 Other methods of diagnosis**

Other methods of diagnosing schistosomosis include intradermal and complement fixation test (Rifaat and Khalil, 1965), enzyme studies where electrophoretic techniques,

commonly starch gel or isoelectric focusing have been employed to the study of schistosomes and have had an effect in their characterisation into species and strains (Rollinson and Southgate 1987). cloned ribosomal RNA gene probes which can differentiate between *S. haematobium*, *S. magrehowiei* and *S. mattheei*. Deoxyribonucleic acid (DNA) from the egg, cercariae and miracidium can be employed in this technique (Walker *et al.*, 1986). Use of cercarial papilla indices (Dufour – Baysaade, 1989), recovery of schistosome eggs retained in tissues can also be made at post mortem. This gives an indication of intensity of infection although the count is not necessarily directly proportional to the adult worm load (Sturrock *et al.*, 1976).

Clinical pathological findings which include hypoalbuminaemia, hyperglobulinaemia hypoproteinaemia and eosinophilia (Saad *et al.*, 1984) may also be used to augment the diagnosis.

### 2.5.3.1 Molecular diagnostic techniques

*Schistosoma haematobium* and the closely related *S. bovis* are sympatric in many African countries such Kenya. Since these two species can inhabit the same *Bulinus* snails, maybe found in the same fresh water habitat and have morphologically similar cercariae better means are needed to differentiate them (Barber *et al.*, 2000).

Epidemiological studies aimed at identifying transmission foci of *S. haematobium* or *S. bovis* are complicated by the fact that cercariae of both of these widely distributed species are produced in bulinid snails and are difficult to distinguish. When using a procedure where hamsters are exposed to cercariae to determine the species identity of the parasites, it takes up to 3 months to determine if the bulinid transmitted schistosomes in a particular water body are human or ruminant parasites, or if both species are represented (Barber *et al.*, 2000).



The second internal transcribed spacer (ITS2) region of the ribosomal gene complex from various schistosomes together with the restriction fragment length polymorphism (RFLP) assay can be used to differentiate species specific cercariae or other life cycle stages in a basic molecular biology laboratory within a day. This can be done by using in turn a 4 hr single PCR amplification, a 1 hr agarose gel electrophoresis to confirm presence of an ITS2 band, and then a 4 hr restriction enzyme (RE) digest with either *Sau3A1* or *Taq I*, followed by a one hr 1.5% agarose gel electrophoresis and visualisation using ethidium bromide staining. By helping to differentiate the schistosome parasite present in a certain water body, the analysis will aid in disease control and in on going epidemiological studies (Barber *et al.*, 2000).

#### 2.5.3.1.1 Schistosome DNA extraction

Genomic DNA can be extracted using the method described by Truett *et al.* (2000). Briefly adult worms preserved in 70% ethanol are soaked in double distilled water for at least one and a half hours before initiating the DNA extraction process. An alkaline lysis reagent containing 25 mM NaOH, 0.2 M disodium ethylenediaminetetraacetic acid (EDTA) at a pH 12 is prepared by dissolving the salts in water without adjusting the pH. A neutralising reagent made up of 40 mM Tris-HCl, at pH 5 is also prepared by dissolving Tris-HCl, in water without adjusting the pH.

Individual worms soaked in distilled water are each placed in a 0.2 ml PCR tube using a fine pair of forceps. A 75 microlitre volume of the alkaline lysis reagent is then added into each tube and the samples heated to 95 °C for one hour with occasional vortexing every 30 minutes to break the worms and after one hour heating, the sample is then cooled to 4 °C and 75 microlitre of the neutralising reagent added into each tube. Thirteen microlitre of the DNA extract is then used in a PCR reaction in a final volume of 50 microlitres (Barber *et al.*, 2000).

### 2.5.3.1.2 Restriction fragment length polymorphisms

Whenever specific closed DNA probe is used to analyse a southern blot of other DNA, a limited number of restriction fragments of specific and characteristic lengths are identified. Because single base mutations can either create additional sites or destroy existing sites, DNA preparations from different individuals frequently exhibit different patterns of size distribution of restriction fragments that hybridise with a particular probe. These differences are called restriction fragment length polymorphisms (RFLP). Restriction fragment length polymorphism analysis has been used widely to detect variation at the restriction sequence (Botstein *et al.*, 1980). The usefulness of RFLP in identifying species has recently been demonstrated (Barber *et al.*, 2000; Rollingson and Southgate, 1987). More importantly however, is the relatively high cost and technically demanding nature of this technique (Jonathan *et al.*, 1998).

### 2.5.3.1.3 Electrophoresis

Two (2) gms agarose powder is weighed and put in a conical flask. This is then mixed with 2 g TAE buffer and 98 ml of distilled water is added. The mixture is then incubated for 2 minutes and brought to a boil. After cooling 0.3 microlitre of Ethidium bromide is added and this is used to make a 2% agarose gel. Ethidium bromide enables proper viewing of the electrophotogram. The cooled mixture is poured into a gel chamber and left for twenty minutes to allow the gel to set. A plastic comb is put in the setting gel so as to make wells for the uncut PCR product and the digested RFLP product. Twenty microlitres of the marker dye and twenty microlitres of the electrophoretic material are mixed on a white tile and after the agarose gel is ready it is put in an electrophoresis chamber.

The 100 base pairs molecular marker is loaded into the first well. The uncut PCR product is loaded in the second well. Then the digested RFLP products are loaded into the next wells depending on their number. The uncut PCR product is loaded into the well after the digested PCR products and the last well is loaded with the 100 base pair molecular marker. Each well is loaded using the 20 microlitre pipette and after loading the wells with the various materials a current of 80 volts is passed through the gel for one hour after which a photogram of the gel is taken. The bands clearly show the position of the PCR and RFLP products.

## **2.6 Snail hosts**

The fresh pulmonate snail genus *Bulinus* is divided into four species groups: *B. africanus* group, *B. forskalii* group, *B. reticulatus* group and the *B. truncatus/tropicus* complex (Brown, 1994). Despite limited morphological divergence within species groups, there is considerable molecular divergence (Jones *et al.*, 2001; Stothard *et al.*, 2001). Within the *B. africanus* group ten species are recognised and distributed throughout the Afro-tropical regions and Madagascar.

### **2.6.1 Identification of snails**

Snails can be characterised by three methods: morphometric analysis of shell measurements, enzyme analysis and analysis with random amplified polymorphic DNA (RAPD) (Stothard *et al.*, 1997).

### **2.6.1.1 Morphometric analysis**

The shell shape and measurements are used to characterise snails. This method is important up to the genus level but the reproductive system of the snails is also used to characterise up to the species level although adult snails are required for this identification which makes it a specialised task (Anonymous, 1985).

### **2.6.1.2 Enzyme analysis**

Enzyme electrophoresis has been used for better snail species identification. This method appears better because it provides characteristics that are objective and are independent of shell size and, results can be obtained within a day (Rollinson *et al.*, 1997).

### **2.6.1.3 Molecular analysis**

In this analysis particular emphasis is given to ribosomal RNA genes, random amplified polymorphic DNA and the mitochondrial gene cytochrome oxidase I (COI). These molecular methods allow for differentiation and identification of species and even strains including individual differences within a population. Some snails are resistant to infection and the concerned genes can be identified using molecular techniques. This can help identify snails that play a major role in disease transmission like schistosomiasis (Rollinson *et al.*, 1997).

## **2.6.2 Snail sampling methods**

Several methods can be used to sample snails. These include: direct counting, scooping, drag scooping, dredging, palm leaf traps, bamboo traps and artificially produced attractants (Anonymous, 1985).

### **2.6.2.1 Direct counting**

This involves collection of snails by use of forceps for a specified time over a specified area. This method is limited by the ability of the collector to detect snails in the habitat and the tendency to collect mainly the larger specimens. This method is usually applicable in shallow waters and is suitable on rocky and stony substrates.

### **2.6.2.2 Scooping**

In this method scooping is done for a certain time over a specified section of the habitat. The collected material can be sorted out in the field, but can be brought to the laboratory especially if distributions and egg laying rates are to be determined.

The effectiveness of this method depends to a great extent on individual collector and fluctuations in the water levels. Results can be recorded as the number of snails collected per scoop or the total number of snails collected during a certain period of time.

### **2.6.2.3 Drag-scoop**

This is an instrument suitable for collection in irrigation canals and drains. It is designed to collect all snails within a strip of 25 cm from the centre of the canal to the edge and at the same time it collects a sample of aquatic vegetation which can be used for inspection of egg masses which gives an idea of the egg laying rate. In a modification of the drag-scoop, the lower edge is equipped with a cutting edge in order to make it more suitable in stout vegetation.

#### **2.6.2.4 Dredging**

The dredge is pulled a number of times for a certain specified distance and the number of snails for each dredging is recorded. If the effectiveness of sampling is known, this method may be used to estimate the absolute density of snails.

#### **2.6.2.5 Other traps**

Palm leaf traps can be placed in the habitat directly although some standardisation may be necessary. Bamboo traps are anchored floating horizontally in shallow waters near the margin of the water mass. Artificially produced attractants like extracts of laboratory snail food are added to a matrix and these traps can also be standardised.

### **2.7 Snail control**

The control of schistosomiasis can be achieved by eliminating the snails with three main methods in use which include chemical, biological and environmental methods.

#### **2.7.1 Chemical method**

This method uses poisons to eliminate snails although resistance to the poison may finally develop. Molluscides can be used though they have proved to be toxic to the environment especially in fish ponds and the cost may be too high (Tucker, 1983).

#### **2.7.2 Biological method**

In this method, biological predators of snails such as tilapia and crayfish can be used to predate on them. They do this by competing for food, disturbing them physically or damaging them. The advantage of the method is that snails are reduced over a longer period of time. there is an increased ability of the area to withstand reinfection and the method is cheap in

terms of management while there is no toxic effect to plants and animal life. The disadvantage of this method is that it is slow in the way it acts and the introduction of exotic species may have an adverse effect on the environment especially in fish ponds (Lee *et al.*, 1982).

### 2.7.3 Environmental method

This method involves modifying the environment to make it unsuitable for snail breeding and development. To achieve this waters level can be fluctuated especially in fish ponds and removing any vegetation within or around the water body so that the snails do not get any place for attachment and breeding.

### 2.8 The genus *Bulinus* distribution in Kenya

In Kenya, the genus *Bulinus africanus* group is represented by *B globosus*, *B. africanus*, *B nasutus*, and *B ugandae*. The distribution of *B globosus* and *B nasutus* overlaps in coastal Kenya e.g., Kwale District and in the Lake Victoria area (Kisumu). *Bulinus africanus* is commonly found in higher altitude areas of central and eastern Kenya like Mwea irrigation scheme and Kitui District. *Bulinus ugandae* is distributed in limited foci in the Lake Victoria area (Barber *et al.*, 2000; Kariuki *et al.*, 2004).

## **CHAPTER THREE**

### **3.0 MATERIALS AND METHODS**

#### **3.1 Study area**

Kwale District is one of the seven districts of Coast Province. It borders Taita Taveta District to the west, Kilifi District to the northwest, Mombasa District and Indian Ocean to the east and the Republic of Tanzania to the south. The district is located in the south eastern corner of Kenya lying between latitudes 3°3' and 4°45' south and longitudes 38°31' and 39°31' east. It has an area of 8260 km<sup>2</sup> of which 62 km<sup>2</sup> is under water. The area excludes the 200 mile coastal strip known as the exclusive tourism zone.

The district is divided into 5 administrative divisions namely Matuga, Kubo, Msabweni, Kinango and Samburu (Figure 2). The district has a bimodal type of rainfall. The short rains occur between October and December while the long rains occur between March/April and July. Total precipitation varies from 900 – 1500 mm per annum along the coast to 500 – 600 mm in the hinterland. Average temperatures ranges from 26.3 °C to 26.6 °C in the coastal lowlands and 24.6 °C to 27.5 °C in the hinterland. The study was carried out between March and September 2005.

#### **3.2 Experimental design**

The study locations were purposively selected abattoirs and various watering points and some selected farms (Figures 3 and 4). The farms visited were in close proximity to the water bodies.





Figure 2. The map of Kenya showing the location of Kwale District (arrow)

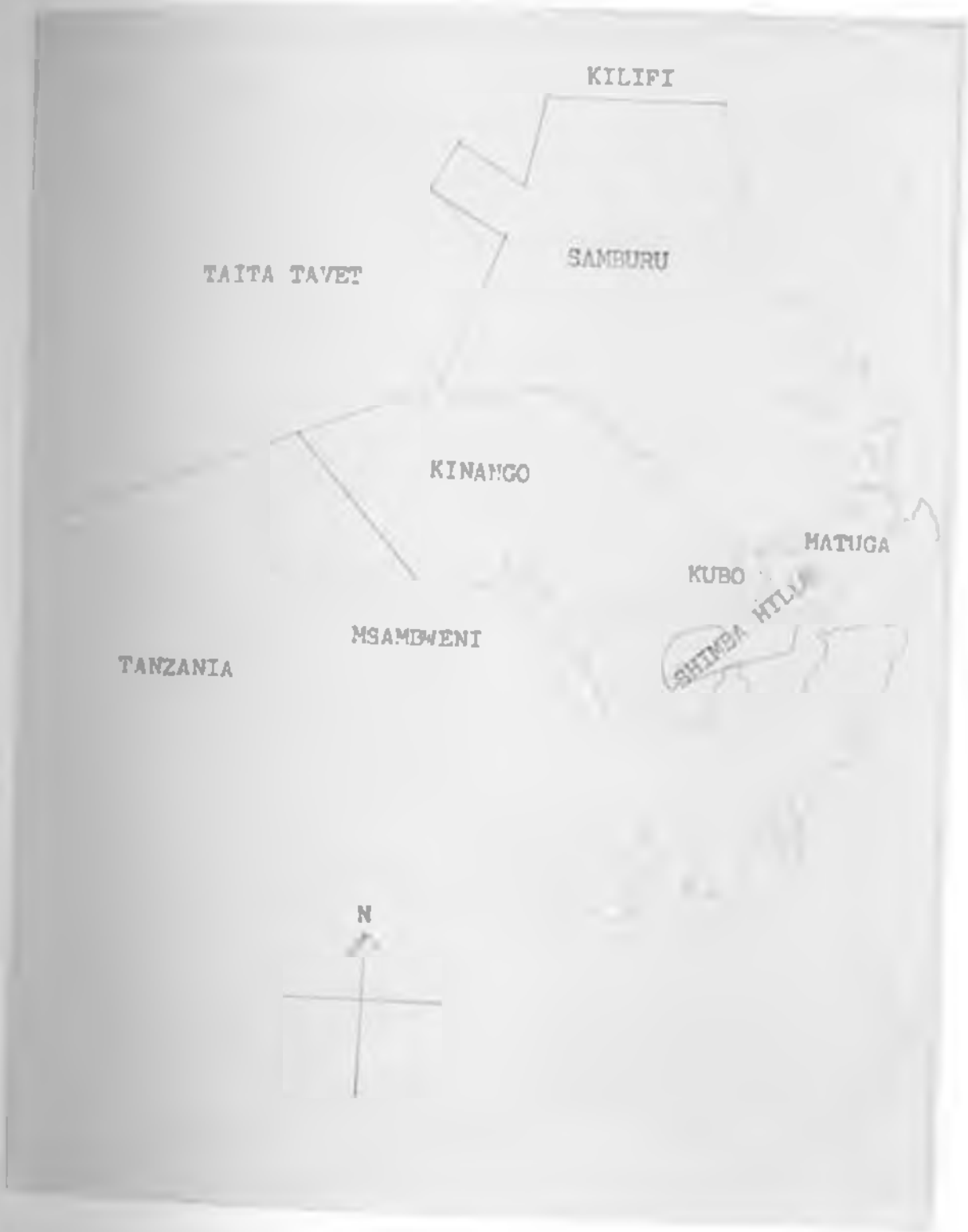


Figure 3. Map of Kwale District showing divisional administrative boundaries



Figure 4. Map of Kwale District showing selected slaughter houses and watering points

### 3.2.1 Collection and examination of cattle faecal samples

Individual rectal faecal samples were collected from herds of cattle in selected farms in Kwale District. These farms were near the various watering points. Each sample was clearly labelled based on animal's age, sex, and date of collection. They were placed in cool boxes and transferred to Kwale KIMRI Laboratory for analysis. Samples were analysed for *S. bovis* eggs using the sedimentation technique (Hansen and Perry 1994). Rectal faecal samples were also taken from slaughtered animals at the abattoir and analysed as above. Any other helminth ova were noted and recorded.

### 3.2.2 Collection of stool and urine samples

Collection and examination of human stool and urine was done under the Eastern & Southern Africa Centre of International Parasite Control (ESACIPAC) school children deworming programme authority. Three schools, one from Msahweni, Matuga and Kinango divisions were visited and standard three pupils with an average age of 9 years were sampled for *S. bovis* and *S. haematobium* eggs.

The school administration was approached and requested to allow stool and urine samples to be taken from standard three pupils. These pupils were chosen because they usually swim in dams, rivers and streams making this age group to be at a higher risk of infection. They were informed that the sample analysis would be free, and the children who were positive for any helminth infection would be given free treatment. The teachers explained to the pupils that the purpose of the exercise was to find out if bovine schistosomosis can be a threat to human health.

The Kwale KEMRI Laboratory staff explained to the children how to get the stool samples. The first long call in the morning was to be sampled and about 50 gm put in a polypot sample container while the urine sample would be put in a different polypot sample container. The Kato-Katz method (Katz, *et al.*, 1972) was used for stool examination and the filtration technique (Bell, 1963) used for urine examination.

### 3.2.3 Examination of laboratory records

Laboratory records at Kwale KEMRI Laboratory were examined for the prevalence of schistosomiasis and other parasites among human patients over the previous two years and during the study period from March to September 2005.

### 3.2.4. Snail sampling studies

Snail sampling studies were carried out at selected animal and human watering points. The sites included Kinango dam, Kandigo stream, Bangoni river, Manola river, Ziwani dam and Zigira dam (Figure 4). The scooping method was used to sample the snails.

Snails that transmit parasites which infect humans and livestock were collected and identified by screening natural populations of snails (Muchemi *et al.*, 2003). Fresh water snails of the genus *Bulinus* were collected and relative populations noted over the study period. Morphological characteristics were used to identify the genus as described by Stothard *et al.* (1997).

### 3.2.5 Abattoir surveys

Sampling was done at selected abattoirs in the district. These included Ngombeni and Kwale in Matuga division and Mwambungo in Msabweni division. Slaughtered animals within

the abattoir were visually examined for the presence of *S. bovis* adult worms in the mesenteric veins. Using the formula  $n = 4p(1-p) / L^2$  where  $n$  is the sample size,  $p$  is the estimated prevalence and  $L$  is the precision a sample size of 336 animals was sampled using an estimated prevalence of 30% and assuming a 95% confidence level.

This number of animals was sampled in the three selected abattoirs where livestock origin and sex were recorded prior to slaughter and a faecal sample was taken. After slaughter, the mesenteric veins were inspected visually for the presence of *S. bovis* worms. The worms were removed and preserved in 70% alcohol for further studies. Polymerase chain reaction (PCR) was used for confirmation of adult *S. bovis* worms. This was done by schistosome DNA extraction followed by its digestion with *sau3A1* enzyme and subsequent agarose gel electrophoresis (Barber *et al.*, 2000).

### 3.3 Data analysis and interpretation

The statistical programme, Instat<sup>®</sup> version 2.51 (Stern *et al.*, 2002) was used for analysing the data. A one way Analysis of Variance (ANOVA) was used to compare the difference in frequencies of the number of animals infected for the different sexes and age groups of the sampled animals and to check for any significance of the differences in frequency of the parasite. A graphical presentation was used to compare frequencies of infections over the study period.

## CHAPTER FOUR

### 4.0 RESULTS

#### 4.1 Rainfall pattern in Kwale District

The rainfall pattern during the study period was much lower than the average rainfall expected (Figure 5). The months of May to July were wet while March/ April and August/ September were dry. The highest rainfall was received in May while March had the lowest. Moderate rainfall was received between July and September.

#### 4.2 Faecal sampling

A total of 492 cattle faecal samples were examined during the study period. Out of these 83 were positive for *S. bovis* which gave a point prevalence of 16.9%. The prevalence varied between the divisions. Kinango division had a prevalence of 14.4%, Msabweni 16.7%, Matuga 30%, Kubo 23.1% and Kwale slaughter house 9.8% (Table 1). The difference in prevalence between the administrative divisions was statistically significant ( $P < 0.05$ ) by division.

The prevalence of *S. bovis* eggs varied with age, the age group below 3 years being 18.1% while the age group between 3 to 6 years had a prevalence of 12.0%. The age group above 6 years had a prevalence of 21.1% (Table 2). However, these differences were not statistically significant ( $P > 0.05$ ).

The prevalence also differed with the sex of the animals. The female animals showed a slightly higher prevalence of (17.4%) than the males which showed a prevalence of (15.3%) (Table 3). However, these were not statistically significant ( $P > 0.05$ ).

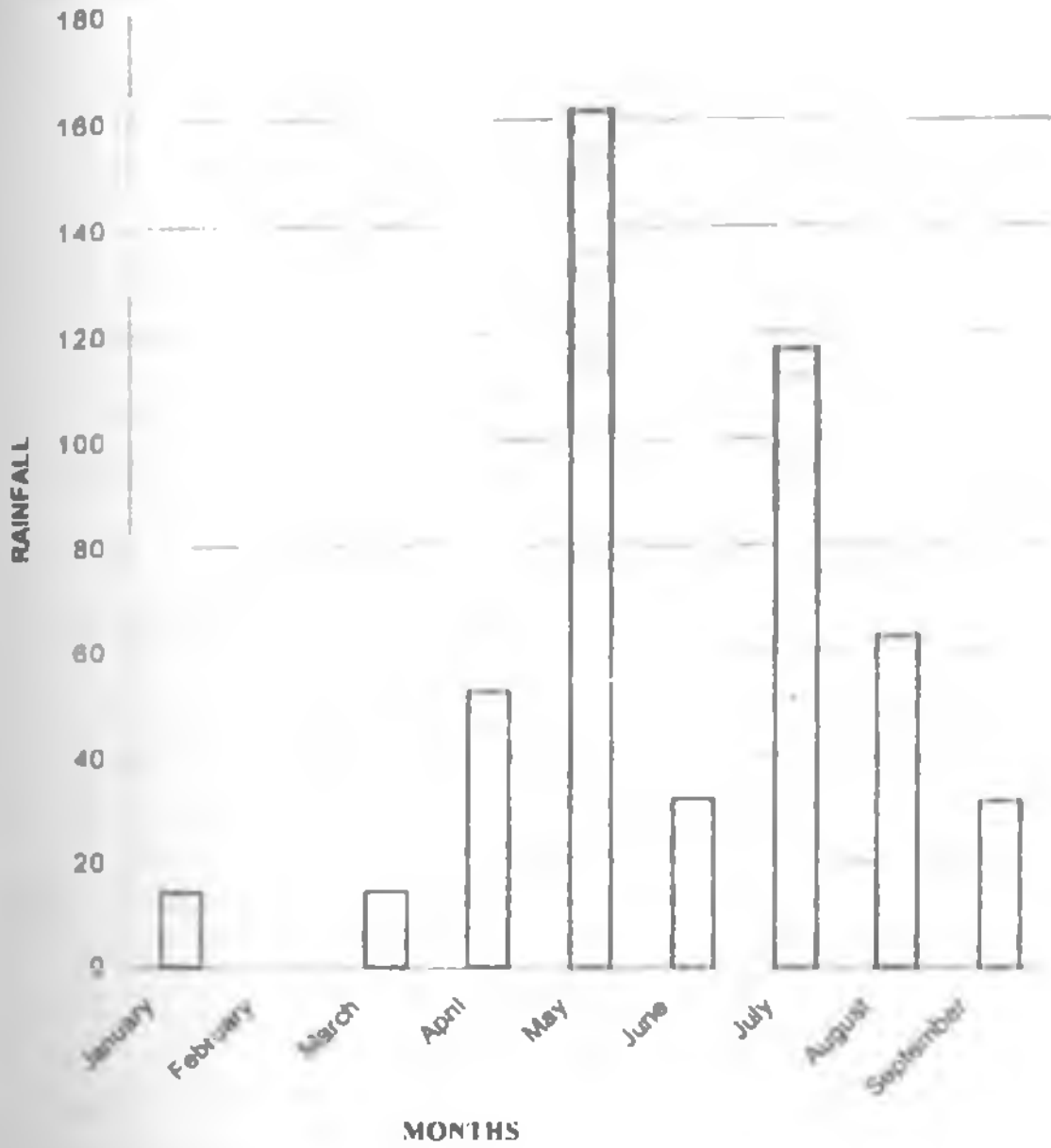


Figure 5. Rainfall pattern in Kwale District over the study period



Table 1. Prevalence of *S. bovis* eggs in faecal samples of cattle from four divisions and one slaughter house in Kwale, coastal Kenya

	<u>No. examined</u>	<u>No. infected</u>	<u>Parasite Prevalence (%)</u>
Division			
Kinango	174	25	14.4
Msabweni	60	10	16.7
Matuga	70	21	30
Kubu	65	15	23.1
Kwale slaughter	123	12	9.8
<hr/>			
Total	492	83	16.9

Table 2. Prevalence of *S. bovis* eggs in cattle faecal samples from Kwale, coastal Kenya

	<u>No. examined</u>	<u>No. infected</u>	<u>Parasite Prevalence (%)</u>
Age			
Less than 3 years	138	25	18.1
Three to six years	183	22	12.0
More than six years	171	36	21.1

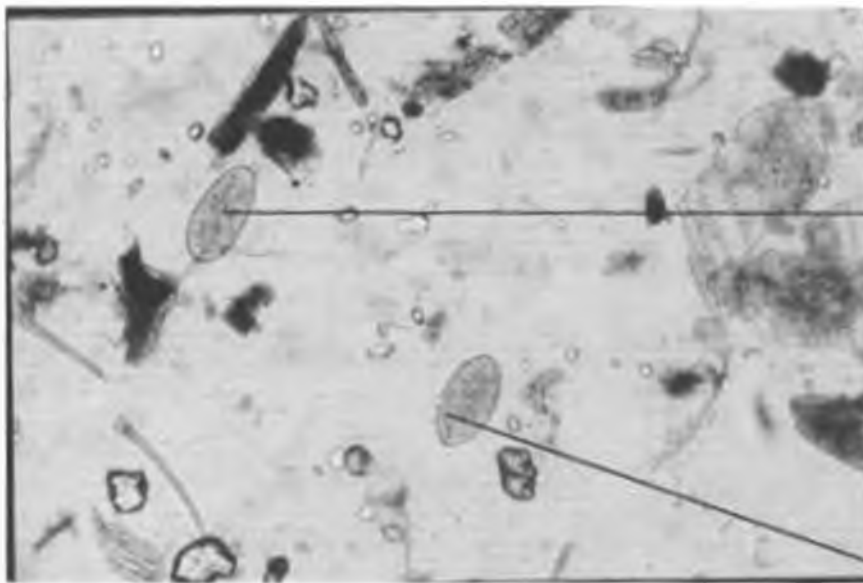
Table 3. Prevalence of *S. bovis* eggs in faecal samples of male and female cattle from Kwale, coastal Kenya

Sex	No. examined	No. infected	Parasite Prevalence (%)
Male	194	30	15.3
Female	270	47	17.4

Three out of 492 specimens were found to have eggs (one egg in each specimen) which could not be identified. These eggs had the shape of *S. haematobium* but the size was bigger than either *S. haematobium* or *S. bovis* (Figure 6). This egg had a golden brown colour like *S. bovis*. Other trematode eggs that were encountered in the faecal sample analysis included: the conical flukes (*Paramphistomum*) and the liver fluke (*Fasciola*) (Figure 7). Paramphistome eggs were transparent with an operculum while the liver fluke eggs had a golden brown to yellow colour and with an operculum.



Figure 6. *Schistosoma bovis* egg in a faecal sample



*Paramphistomum* egg

*Fasciola* egg

Figure 7. A faecal sample showing *Fasciola* and *Paramphistomum* eggs

NB: *Fasciola* egg is golden brown and *Paramphistomum* egg is transparent

### 4.3 Abattoir survey for adult *S. bovis*

Cattle slaughtered in three abattoirs located in the Kwale area were examined for adult *S. bovis* in the mesenteric veins (Figure 9). The abattoirs included Ngombeni where *S. bovis* prevalence was 16.3%, Kwale had a prevalence of 28.7% and Mwambungo had a prevalence of 10.5%. On treatment of the adult worms with lactic acid the dark areas representing the female fluke was clearly visible. This illustrates the pairing of the schistosomic adult worms (Figure 10). The overall prevalence in the three abattoirs was 25.1%. Interestingly, statistically significant differences in prevalences were observed between slaughter house ( $P < 0.05$ ).

The parasite prevalences also varied between study months (March to September) with highest prevalences being observed in May and the lowest in September (Figure 8). The differences were not statistically significant  $P > 0.05$ . During the abattoir survey other trematodes were also recovered from slaughtered cattle, and these included conical flukes (Paramphistomes) (Figure 11) in the rumen and liverflukes (*Fasciola gigantica*) (Figure 12) in the liver. Adult conical flukes were more common than either liver flukes or blood flukes.

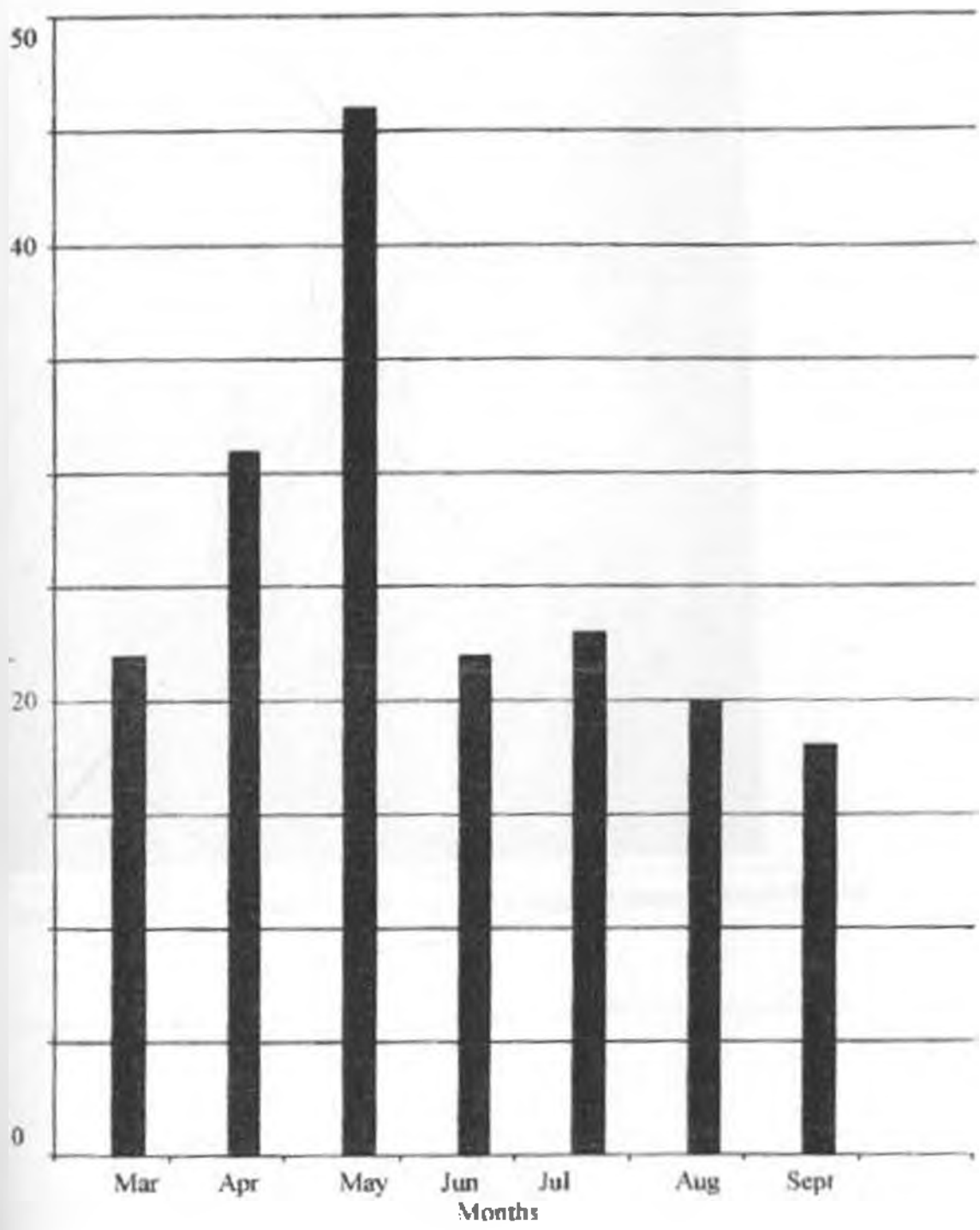


Figure 8. The incidence of *S. bovis* over the study period in slaughtered cattle



Figure 9. Examining for adult *S. bovis* worms at a slaughter house in Kwale District

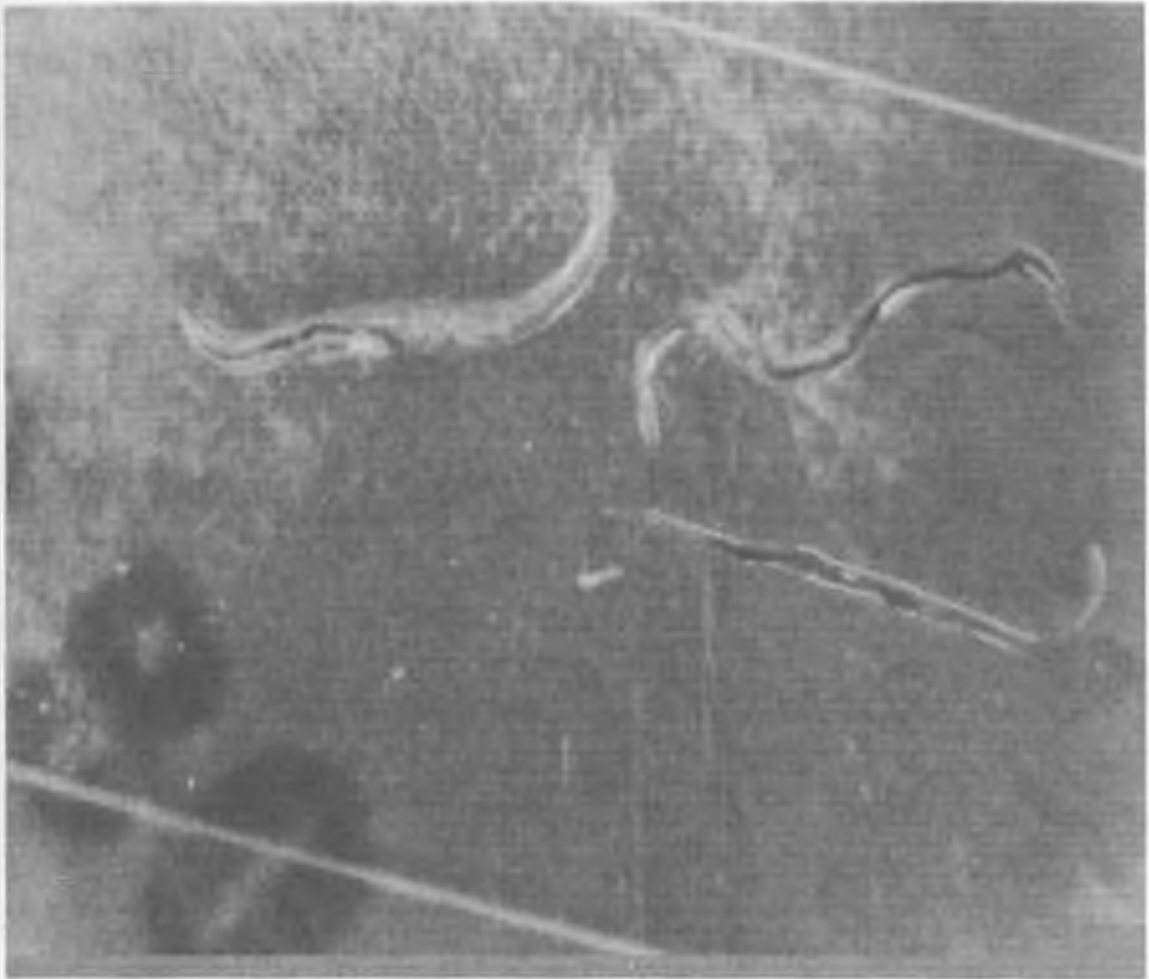


Figure 10. *Schistosoma bovis* worms after treatment with lactic acid



Figure 11. Conical flukes (Paramphistomes) from the rumen of a carcass





**Figure 12. Liver fluke (*Fasciola*) from the liver of one the carcasses**

#### 4.4 Snail sampling survey

When livestock came to the watering sites usually between 11.00 a.m and 3.00 p.m they got into the water giving the cercariae a chance to penetrate their skin (Figure 13). To determine the presence of the snail intermediate hosts of *S. bovis*, the water bodies were sampled using a snail scoop (Figure 14). In Manola and Bangoni rivers during the dry season snails were seen only after digging into the sandy riverbeds while, at Zigira and Kinango dams no snail of the genus *Bulinus* were found. Kandingo stream showed a higher population of the snails (Tables 4 and 5). During the wet period the snail population increased in the water bodies (Figure 14 and 15). Other species of snails found in the study water bodies in Kwale included the genera *Lunistes*, *Melanoides* and *Cleopatra*.

#### 4.5 School children stool and urine samples

Varying numbers of sample containers were received from different schools, 40 samples were received from Nguzini primary school in Kinango division, 28 samples from Pungu primary school in Matuga division while 17 samples were received from Muhaka primary school in Msabweni division. All the stool samples were negative for *S. bovis* eggs. Three urine samples from the pupils were positive for *S. haematobium* eggs (Table 6).

#### 4.6 Laboratory records

Two year 2003-2004 laboratory records at KEMRI Kwale Laboratory were examined for any cases of *S. bovis* eggs. No *S. bovis* eggs were identified in stool samples from humans over the study period. Other parasites identified and recorded in the study area included malarial parasites, helminths parasites like *Ascaris lumbricoides* and *Trichuris trichuria*



Figure 13. Cattle watering at one of the sampling points



Figure 14. A field assistant scooping snails

Table 4. Relative abundance of *Bulinus* snails in Kwale water bodies during the study period

Month	Total
March	70
June	252
July	218
September	156
Total	696

Table 5. Relative abundance of *Bulinus* snails in the different water bodies surveyed in Kwale

Water body	No. of snails per water body
Kinango dam	0
Zigira dam	352
Ziwani dam	0
Manola river	74
Bangoni	50
Kandigo stream	320
Total	696

**Table 6** Number of *S. haematobium* in urine samples and other helminth parasites in stool samples of the school children in three divisions

Division	School	Total	<i>S. haematobium</i>	Others
Matuga	Pungu	28	3	18
Msabweni	Muhaka	17	0	7
Kinangu	Ngozini	40	0	1
<b>Total</b>		<b>85</b>	<b>3</b>	<b>26</b>

Other helminth parasites identified in stool samples included *strongyle* spp, *Ascaris* spp, hookworms and *Trichuris* spp.

#### 4.7 Confirmation of the identity of the adult schistosomes recovered from slaughtered cattle in abattoirs

The identity of the adult worms recovered from bovine carcasses was confirmed by the PCR-RFLP assay described by Barber *et al* (2000). In this assay schistosome genomic DNA was extracted and amplified by PCR using primers targeting a 480 base pair portion of the second internal transcribed spacer (ITS2) region of the ribosomal gene. Restriction fragment length polymorphism (RFLP) analysis of the 480 bp fragment of the ITS2 using the restriction enzyme *Sau3A1*, yielded fragment patterns on an agarose gel after electrophoresis and staining with ethidium bromide indicated the worms were *S. bovis* (Figure 15). In this assay *S. bovis* can be differentiated from *S. haematobium*. These results showed that the adult worms that were recovered from slaughtered cattle in Kwale district were *S. bovis* which confirmed that cattle in this area are infected with *S. bovis*.

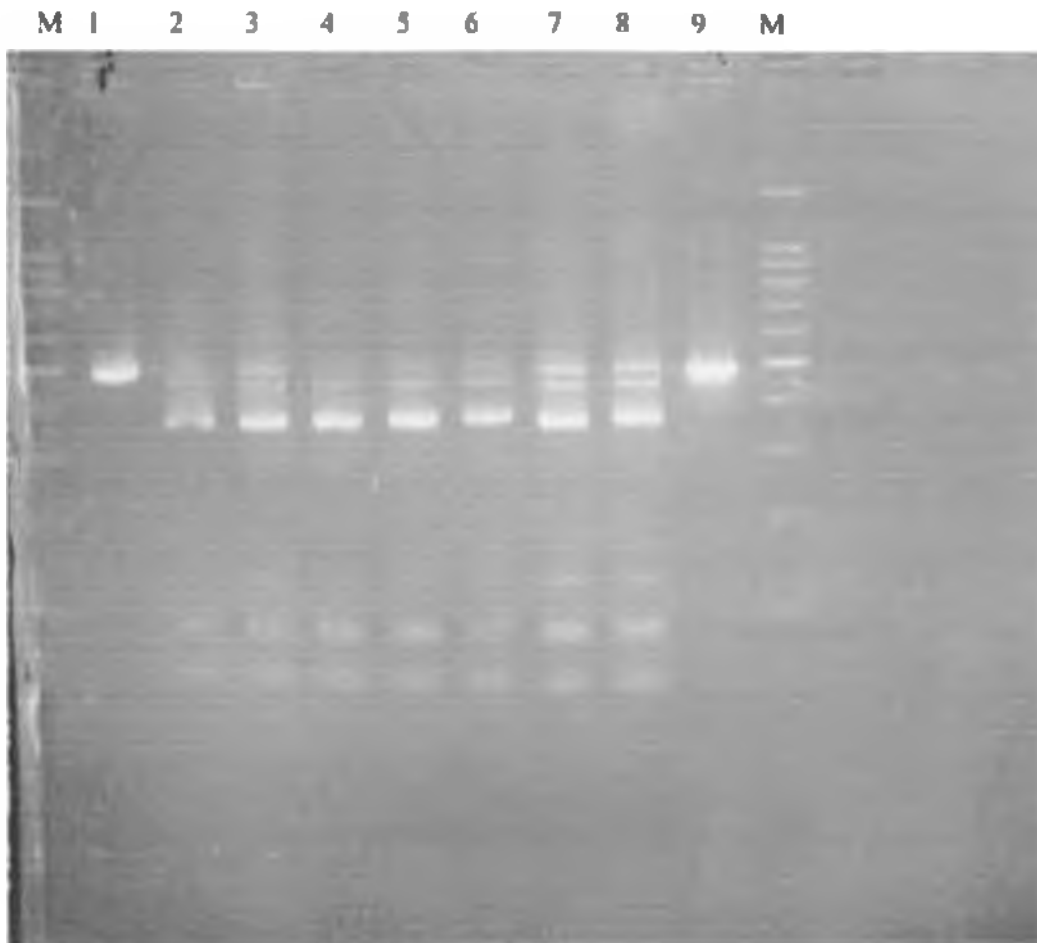


Figure 15. Agarose gel picture showing RFLP patterns produced by electrophoresis.

Figure 15B: The picture after the 480 bp PCR products amplified from genomic DNA of several individual bovine schistosomes obtained from slaughtered cattle in Kwale were digested with the restriction enzyme *Sau3A1*. Lanes labelled M represent the 100bp DNA marker, 1 and 9 represent undigested PCR products of reference schistosomes, 2 and 8 represent *Sau3A1* digested PCR products of reference schistosomes and 3-7 represent PCR products of individual bovine schistosomes recovered from slaughtered cattle.

## CHAPTER FIVE

### 5.0 DISCUSSION AND CONCLUSIONS

#### 5.1 Discussion

*Schistosoma bovis* is present in cattle in Kwale District and its prevalence in the present survey was 16.9% in faecal samples. Cattle below 3 years age and above 6 years old had higher prevalences of infection than those between 3 to 6 years. These findings closely agree with those of Majid *et al.* (1980) who examined cattle in Southern Sudan and those of Makundi *et al.* (1998) who was studying bovine schistosomosis in Iringa District, Tanzania.

It has been suggested that immune suppression operating in animals observed as lowered fecundity in worms between 3-6 years of age may be the mechanism involved in the development of acquired resistance to *S. bovis* infection in cattle in this age group (Bushara *et al.*, 1980). The higher prevalence in the younger age groups (< 3 yrs) does suggest that this age group is more susceptible to schistosome infection and lacks a fully developed immune system against the trematode infection. The rise in the faecal egg excretion in the older cattle of over 6 years is probably due to a "breakdown" in the immune system in this age group (Makundi *et al.*, 1998).

Relative snail abundance varies from place to place and from season to season. No snails were present in the water habitats in the drier months of the year hence a lower prevalence of the parasite in these months than the wet ones. Snails occur in great abundance during the wet months, and that is the time infected snails are likely to be present. On the other hand during the dry months fewer snails are present and the possibility of getting infected snails is also much lower. During the drier months the sporocyst development and maturation in the snail is arrested as the snails aestivate. Development of sporocyst resume during the wet season when conditions are more favourable for transmission (Niel *et al.*, 1980).



The temperatures during the wet season are more appropriate for miracidial and cercarial emergence from the eggs and snails, respectively and their survival while during the dry period the miracidia and cercariae survival is minimal (Niel *et al.*, 1980). This may also explain the difference in prevalence of the parasite over the wet and dry seasons. The fecundity of the female worms is higher over the wet period than over the drier periods and hence the higher prevalence of the parasite in the wet season (Niel *et al.*, 1980). The difference in prevalence of infection between sexes was not statistically significant and therefore sex may not be a factor associated with parasite infection as Makundi *et al.*, (1998) found when investigating the prevalence of schistosomiasis in cattle in Iringa District, Tanzania.

The presence of ova resembling those of the human pathogen *S. haematobium* in cattle was puzzling and interesting and further investigations are necessary to verify this observation. Hybridisation between *S. haematobium* and *S. bovis* is possible under experimental conditions (Taylor, 1970) but it is unlikely to occur in bovines as they are not permissive hosts of *S. haematobium*. Apparently the *S. haematobium* like eggs appeared to be much larger than typical *S. haematobium* or *S. bovis* eggs. It has been suggested that eggs of *Schistosoma* species may change shape when in unnatural hosts due to differences in diet (Rollinson, *et al.*, 1987). Nevertheless the possibility that the parasite eggs recovered from bovines in Kwale were indeed *S. haematobium* cannot be completely ruled out.

The presence of *S. haematobium* in the South African buffalo has been reported by Bason and Kruger (1970) and since cattle is in the same family as the buffalo, these eggs may actually have been those of *S. haematobium*.

The prevalence of adult *S. bovis* worms in slaughtered carcasses in Kwale district was 25.1%, and this prevalence compares with those observed by Dinnik and Dinnik (1965), in abattoir

surveys on *S. bovis* who recorded a prevalence of 23% in Masailand (Kajiado and Narok districts) and 25% in Ukambani (Machakos and Kitui districts ).

During the study period from March to September 2005, Kwale experienced dry months in March, April, June, and September and wet months in May, July and August. The prevalence of infection was low during the dry months and higher during the wet months.

The prevalence increased with the onset of rains in April and peaked in May when the rains were heavier but progressively decreased with lowered rainfall towards September. This pattern can be explained by the host parasite relationship where, the parasite adapts itself according to the host internal environment. When there is abundant feed the parasite reproduces more of its offspring as there are better chances of survival and reproduction is low when feed availability is low (Niel *et al.*, 1980).

Further analysis of adult worms using molecular techniques indicated that the adult parasites recovered from slaughtered cattle in Kwale district were *S. bovis*. These results agree with those of Barber *et al.* (2000) who observed that worms recovered from slaughtered bovines in different parts of Kenya were *S. bovis*.

The snail intermediate hosts of *S. bovis* the pulmonate snails of the genus *Bulinus* were present in the water bodies studied in Kwale District in large numbers in some of the sites, while they were absent in others. In general snail abundance showed seasonal variations. During the dry months, some river beds were completely dry and snails could only be recovered after digging into the sand in riverbeds, where they were buried under the sand in aestivation. In habitats that retained water throughout the study period (dams and streams) snail abundance was lower during the dry months but higher during the wet months and often would be found attached to vegetation. Snail populations therefore, increased with the onset of the wet months, and declined during dry months when the snails aestivated to avoid extreme

weather conditions (Niel *et al.*, 1980). These findings are in agreement with those made previously by Noda *et al.* (1994) who observed seasonal variations in snail population in Kwalu.

The absence of *Bulinus* and other pulmonate snails in some water bodies could in part be attributed to the presence of prosobranch snails that are thought to be predators and competitors of pulmonate snails, and therefore, potential biological control agents for schistosome transmitting snails (Hofkin *et al.*, 1991; Mkoji *et al.*, 1992, 1998; Loker *et al.*, 1993).

The mesenteries are usually sold at the local markets as "matumbos" (tripes) whether infected or not. This then makes *S. bovis* to be of Public health importance because infected meat is passed for human consumption which may cause either transient infection (Raper, 1951) or spurious infection (Kinoti *et al.*, 1988) and if the public finds the *S. bovis* aesthetically not acceptable they may not purchase the intestines which may have an economic implication. From the laboratory records, there were no recorded cases of *S. bovis* eggs in human stool samples. Similar results were recorded from school children and therefore it seems that humans may not be necessary for the transmission and maintenance of *S. bovis*. Humans seem to be refractory and any *S. bovis* eggs present in stool samples are most likely spurious (Kinoti *et al.*, 1988). Further investigations are therefore needed to determine if *S. bovis* does infect humans at all. An area that might be of interest is the possibility of *S. bovis* being able to break species barriers in HIV infected individuals.

## 5.2 Conclusions and recommendations

1. The prevalence of *S. bovis* in Kwale district was 16.9% in faecal samples and 25.1% in slaughtered cattle. The age groups below 3 years and above 6 years were shedding more eggs and therefore require more attention when carrying out any helminths treatment programme at the farm level and since the prevalence of the parasite is also high during the rainy season its important to treat the animals strategically just before the rains.
2. The egg resembling *S. haematobium* was found in three out of 492 rectal faecal samples, it may therefore be necessary to carry out more work in this study area to confirm its identity.
3. The adult *S. bovis* worms were found in the mesenteric veins of the slaughtered cattle. This may present a public health importance as the infected mesenteries are sold for human consumption. Thorough meat inspection is therefore necessary so that the organs passed for human consumption are free from the parasite.
4. The snail intermediate host, genus *Bulinus*, is present in various water bodies in the district and therefore measures can be instituted to control the snails either chemically, biologically or through environmental modification. The biological method of control can be investigated further to see if it is operational in the various water bodies in Kwale District.
5. Since *S. bovis* share the intermediate hosts with *S. haematobium* then control of this snail may control both *S. bovis* and *S. haematobium* and this would be beneficial from both a public health and economic point of view.

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