EPIDEMIOLOGY AND CONTROL OF BOVINE FASCIOLOSIS AND SCHISTOSOMOSIS IN THE SOUTHERN HIGHLANDS OF TANZANIA

BY

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ABSTRACT

The main purpose of this study was to determine if anthelmintic treatment of early patent primary fasciolosis and schistosomosis would prevent development of acute disease and would improve productivity of calves which continue grazing high potential natural transmission areas, without causing unwanted side effects or interfering with the development of immunity. Aspects of transmission biology and host parasite relationship were also investigated. Field studies, including cattle and snails, were performed at Lulanzi dairy farm where the trematode infections were highly prevalent. In addition experimental *Schistosoma bovis* infections were carried out in confined calves. The results from snail studies showed that *Bulinus natalensis* was the most abundant freshwater snail, which was responsible for the transmission of *Schistosoma bovis* mainly towards the end of the rainy season. Results of the field study, where cattle were naturally exposed, showed that acquisition of trematode infections was gradual. It took five months for the peak egg excretion to be reached, followed by a gradual decline to a very low level. Both triclabendazole and praziquantel drugs were highly efficacious and reduced *Fasciola* and *Schistosoma* worms by 100% and 95.6% respectively; while for *S. bovis* faecal and tissue eggs the reduction was 98.9% and 79-96%, respectively. Treatment kept the faecal egg excretion of both parasites species at a very low level for more than seven months and did not affect the development of immunity. Deterioration of the body weights and haematological parameters were only seen during the dry season and it was more severe in the untreated compared to the treated, challenged calves. More severe pathological lesions, mainly fibrosis of the liver, were observed in the treated experimentally infected animals than in the untreated ones. However, no clinical

signs were associated with such changes and there was a gradual resolution of the pathological lesions. In the experimental *S. bovis* infections it was shown that, along the small intestine, eggs were mainly deposited in the anterior part at week seven, in the central part at week 18 and evenly distributed at week 32 post infection. The key findings from the present study are that *B. natalensis* for the first time was proved to transmit *S. bovis* and that such an infection occurred in the snails that had an existing amphistome infection. The animal studies have demonstrated that natural *Fasciola* and *Schistososma* infections in cattle are mainly sub-clinical and their impact is seen mainly during the dry season when feed supply is limited. Although treatment of early infections transiently causes additional liver fibrosis, treatment prevents development of acute disease and does not interfere with the development of immunity. Furthermore, treatment prevents further deterioration of the health of the cattle during the dry period. In addition, the observed reduction in faecal egg excretion has an epidemiological impact of reducing the transmission of these infections. In general, the present findings raise the possibility of strategic use of anthelmintics for preventing the development of acute trematode disease in young ruminants, improving their productivity and reducing the transmission intensity in endemic areas.

DECLARATION

I, ASANTELI ELIANGIKUNDI MAKUNDI, do hereby declare to the Senate of the Sokoine University of Agriculture that this thesis is my own original work and has not been submitted for a degree award in any other University.

Signature.....

Date.....22/11/2001.....

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DEDICATION

This dissertation is dedicated to my children, Haikaeli, Felix and Emmanuel

Remember the saying of the famous parasitologist of the 20th century

"I am great believer that when you are young you should struggle in acquiring experience and not thinking about money. I suppose, I could have made a lot of money than what I have done, but somehow learning seemed more important, learning so that others could learn more."

Professor Robert Thomson Leiper

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ABBREVIATIONS

ADCC	Antibody-dependent cell-mediated cytotoxicity
ADP	Average pore diameter
ANOVA	Analysis of variance
Bwt	Body weight
CAA	Circulating anodic antigen
CCA	Circulating cathodic antigen
DANIDA	Danish International Development Agency
DBL	Danish Bilharziasis Laboratory
DNA	Deoxyribonucleic Acid
ELISA	Enzyme linked immunosorbent assay
ENS	Enteric nervous system
EPGF	Egg per gram of faeces
EPGT	Egg per gram of tissue
GIT	Gastro intestine
GLM	General linear model
GST	Glutathione-s-transferase
H & E	Haematoxylin and eosin
Hb	Haemoglobin
IFN	Interferon
Ig	Immunoglobulin
IL	Interleukin
IU	International units

KDa	Kilo Daltons
Log	Logarithm
LSD	Least significant difference
p.i.	Post infection
PCR	Polymerase chain reaction
PCV	Packed cell volume
PZQ	Praziquantel
RAPD	Random amplified polymorphic DNA
RNA	Ribonucleic acid
RVAU	Royal Veterinary and Agricaltural University
SAS	Statistical analysis software
SEA	Serum egg antigen
SUA	Sokoine University of Agriculture
TFN	Tissue necrosis factor
TGF	Transforming growth factor
Th	T-helper cells
VG	Van Gieson
WHO	World health organization

CHAPTER ONE

INTRODUCTION

Trematode infections are widespread among domestic ruminants in Tanzania causing major diseases and resulting lowered productivity (Hammond, 1965; Mahlau, 1970; Msanga, 1985; Kassuku *et al.*, 1986; Makundi *et al.*, 1998). Previous cross-sectional studies have shown that bovine fasciolosis and schistosomosis are very common in Iringa District in the southern tropical highlands of Tanzania and in some farms the prevalence is close to 100% (Dinnik and Dinnik, 1965; Mahlau, 1970; Makundi *et al.*, 1998). However, the economic impact of these infections and basic factors influencing their epidemiology under prevailing local conditions such as parasite and intermediate host spectra and the immune response of cattle to these infections, is not well established. So far, very little has been done on the control of these infections except for selective treatment of fasciolosis in the most severely affected herds or animals (Mahlau, 1975; Kassuku *et al.*, 1991; Maingi and Mathenge, 1995).

Transmission of *Fasciola* and *Schistosoma* parasites depends on the existence of compatible freshwater snail species (Christensen *et al.*, 1983). In East Africa *F. gigantica* is the predominant *Fasciola* species infecting ruminants and is transmitted by *Lymnaea natalensis*. On the other hand, transmission of *Schistosoma bovis*, which is the predominant bovine *Schistosoma* species in East Africa, depends on a wide number of bulinid snails, particularly *Bulinus africanus* and to a less extent *B. forskalii* (Christensen *et al.*, 1983, Kassuku *et al.*, 1986; Mwambungu, 1988).

Recently, *B. natalensis*, which had not been reported to serve as intermediate host for *S. bovis* in Tanzania, seemed to be the only bulinid snail serving as intermediate host for bovine schistosome infection at Lulanzi dairy farm in Iringa District (Makundi, 1993). In general there is limited information concerning the intermediate host snail and bovine schistosome species spectra in the southern highlands of Tanzania.

Clinical bovine fasciolosis and schistosomosis occur mainly in calves following primary infection with large numbers of metacercariae and cercariae respectively. Under natural conditions, acute clinical cases of schistosomosis and fasciolosis are relatively uncommon (Dargie, 1980). However, under certain favourable conditions epizootics may occur (Reinecke, 1970; Van Wyk *et al.*, 1974; Markovics *et al.*, 1993; Maingi and Mathenge, 1995). Epizootiological observations and experimental infections have shown that cattle are capable of developing resistance to these infections (Boray, 1969; Hammond and Sewell, 1974; Majid *et al.*, 1980a; Bushara *et al.*, 1980, 1983a, 1983b). However, such immunity in the herd is probably achieved at the expense of severe morbidity and mortality in the young calves (Hussein, 1980).

Treatment of an early patent primary infection would reduce the pathogenic effect by preventing clinical disease and mortalities. Most of the anthelmintic treatments carried out against trematode infections in cattle have been based mainly on determination of the efficiency of killing of worms (Bushara *et al.*, 1982; Kassuku *et al.*, 1991). Furthermore, treatment of hamsters against *Schistosoma haematobium, S. japonicum, S. mansoni, S. intercalatum and S. mattheei* experimental infections with praziquantel has shown that the drug was highly efficacious (Webbe and James, 1977) and that it releases or exposes concealed parasite tegument antigens that are

potent immunogens (Pearce *et al.*, 1991; Day *et al.*, 1992). Such results suggest that praziquantel may be used for both curative and prophylaxis purposes. However, there is limited information on the long-term consequences of such treatments on the pathology, immunity and productivity in animals, which continue grazing high potential transmission areas such as the southern tropical highlands of Tanzania.

Therefore, the overall objective of this study was to contribute local epidemiological knowledge that may be used to plan for a control strategy of trematode infections in livestock in the southern highlands of Tanzania.

The specific objectives of the present study were:-

1. To determine the immediate and longterm effects of challenge infection in calves treated against primary *Fasciola* and *Schistosoma* infections on pathology, immunity and productivity under natural and experimental conditions.
2. To determine the population dynamics of *Bulinus natalensis* snail which seemed to serve as an intermediate host for *S. bovis* at Lulanzi dairy farm and seasonality of transmission of *S. bovis* by this snail at Lulanzi dairy farm.

CHAPTER TWO

LITERATURE REVIEW

2.1 Bovine schistosomosis

2.1.1 Aetiology and taxonomy

Schistosomes are obligate parasites of the vascular systems of birds and mammals and unlike most of the digeneans, which are hermaphrodites, they are dioecious. The slender female is held within the gynaecophoric groove formed by lateral overgrowths of the male. The presence of the male is necessary for the female development and maintenance of her mature state (Southgate *et al.*, 1998). Morphological characters, especially those of the female such as size, number, shape and size of the eggs in the uterus, are essential in species identification. The female *S. bovis* are 13-34 mm long with 5-62 (mean 29) uterine eggs (Loker, 1983; de Bont and Vercruysse, 1998). In males, scanning electron microscopy studies have shown that the surface of the male bears structures such as tubercles that are useful for taxonomy (Southgate *et al.*, 1986; Fournier *et al.*, 1989). Apart from the morphological characteristics of adults, the characteristics of the cercariae such as chaetotaxy may be used (Bayssade-Dufour *et al.*, 1989; Cabaret *et al.*, 1990; Kigadye, 1998) for identification purposes. Recently, such studies on morphological characteristics have been improved by applying computer-assisted methods (Chau *et al.*, 1996).

Therefore, based on morphological characteristics, geographical location and the host they infect, mammalian schistosome species are subdivided into four

subgroups, *i.e.* the *haematobium* group (with terminal spined eggs), the *mansoni* group (with lateral spined eggs), the *indicum* group (species occurring in the Indian subcontinent) and the *japonicum* group of East and South East Asia (de Bont and Vercruysse, 1998; Southgate *et al.*, 1998). *S. bovis* belongs to the *haematobium* group together with four other species, *S. mattheei, S. currasoni, S. leiperi* and *S. margrebowiei*, that also infect domestic and/or wildlife ruminants, and two schistosome species, *S. haematobium* and *S. intercalatum*, that infect humans.

Species identification of *S. bovis* based on the morphological characteristics of the female alone is rather difficult due to its resemblance to the closely related species such as *S.* mattheei and *S. leiperi* (Loker, 1983; Dinnik and Dinnik, 1965; Touassem, 1987). Likewise, there are great intra-specific variations in morphological characteristics of the male, such as the presence or absence of tubercles, size, shape and degree of spination (Southgate *et al.*, 1981, 1986). The difficulties in species differentiation in bovine schistosomes are further aggravated by the inter-specific sexual interactions leading to hybridization with offspring with intermediate characters (Taylor, 1970). For instance, while miracidia of *S. bovis* are refractory in developing in *B. globosus* snails, the hybrids from *S. bovis* X *S. haematobium* had eggs similar in size and shape to those of *S. bovis*, and the miracidia produced can develop in *B. globosus* (Taylor, 1970). Also, it has been observed that the cercarial emergence rhythms in F_1 and F_2 resulting from *S. mansoni* (with diurnal peak) and *S. rhodhaini* (with nocturnal peak) were characterized by two unequal peaks, one diurnal and another nocturnal (Théron, 1989). The ability of each trematode parasite to conserve its cercarial emergence pattern is also maintained at strain level as observed in *Biomphalaria glabrata*, where dual infection with the two *S. mansoni*

chronobiological strains led to two shedding patterns, one with an early and the other with a late shedding pattern (Théron *et al.*, 1997). Similarly, scanning electron microscopy studies have led to postulation that the presence of tubercle spines observed in *S. mattheei* populations is a characteristic inherited from *S. haematobium* as a result of sympatry (Kruger *et al.*, 1986).

Recently, there have been rapid developments in molecular biology, which seem to solve the difficulties related to species identification based on morphological characteristics. In these studies, particular attention has been focussed on the detection and analysis of parasite variation in ribosomal RNA genes, mitochondria DNA and random amplified polymorphic DNA (RAPD) (Rollinson *et al.*, 1986). Phylogenic analysis of the *S. haematobium* group species by Kaukas *et al.*, (1994) based on RAPD studies has shown that *S. bovis* and *S. curassoni* are sister groups, *S. leiperi* and *S. mattheei* form a separate lineage, while polymorphism was revealed in *S. haematobium* (3 strains) and in *S. mansoni* (2 strains). Therefore, the molecular techniques may be used for both strain and species differentiation and may be used to differentiate bovine schistosome species present in the Topical Highlands of Southern Tanzania.

2.1.2 Life cycle

Like that of most of the digeneans, the life cycle of schistosomes is complex and they demonstrate alternation of reproduction with the asexual stages in the freshwater snails while the sexual stage occurs in vertebrates. The schistosome egg, when voided in the faeces of the definitive host, has a fully developed miracidium that hatches rapidly if it is exposed to favourable physicochemical conditions such as

osmolarity and temperature (24-28° C). The newly hatched miracidia have a short life span (6-8 h). However, their swimming speed (2 mm/sec), together with being positively phototactic and negatively geotactic enables them to locate the snails that are mainly found near the surface of water. The role played by chemo-attractants "miraxone" released by the snails (Shiff and Kriel, 1970; Etges *et al.*, 1975) has in the past been debatable. However, recent studies have shown that schistosome miracidia can discriminate between snail strains (Haas *et al.*, 1995) as observed in *S. bovis* (Toaussem and Combes, 1985). The miracidia will develop into mother sporocysts, then to several daughter sporocysts and finally cercariae. However, the intra-molluscan development of schistosomes is complex and in *S. bovis*, Touassem and Therón (1986) have demonstrated three patterns of sporocystogenesis by daughter sporocysts. For optimal intra-molluscan development, optimal environmental factors such as temperature (22-28° C) and food is necessary. The development period in the snail for *S. bovis* in *B. africanus* in Tanzania is about 30 days at 22-25° C under laboratory conditions. However, studies have shown that the development period was inversely correlated to the temperature (Touassem and Jourdane, 1986).

Infection of the final host by the cercariae is via the skin and like most of the schistosome species, *S. bovis* transmission depends on large mammals with a long life span like cattle, where the sexual part of their life cycle occurs (Loker, 1983). Apart from skin, the oral route can also play a significant role in cattle (van Wyk *et al.*, 1974) and in goats (Kassuku *et al.*, 1985a; Boulanger *et al.*, 1999). Upon penetration, the cercaria undergoes transformation into schistosomulum as a way of adapting to the new physicochemical changes such as oxygen and carbon dioxide

tension and glucose concentration (de Jong-Brink, 1995). The schistosomula will then enter the blood stream and after circulation will reach the lungs where they are transformed into slender forms before finally inhabiting the liver, via the circulatory system, where development to mature stages takes place. Pairing of male and female takes place at about five weeks and they descend down into the mesenteric veins. The female leaves the male temporarily and migrates to the fine venules, where deposition of eggs occurs. The eggs will finally enter the gastrointestinal lumen and are voided with faeces. It has been proposed that different mechanisms such as mechanical, chemical and inflammatory reactions are involved in the trans-mucosal passage of the eggs (Semuguruka, 1992). Some of the eggs are transported by the blood into the liver, after which they play no part in propagating the parasite. However, there are few alternative routes of egg excretion, *e.g.* urinary bladder (Hussein *et al.*, 1984) and gall bladder (Makundi, 1993). Numerous *S. bovis* eggs were observed in the mucosa of a 10 years old cow from Lulanzi village in Iringa district, Tanzania that was naturally infected during cross-sectional surveys (Makundi, 1993). Furthermore, the recent findings of vertical transmission of *S. japonicum* in pigs (Willingham *et al.*, 1999) is a clear suggestion that this route may equally occur in domestic ruminants taking into account that ruminants have much simpler placenta (three-layer fetal and two-layer maternal placenta membranes) than that of pigs, where both fetus and maternal placenta have complete (three) layers. The pre-patent period for *S. bovis* in cattle, sheep and goats is 6-8 weeks (Massoud, 1973; Hussein *et al.*, 1976; Kassuku *et al.*, 1986).

2.1.3 Epidemiology

2.1.3.1 The intermediate host spectra

As regards the asexual reproduction cycle, *S. bovis* has a narrow intermediate snail host spectrum, mainly the tetraploid *Bulinus truncatus* in North and West Africa (Graber and Daynes, 1974; Diaw *et al.*, 1998) but in East Africa (Kenya and Tanzania) the transmission depends on a wide number of freshwater bulinid snail species, in particular those of the *B. africanus* group (Mutani *et al.*, 1983; Southgate *et al.*, 1985; Kassuku *et al.*, 1986). Limited studies in the Southern Highlands of Tanzania have shown that *B. africanus* is the most important intermediate host snail for *S. bovis* (Kinoti, 1964; Sturrock, 1964; Kassuku *et al.*, 1986) and that in some focal areas *Bulinus forskalii* may contribute significantly to the transmission (Southgate and Knowles, 1975; Mutani *et al.*, 1983; Southgate *et al.*, 1985; Mwambungu, 1988). Similarly, in neighbouring countries like the Democratic Republic of Congo (formerly Zaire), *B. africanus* and *B. forskalii* are the main intermediate hosts for *S. bovis* (Chartier *et al.*, 1990, 1993). Although *B. globosus* and the tetraploid *B. truncatus* in East Africa may serve as intermediate hosts for *S. bovis*, they play a very limited role. On the other hand, *B. nasutus, B. abyssinicus* and the diploid *B. tropicus/truncatus* snails are considered to be refractory (Brown, *et al.*, 1971; Mutani *et al.*, 1983). Beyond North Africa, *B. truncatus* continues to be the most important intermediate host snail for *S. bovis* in the Middle East and Mediterranean countries (Mouahid and Mone, 1990). However, in the Iberian peninsula (Portugal and Spain) *Planorbarium metidjensis* dominates in addition as the intermediate snail host for *S. bovis* (Ramajo and Martin, 1972; Ramajo and Simon, 1988). Other snails such as *B. contortus* (Markovicks *et al.*,

1993) and *B. wrightii* (Arfaa, 1976) also play a role in the transmission of *S. bovis* in the Middle East. Therefore, the distribution of schistosomes is highly related to their intermediate host snails, since the presence of compatible intermediate hosts is the singly most important limiting factor (Preston and Southgate, 1994).

2.1.3.2 The definitive host spectra

Most of the 23 known mammalian schistosome species rely on large-bodied mammals with a long life span, such as primates, ungulates and proboscides for their transmission, and only one species, *Schistosomatium douthitii*, which depends exclusively on rodents (Loker, 1983). The Artiodactyla are the most important definitive hosts hosting 12 out of 23 mammalian schistosome species (Loker, 1983; de Bont, 1995). It is highly debatable as to which schistosomes are valid bovine species in Africa (Christensen *et al.*, 1983). Only *S. bovis, S. mattheei, S. curassoni, S. leiperi* and *S. margrebowiei* have been accepted as valid African bovine species. In East Africa, *S. bovis* is the most predominant bovine species and it accounted for 99.9% of the 60% of cattle slaughtered at lake regions, while the remaining animals were infected with *S. mattheei* (Dinnik and Dinnik, 1965). *S. bovis* also occurs in domestic small ruminants (Majid *et al.*, 1983; Kassuku *et al.*, 1986), but the reluctance of small ruminants to enter water, leaves cattle as the most important definitive host. Occasionally, *S. bovis* has been reported in a number of rodents (Malek, 1969; Lo and Lemma, 1975) and wild ruminants (Sobrero, 1975; Ngendahayo *et al.*, 1987). However, their role in the transmission of this parasite is considered insignificant from an epidemiological point of view (Christensen *et al.*, 1983). For instance, studies on parasitic infestation of wild herbivores in Serengeti

and Rukwa regions in Tanzania showed that none of the 350 different species of animals examined was infected with *S. bovis*, although this infection was highly prevalent in cattle and goats in the vicinity of the Serengeti National Park (Sachs and Sachs, 1968). The few reported human cases (Teesdale, 1976; Mouchet *et al.*, 1988) of *S. bovis* infection are considered to be spurious. The southern part of Tanzania marks the end of the distribution of *S. bovis* southwards and in this area it overlaps with *S. mattheei* and *S. leiperi* (Dinnik and Dinnik, 1965). However, unlike *S. bovis*, *S. mattheei* is not considered to be specific to domestic ruminants as it also occurs in a number of wildlife ungulates, primates and man (Pitchford *et al.*, 1974; Hira, 1975; van Wyk, 1983; van Wyk *et al.*, 1997). However, limited studies have shown that pure *S. mattheei* infections in man may not result in the production of viable eggs (Wolmarans *et al.*, 1990). On the other hand, *S. leiperi* predominantly occurs in wildlife in particular antelopes, *Kobus lechwe* and *K. vardoni* (Pitchford, 1976; Malek and Ongom, 1984). Theoretically, the limited reports of *S. bovis* in wild ruminants could be due the lack of studies in the area rather than the absence of the parasite. For example in Tanzania, Rehani (persona-communication) has seen schistosmes in the mesenteries of an antelope at Mvomero in Morogoro while Balemba (personal-communication) has seen S. *bovis* eggs in the intestinal muscosa of a wildebeest originating from Kisaki in Morogoro.

2.1.3.3 Geographical distribution

The distribution of important African bovine schistosome species depends mainly on their primary intermediate and definitive hosts. *Schistosoma bovis* is predominant in domestic ruminants in Africa north of latitude 10° S (Makundi *et al.*,

1998; de Bont and Vercruysse 1998; Diaw *et al.*, 1998). *S. bovis* is more widespread in the equatorial and the sub-Sahelian regions than in the Sahelian and Sahara desert regions, presumably due to water availability. Therefore, it is highly prevalent in Tanzania (Kassuku *et al.*, 1986; Makundi *et al.*, 1998), Democratic Republic of Congo (former Zaire) (Chartier *et al.*, 1991, Bagalwa *et al.*, 1996), Sudan (McCauley *et al.*, 1983; Aradaib *et al.*, 1995), Ethiopia (Negesse, 1994), Nigeria (Olusi, 1996) and Senegal (Diaw *et al.*, 1998). The paucity of reports of this infection in other East African countries like Uganda and Kenya is largely due to the lack of studies regarding the parasite rather than its absence (Magoma *et al.*, 1999). In North Africa, *S. bovis* is less common in Morocco (Freton *et al.*, 1989) and is possibly absent in Egypt and neighbouring countries such as Tunisia and Libya.

Beyond North Africa it extends into the Middle East countries (Massoud, 1973; Arfaa, 1976; Markovics *et al.*, 1993), and Mediterranean countries such as Italy (Mouahid and Mone, 1990), and the Iberian peninsula (Ramajo and Martin, 1972). Other reports of the occurrence of *S. bovis* in Asian countries such as Pakistan (Anwar and Gill, 1990; Javed *et al.*, 1993) and Bhutan (Win *et al.*, 1991) are debatable in view of the absence of the intermediate snail hosts in these areas (Hussein, 1973). Studies have shown that *Bulinus* snails from Yemen were not compatible with *S. bovis* (Orecchia *et al.*, 1973). Similarly, although *S. bovis* has been reported in imported ruminants in Saudi Arabia it has not been reported in the indigenous cattle (Ghandour, 1991). Furthermore, Brown (1997) has shown that the snails which were thought to be *Bulinus indicus* were in fact *Ameriana carinata* which are refractory to schistosomes. In Africa, south of 10° S, *Schistosoma mattheei* predominates as the domestic ruminant schistosome. Hence, *S. mattheei* is the most

important bovine schistosome species in Zambia, Zimbabwe and South Africa (de Bont, 1995; van Wyk, 1983). Other ruminant *Schistosoma* species such as *S. leiperi* and *S. margrebowiei* are confined mainly to Zambia, northen of Botswana and Namibia along the Caprivi strip, due to the restricted distribution of their primary definitive hosts, *Kobus lechwe* and *K. vardoni* respectively (Pitchford, 1976). Outside this region *S. leiperi* has been reported in cattle in the southern part of Tanzania (Dinnik and Dinnik, 1965) and in Uganda (Malek and Ongom, 1984). Therefore, in Tanzania *S. bovis* is the most predominant species (Dinnik and Dinnik, 1965, Southgate, 1980; Kassuku *et al.*, 1986; Makundi *et al.*, 1998). It has been suggested that in the southern highlands of Tanzania, it overlaps with *S. mattheei* and *S. leiperi.* The previous report of *S. bovis* occurring in Zambia is debatable in view of the recent studies carried out by de Bont (1995). Also the recent reports of the absence of *Bulinus africanus* in Zambia, the primary intermediate host for *S. bovis,* further dismisses the possibilities of its occurrence in Zambia (Brown and Rollinson, 1996). The newly accepted bovine schistosome species, *S. curassoni*, occurs mainly in West African countries and is an important parasite of small ruminants rather than cattle (Vercruysse *et al.*, 1984, 1994; Diaw *et al.*, 1998).

2.1.3.4 Transmission biology

2.1.3.4.1 The intermediate host snails

The transmission of trematode infections in domestic animals and human beings depends on interaction between the parasite, the definitive host and the intermediate freshwater snails and is influenced by a complex of environmental factors, in particular the climate (Boray, 1969; Chejina, 1994). However, regarding

ruminant fasciolosis and schistosomosis, the type of management system plays the most significant role (Silangwa, 1973, Kassuku *et al.*, 1986).

The successful transmission of *S. bovis* depends on the existence of the compatible freshwater snail as described above. The survival of intermediate host snails in a true tropical climate depends on the availability of fresh stagnant or slow-moving water enriched with required nutrients (Chejina, 1994). Therefore, in the permanent habitats in the tropics, except for the tropical highlands, temperature is not a limiting factor and transmission may occur throughout the year, although fluctuations in the number of snails may occur with fluctuations in water levels (Kassuku *et al.*, 1986; Ramajo and Simon, 1988; Diaw *et al.*, 1998). Low temperatures may slow down the rate of development of the larval stages in the intermediate snails.

Studies in Tanzania have shown that the traditional management method of utilizing limited water resources for watering large numbers of herds of cattle creates favourable conditions for intensive transmission (Kassuku *et al.*, 1986). The southern tropical highlands of Tanzania have an undulating landscape with hills and valleys, which are transacted by a maze of seasonal/permanent water springs, ponds, tributaries, streams and rivers, thus forming favourable habitats for snails. Along the river valleys there are numerous burrows that for many years have been created by man while making earth bricks. The rivers flood these burrows during the rainy season and as the water subsides, pools of water remain which not only are favourable for snail breeding but also for watering cattle. Most of these dry out as soon as the dry season starts. Thus transmission of bovine schistosomosis is seasonal with the highest intensity at the end of the rains (Reinecke, 1970; Kassuku *et al.*,

1986). However, a few of these water bodies never dry out completely and towards the end of the dry season they maintain large numbers of snails acting as "breeding pockets" for the next season (Marti *et al.*, 1985). The onset of torrential rains and floods disperse them to considerable distances and inhabit the newly formed pools. Even in those ponds which dry out, the freshwater snails are often capable of aestivating, and as the rains start they emerge and due to their relatively higher growth and reproduction rates than in non-aestivated snails, they potentially serve as breeding stock for the next generation (Oyeyi and Ndifon, 1990; Ngonseu *et al.*, 1991). Biological factors such as predators, pathogens and competitor snails may also influence the population of freshwater snails (Madsen, 1990; Estuningish, 1998). However, there is little evidence that they are of practical value under field conditions (Chejina, 1994). Therefore, the population dynamics of freshwater snails in the tropics is largely influenced by the rainfall pattern as it has a profound effect on water speed and feed availability.

2.1.3.4.2 The definitive host

For successful initiation of the vegetative reproduction of schistosomes in the intermediate host snails, it is essential that the schistosome eggs voided with faeces are able to reach water. In this respect, the type of animal management system (traditional agricultural practice) has a major influence as it determines the water contact patterns of the animals. In the traditional animal management systems in tropical Africa, in particular in Tanzania (Masanja, 1986), animals graze extensive communal areas with limited watering facilities and poor unimproved grass. Therefore, during the rainy season when there is plenty of grass and water there is

limited movement of animals. However, during the dry season, as observed in Zambia, large numbers of cattle are forced to share limited grazing and water resources in the lowland or flood plain areas (Silangwa, 1973, 1974). The water bodies, which remain in these flood or lowland areas after the rains, harbour relatively large numbers of freshwater snails. These freshwater snails shed trematode cercariae some of which encyst on the grass as metacercariae (for paramphistome and *Fasciola* species) which later are eaten by the animals. Also cattle may be infected directly through the skin or oral mucosa by schistosome cercariae when they drink or enter water (Kassuku *et al.*, 1985a; Boulanger *et al.*, 1999). Observations in farms in the southern tropical highlands of Tanzania have shown that even when there is an alternative artificial source for watering animals, the cost of maintaining pumped water is exorbitant and most of the time the animals are deliberately watered from the available natural water bodies (Makundi *et al.*, 1998). Furthermore, even when such facilities are inexpensive it is common for animals to utilise natural and artificial water bodies simultaneously and in some farms, breeding of snails in water troughs is not an uncommon finding (Lawrence and Condy, 1970; van Wyk *et al.*, 1974). At the Training for Rural Development Centre (TRDC) farm in Iringa Tanzania, thousands of *L. natalensis* snails were found breeding in the water trough that was build as one of the ways of controlling trematode infection (Kassuku-personal communication). Therefore, the sedentarily kept animals in farms and villages, which are deliberately forced to use limited water resources, are at higher risk than those kept by nomads. However, when there is disruption of animal movement patterns, for instance during severe droughts, wars or in periods of excessively high rainfalls, heavy mortalities may occur in both systems, as observed

recently during El Nino rains of 1997 in Tanzania (Makundi *et al.*, in preparation). Whereas studies on the water contact patterns of ruminants are limited, experience has shown that cattle enter water easily and may spend a long time eating only from available lush pastures during the dry season., Also cattle to defecate directly into the water while drinking water. Such behaviour together with its faecal form, further suggests that cattle are the primary definitive host. Among different groups of cattle, the young age group produces a relatively higher number of eggs in faeces than the older animals (Majid *et al.*, 1980a; Kassuku *et al.*, 1986; Makundi *et al.*, 1998) and theoretically, they should be the major source for contamination of the environment. However, there is a lack of data on the composition of different age groups in cattle populations, which would enable establishment of the relative index for potential contamination by different age groups. More often, farmers would prefer to keep much older animals, even if they are castrates as the old animals have already attained immunity against most killer diseases in the area. Possibly, in traditional herds, the older animals may have the highest index of potential contamination although they have low faecal egg counts in comparison to immature ones, because they constitute most of the herd. In general, the transmission of ruminant schistosomosis is influenced greatly by the type of grazing management and any control scheme should focus on its improvement.

2.1.3.4.3 The parasite

In order for schistosome species to survive, they have developed mechanisms during their life cycle, such as high reproduction potential in the snails (asexual) and in the vertebrates (sexual), to compensate for the losses occurring

during egg, miracidial and cercarial stages. Eggs deposited outside water will not survive except during rainy season, and those deposited in water require a combination of several physico-chemical factors in order to hatch. Since the miracidial lifespan is very short, the miracidium has to come in contact with a compatible host immediately, and due to the nutritive value of ruminant faeces deposited continuously at the cattle watering sites, snails tend to aggregate at the site (Kassuku-personal communication), thus facilitating high transmission (Kassuku, personal-communication). The development of different stages in snails also requires favourable physico-chemical factors. Under optimal conditions the development period for *S. bovis* in *B. africanus* is about 30 days (Mutani *et al.*, 1983). In addition, the digenean larval stages are able to survive in aestivated snails during adverse conditions guaranteeing their successful transmission.

The released cercariae do not feed and may survive in the water for almost 24 hours. In order for the cercariae to obtain contact with the proper host, schistosomes have developed emergent rhythms from the snails that are unique for each species. With regard to bovine schistosomes, *S. bovis* and *S. leiperi* have photophatic circadian rhythm (peak around noon) (Ramajo, 1972), while *S. mattheei* demonstrates photophatic ultradian rhythm (one early in the morning and the second late in the evening) at certain times of the year (Pitchford and Du Toit, 1976). On the contrary, *S. margrebowiei* demonstrates a clear-cut ultradian rhythm, which coincides with the water contact behaviour of its primary definitive host, *Kobus lweche* and *K. vardoni* (Pitchford *et al.*, 1969; Raymond and Probert, 1991). The mechanisms involved are not well known. For instance, it has been demonstrated that the hybrids of *S. mansoni* (photophatic-circadian) and *S. rhodhaini* (scotophatic-

circadian) exhibit ultradian cercarial shedding, one in the daytime and the other in the night.

Host location by the cercariae is triggered by shade, water turbulence and chemo-attractants released by animals. The inherent active upward and downward movement of cercariae around the water surface allows schistosomes to come in contact with their host. The lifespan of the cercaria in the environment is short and the optimal temperatures are between 15-35°C beyond which there are high mortalities (Lawson and Wilson, 1980)). As stated earlier, temperature is very important as it have an effect on the metabolic activity of the snails and the parasite as well as rainfall pattern of any given habitat. Therefore, apart from the temperature casing fluctuation of cercariae with season, the influence of temperature on rainfall pattern indirectly influences the observed seasonality of transmission by affecting the density of the snails (Dazo *et al.*, 1966; Utziger and Tunner, 1986). Perhaps temperature may be of epidemiological importance in the Southern Highlands of Tanzania, where apart from moisture, the low temperature observed during the dry season may account for low *S. bovis* transmission even in permanent water bodies (Kassuku *et al.*, 1986). Similarly, studies in the high veld in Zimbabwe which is dominated by a climate like that of the Southern Tropical Highlands of Tanzania, have shown that transmission of *S. mattheei* was very low during the cool and dry season compared to the warm and rainy period (Chandiwana *et al.*, 1987).

In the final host the schistosomes are capable of surviving for many years intra-vascularly although the vertebrate hosts are capable of developing anti-parasite immune responses. This is a result of the ability of schistosomes to develop various immuno-evasion mechanisms such as reduction of their own antigenicity, *e.g.*

molecular mimicry, acquisition of host antigens (masking), antigenic variation and tegument shedding (McLaren *et al.*, 1975; Pearce and Sher, 1987; Pearce *et al.*, 1990; de Jong-Brink, 1995). Other ways in which schistosomes may evade the host immune system is by secreting detoxifying and antioxidant enzymes against host-effectors of oxygen radicals and modulation of the immuno-competence of the host by producing cytokines, which interfere with the function of the immuno-competent cells (Mosmann and Subash, 1996; Capron, 1998). Due to such formidable ability of schistosomes to evade the host immune system, it has been suggested that the development of effective vaccine should be geared towards attacking the juvenile stages, which are more vulnerable than the adult stages.

2.1.4 Pathogenesis, clinical signs and clinical pathology

The pathogenesis and the clinico-pathological changes due to African bovine schistosomosis are well documented (Dargie, 1980; Saad *et al.*, 1980; Aradaib *et al.*, 1995) and observations are not different from those seen in small ruminants (Monrad *et al.*, 1981, 1990; Kassuku *et al.*, 1986, Johansen *et al.*, 1997b). Unlike fasciolosis, where the immature fluke and the adult parasite have a direct effect on the clinico-pathological changes, in schistosomosis the clinico-pathological changes are associated with the eggs. Therefore, in African bovine schistosomosis the disease may occur as an acute intestinal form or a chronic hepatic syndrome. The acute intestinal form is rarely observed under natural conditions, and these occur mainly in naive animals exposed to heavy primary infections. The acute form of the disease is seen mainly at the onset of egg laying and is characterised by foetid haemorrhagic diarrhoea, anaemia, hypoalbuminaemia, eosinophilia and weight loss

or poor weight gain (Lawrence, 1977a; Saad *et al.*, 1980; van Wyk *et al.*, 1997). The observed anaemia and hypoalbuminaemia during acute disease are directly related to the loss of blood through haemorrhagic enteritis and direct plasma protein loss across the damaged mucosa (Dargie, 1980; Saad *et al.*, 1980). On the other hand, the mechanisms associated with diarrhoea during acute schistosomosis in man and animals are not well established. It has been hypothesised that factors other than size of worm burden, for instance host reactivity, constitute important pathogenic elements in the manifestations of the acute phase of schistosomosis, *e.g.* diarrhoea (Rocha *et al.*, 1995). This is clearly manifested by observation that diarrhoea occurred before the onset of egg excretion and diminished while faecal EPG was still high (Hussein, 1971; Lawrence, 1977a). Recently, studies by Balemba *et al.*, (2000) have suggested that the complex interactions between schistosome egg excretion, immune system cells, the enteric nervous system (ENS) and epithelial cells may be the cause of diarrhoea.

Experimental studies using moderately high single primary infections have shown that the acute syndrome is transient and that the observed acute phase changes diminish gradually and are accompanied with decline of faecal egg excretion, improvement of body weight gains and haematological parameters (Lawrence, 1977a; Saad *et al.*, 1980; Monrad *et al.*, 1995). However, there is limited information regarding the onset and consequences of natural infections and studies in this area are desirable. Van Wyk *et al.*, (1997) demonstrated that calves exposed to very high primary infections (247 cercariae/kg bwt) develop drastic, rapid loss of body mass, severe foetid diarrhoea mixed with blood clots, straining and marked abdominal pain and death. These results validate the observed field outbreaks of the disease (van

Wyk *et al.*, 1974; Markovics *et al.*, 1993) and support the view by Hussein (1980) that the observed herd immunity (Majid, 1980a; Kassuku *et al.*, 1986; Makundi *et al.*, 1998) is probably achieved after severe illness. Following the acute phase manifestations, infected animals enter a lifelong period of chronic infection. Although there is decline in faecal egg output, eggs continue flowing into the liver and studies have shown that the proportion of eggs retained in the liver increases with the age of the infection (Hussein *et al.*, 1975). The eggs lead to continuous formation of egg granulomas, which are replaced by fibrous tissue as they heal resulting in severe portal and peri-portal fibrosis and destruction of the blood vessels. Eggs continue to reach the intestines, too, causing continuous ulcerative gut lesions, though not as severe as during the acute phase. However, during chronic stage of the infection there is significant increase in egg granulomas in the submucosa than during the acute infection (Saad *et al.*, 1980). The observation by Balemba *et al.*, (2000) that egg granuloma in the submucosa can damage the nerve ganglia, neurons and nerve fibres of the enteric nerve system (ENS) in the sub-mucosa suggest that chronic schistosomosis may undermine physiological function of the intestines. The main characteristics of African bovine chronic schistosomosis are emaciation, weakness, anaemia and hyper-gammaglobinaemia. While the cause of anaemia is by blood loss ulcerative gut lesions, the cause of emaciation is loss of plasma protein and electrolytes (Dargie, 1980; Mbassa and Willeberg, 1991). The damage of the ENS may be responsible for reduced gut motility and food absorption. Unlike in human schistosomosis mansoni and japonicum, the observed severe consequences of chronic syndrome such as splenohepatomegally, the *caput medusae* or dilated abdominal collateral veins, ascites and haematemesis due to oesophageal variceal

bleeding are rare in cattle, presumably due to anatomic differences (Hussein, 1973). In bovine schistosomosis, van Wyk *et al.,* (1974) and Lawrence (1977b) have demonstrated that chronically infected cattle challenged with relatively high *S. mattheei* infection may develop drastic loss of body weight accompanied by nervous signs and severe peri-portal hepatic fibrosis akin to Symmer's pipe-stem fibrosis seen in humans which is of immunological origin. Such results have not been documented in cattle infected with *S. bovis,* however, observations in the Sudan, Mali and Tanzania suggest that it may be very common (Makundi, 1993; Kaboret *et al.*, 1993).

2.1.5 Pathology

Major pathological changes in the course of schistosomosis result from delayed hypersensitivity reactions against the eggs in various organs and are largely related to the level and possibly the duration of infection although other factors may also be involved (Hussein *et al.*, 1975, Jordan *et al.*, 1993). Apart from eggs, dead worms cause severe pathological lesions too. Experimental infections of cattle with either 5 000-11 000 *S. bovis* cercariae has shown that from day 120 p.i. there was development of papiliform intimal projections, subintimal eosinophilic infiltration and extensive medial hypertrophy and hyperplasia and concentric perivascular fibrosis and angimatoids in particular in the medium and large portal veins (Hussein, 1971). Adult worms were implicated for causing such lesions and not eggs since no eggs were found in the vicinity and also these lesions were absent in the small portal veins where the eggs were lodged. Similar pathological lesions were seen in naturally *S. bovis* infected cattle in the Sudan where in the severely affected animals, thrombi in the portal veins were found mixed with masses of degenerated adult worms

(Hussein *et al.*, 1975). Similarly, it has been established that, lesions of blood vessels in the liver and intestines particularly during chronic *S. mattheei* infections in cattle were directly attributed to the presence of dead worms (Lawrence, 1977b: 1978). In the description of the pathology of *S. bovis* in cattle, it is desirable to distinguish between the acute phase changes related to intestinal lesions seen during the early patency of infection in animals exposed to massive primary infections, and the chronic phase, "chronic hepatic syndrome" related to the liver lesions seen in longstanding illness. The acute schistosomosis "Katayama fever" in Japan or "toxaemic fever" in Brazil, a serum sickness-like syndrome reported in humans (von Lichtenberg, 1987) has not been reported in cattle.

2.1.5.1 Acute intestinal syndrome

During the acute intestinal syndrome following experimental exposure to moderately high, 10 000 - 14 000 *S. bovis* cercariae (Hussein, 1971) or 25 000 *S. mattheei* cercariae in cattle (van Wyk *et al.*, 1997), the main gross liver pathological lesions included minute, greyish pseudo-tubercles beneath the capsule and in the parenchyma and fibrinous streaks on the surface (Hussein, 1971, van Wyk *et al.*, 1997). In the intestines there was large numbers of schistosomes in the mesenteric veins and enlargement of the corresponding mesenteric lymph nodes, while intestinal mucosa lesions were dominated by disseminated petechial and echymotic haemorrhages, ulcerations and congestion (Hussein, 1971, van Wyk *et al.*, 1997). The ulcerations were characteristic for bovine schistosomosis which is the main cause of anaemia and the situation is different in human schistosomosis mansoni, where ulcerations and anaemia are not essential features (Jordan *et al.*, 1993).

However, in moderately low *S. bovis* infection (5 000 *S. bovis* cercariae) Semuguruka (1992) reported only few punctuate foci of congestion on the intestinal mucosa of calves at 7 to 11 weeks after exposure.

Histopathologically the main liver lesions during the acute phase of infection consist of, schistosome egg granulomas (with 1-16 eggs surrounded by a zone of eosinophils, mononuclear cells, a few epitheliod and giant cells) in the portal areas (Hussein, 1971). Mostly, these egg granulomas exist as a subendothelial polypoid mass, occluding the small intra-hepatic portal veins. These changes are accompanied by limited portal fibrosis and degeneration of adjacent parenchyma. In baboons infected with *S. mansoni* the acute portal lesions is mainly peri-oval granuloma (Farah *et al.,* 1997). The major intestinal histopathological lesions associated with acute phase of *S. bovis* infection include the presence of numerous schistosome eggs in the intestinal mucosa and submucosa. Of these eggs, some are free without inflammatory cell cuffs, while some are florid egg granulomas (with a wide zone of eosinophil and mononuclear cells, mainly lymphocytes, macrophages and plasma cells). The location of eggs or egg granulomas can be extra or intra-vascular. In some sections it is common to see eggs in tandem crossing from submucosa through mucosa into the lumen (Hussein, 1971; Semuguruka, 1992; Makundi, 1993). In addition desquamation of epithelial mucosa, increased goblet cell activity and cystic dilatation of the intestinal crypts filled with degenerated cells and eggs were also observed (Makundi, 1993). Goblet cell hyperplasia is a common finding in baboons infected with *Schistosoma mansoni* (Farah and Nyindo, 1997). Balemba *et al.,* (2000) demonstrated that at 7 weeks post infection there were infiltrations of mast cells, eosinophil and lymphocyte into the ENS ganglia and

neuronal perikarya and this could affect the function of the ENS and might partly account for the observed diarrhoea.

2.1.5.2 Chronic hepatic syndrome

In an enzootic area, low or high primary bovine *S. bovis* infection will eventually end up in longstanding chronic disease and an account of the observed pathological lesions due to *S. bovis* in cattle is well documented under both experimental and natural conditions (Hussein, 1971; Semuguruka, 1992; Makundi, 1993, Kaboret *et al.*, 1993). The liver is the most affected organ. The most observed gross pathological lesions in severe chronic infections includes discolouration (dark-brown-black), irregular surface, fibrosis and thickening of portal tracts, extensive hardening of the liver with areas showing multiple elevated purplish or grey nodules (about 1 cm in diameter). The left lobe being the most affected (Hussein *et al.*, 1975; Kaboret *et al.*, 1993). In the intestines, the associated gross pathological lesions include presence of large numbers of worms in the mesenteric veins and tortuousness of the mesenteric veins engorged with blood. In addition, granulomatous thickening of the mesenteric veins (a short distance from the attachment of the mesentery to the intestine) is a constant feature in *S. bovis* infection in cattle (Semuguruka, 1992). However, the extensive *S. mattheei* granulomatous lesions (of about 5 mm in thickness) in the omental veins in cattle reported by van Wyk *et al.*, (1997) comparable to bilharzioma (schistosomal tubercles found spread over the peritoneum) in humans has not been reported in *S. bovis* infection in cattle. Histopathological observations in the chronic phase of *S. bovis* infection in cattle differ from the acute phase in the degree and extent of fibrosis. Studies in goats have

shown that higher frequencies of fibrotic inflammatory foci were observed in the liver during the chronic stage of *S. bovis* infection in comparison to the acute stage (Lindberg *et al.*, 1997). In advanced chronic *S. bovis* infection, the commonly observed histopathological lesions in the liver include extensive fibrosis of the portal and peri-portal areas involving adjacent parenchyma and marked increased number of Kupffer cells laden with schistosome pigment (Hussein, 1971; Hussein *et al.*, 1975). The characteristic vascular changes in the small portal veins include occlusion by egg granulomas, proliferative endophlebitis, sub-intimal and peri-vascular eosinophilic infiltrations, while in the larger veins, intimal proliferation, medial hypertrophy and granulomatous thrombosis are predominant histopathological lesions. In baboons naturally infected with *S. mansoni*, the chronic lesions were periportal fibrosis. (Njenga *et al.*, 1998). However, compared to observations in ruminants, baboons do not develop significant liver fibrosis even in prolonged experimental *S. mansoni* infections (Sturrock *et al.*, 1988).

2.1.6 Immunity

Although the prevalence of bovine schistosomosis due to *S. bovis* and its close relative *S. mattheei* is very high, the mortality is relatively low due to the ability of cattle and small ruminants to mount strong immunity against the parasites. The ability of these animals to develop resistance has been established following series of experiments involving natural and artificial infections followed by challenge infection. Resistance is then measured as the ability of the pre-exposed animals to suppress challenge worm establishment, survival, fecundity and

associated clinico-pathological changes (Woolhouse, 1994; Monrad *et al.*, 1990, 1999).

Under experimental (artificial) infections, it has been established that cattle (Saad *et al.*, 1980) and goats (Saad *et al.*, 1984b; Monrad *et al.*, 1990, 1995; Johansen *et al.*, 1997b) are capable of developing immunity following moderately high single primary infection with *S. bovis.* In these studies it has been shown that challenge infection neither induce additional faecal egg excretion nor adverse clinical signs and clinico-pathological changes observed during the acute phase (Monrad *et al.*, 1995, 1999). Similar findings have also been reported in *S. mattheei* infections in cattle (Lawrence, 1973). On the contrary, the results obtained from sheep are controversial. Saad *et al.,* (1984b) observed development of strong resistance in sheep against primary experimental *S. bovis* infection even higher compared to goats. However, doper lambs experimentally exposed to 3 000 *S. mattheei* cercariae did not develop resistance at all (Lawrence, 1980). Studies have, however, shown that the development of immunity is influenced by the dosage of the primary infection. Studies on *S. mattheei* infections in cattle have shown that massive primary infections may become lethal (van Wyk *et al.*, 1997) and deaths may occur before the host animal has developed protective immunity. Furthermore, the use of low *S. bovis* infections in goats did not provide full protection against subsequent moderately high challenge infections (Monrad *et al.*, 1995).

Apart from the experimental (artificial) infections, natural study designs have also shown that cattle are capable of mounting strong regulatory response following primary natural infections. Epizootiological studies in the Sudan (Majid *et al.*, 1980a) and in Tanzania (Makundi *et al.*, 1998) have shown that there is an age-

related intensity of infection in cattle with high faecal egg excretion being observed mainly in the 1-3 year age group followed by a gradual decline to a very low level in the older animals. In order to test the immune status of mature cattle in areas of high endemicity, Bushara *et al.*, (1980) challenged naturally immune cattle with massive doses of *S. bovis* cercariae (70 000 per animal) and found no additional faecal egg excretion or adverse clinical signs. Similarly, using tracer calves, Kassuku *et al.*, (1986) and de Bont (1995) have demonstrated that while the mature animals continue to excrete low levels of faecal *S. bovis* and *S. mattheei* eggs respectively, the tracer calves had relatively high faecal egg excretion. Such field studies have not been carried out in goats. However, similar studies in sheep in the same area in the Sudan showed that sheep did not develop significant immunity (Majid *et al.*, 1983). In general, cattle and goats are capable of acquiring protection against the pathogenic effects of homologous schistosome challenge infections.

Apart from the homologous immunity observed in ruminant schistosomosis, heterologous immunity is also exhibited between trematode parasites. Substantial resistance has been observed in sheep harbouring non-patent and newly patent *S. bovis* infection against *F. hepatica* (Monrad *et al.*, 1981). Similarly, heterologous resistance has been observed in cattle between *S. bovis* and *F. hepatica* (Sirag *et al.*, 1981) and *S. bovis* and *F. gigantica* (Yagi *et al.*, 1986). The observed development of immunity following trematode infection increased the need for studying the mechanisms involved in order to design control methods such as vaccination.

As in cattle, epidemiological and re-infection studies have shown that humans can develop immunity against schistosome infections. Studies on *S. haematobium* and *S. mansoni* infections in humans in Tanzania, Nile Delta in Egypt

and in Gambia have shown that parasite occurrence or absence (prevalence) and abundance (intensity) tends to rise during childhood, reaches peak during teens age or early old age and declines thereafter (Bradley and McCullough, 1973; Wilkins *et al.*, 1987). Furthermore, it was shown that the convexity of the age related prevalence and intensity patterns of human schistosomosis in endemic areas were stable in most foci but may vary from year to year depending on the rainfall pattern (Wilkins, 1989). Therefore, in areas of high transmission intensity the peak in egg excretion in the urine or faeces in *S. haematobium* and *S. mansoni* infection respectively is achieved at much early age compared to the areas of low transmission. Since the transmission intensity has a marked influence on the results obtained from intensity and prevalence patterns during epidemiological studies on human schistosomosis, treatment and challenge infections were essential in the validation of the development of immunity in humans. Studies by Butterworth *et al.*, (1985) in Kenya have shown that a proportion of children cured of natural *S. mansoni* using hycanthone fail to be re-infected. Furthermore, Wilkins *et al.*, (1987) found that in children that were cured of natural infections in Gambia, re-infection rate was higher in the under 10 years than in the 10-14 years age group despite of the similar water contact patterns.

For a long time, the observed development of immunity against schistosome infections in humans based on epidemiological and re-infection studies were considered circumstantial rather than clear evidence as demonstrated in some animal models. For instance, with schistosomosis mansoni, the primary infections in rat can be terminated by immunity. Rhesus monkeys can develop substantial immunity while mice and baboons show less resistance to challenge infections

(Damian *et al.*, 1976; Peck *et al.*, 1983; Nyindo and Farah, 1999). Both permissive (worms complete their life cycle) and non-permissive (worms never reach maturity) animals have been used to elucidate the protective mechanisms of the development of immunity. Although these animals may not mirror the true situation in the primary definitive host such humans for *S. mansoni* and bovines for *S. bovis*, it was anticipated that they might have unique protective system. Therefore, several mammal species have been used in experimental infections such as inbred rodents, monkeys, baboons and useful information regarding different aspects of the disease such as pathology, immune response and efficacy of drugs has been achieved.

Recent studies have shown that non-human primates such as baboons (olive baboon-*Papio anubis* and yellow baboon-*P. cynocephalus cynocephalus*) are excellent models for studying regulatory mechanisms on immunity and pathology in schistosomosis in humans (Nyindo and Farah, 1999). Baboons are preferred as a model for humans compared to other animal species since anatomically, genetically and immunologically are closely related to humans. Studies have shown that baboons acquire natural schistosome infections and develop similar clinical symptoms, pathology and immunity as humans (Nyindo and Farah, 1999). Studies in five troops of olive baboons in Gombe Stream National Park in Kigoma, Tanzania have demonstrated an age specific prevalence and intensity of infection with *S. mansoni* comparable to what is observed in humans (Muller-Graff *et al.*, 1997). Also experimental exposure and challenge studies have demonstrated that, baboons can develop strong resistance to re-infection with *S. haematobium* (Webbe *et al.*, 1976). Similarly, primary exposure of rhesus monkey (*Macaca mulatta*) wit 200 *S. mansoni* cercariae (Kenyan strain) protected them against challenge with 2 000 cercariae of

the same strain at week 16 post infection (Damian *et al.*, 1976). Although previous studies have shown that baboons were unable to develop significant liver fibrosis even in prolonged *S. mansoni* infection (Sturrock, *et al.*, 1988), recent experimental and natural infection studies have demonstrated development of appreciable classical peri-portal liver fibrosis similar to observations in chronically infected humans (Njenga *et al.*, 1998, Farah *et al.*, 2000). Such results suggest that baboon model closely mimics the pathogenesis observed in humans.

Unlike in domestic ruminants and humans, evidence on the development of immunity to schistosome infections in rodents have depended mainly on re-infection experimental studies rather than epidemiological ones. With the use of normal, transgenic (induced to over produce particular protein) and knockout (targeted depletion of a particular gene) rodents, it has been possible to demonstrate various aspects of the mechanism underlying the development of immunity in *Schistosoma* infections (King *et al.*, 1997; Lucas, *et al*, 1997). Studies in mice have shown that in chronically *S. mansoni* infections there was development of acquired resistance that was associated with suppression of formation and downsizing (modulation) of egg granulomas (Warren and Domingo, 1970). Furthermore, such immune responses were accompanied with decrease in hepato-splenomegally and disappearance of oesophageal varices (Warren, 1977). In general, studies in mice have been mainly directed in elucidating the mechanisms involved in the development of immunity.

Due to the complex nature of the different developmental stages (schistosomulum, adult and egg) of schistosomes in the host, a wide number of immune responses have to be developed by the host. In most of the animal models studies, adult schistosomes seem to be protected against the effector mechanisms.

What is quite common is an anti-fecundity that is seen in cattle and goats infected with *S. bovis* and *S. mattheei* (Lawrence, 1973; Bushara *et al.*,1980; da Costa *et al.*, 1999). Most of the effector mechanisms are directed against the penetrating juvenile flukes and this is well demonstrated in "swimmers itch" where avian schistosmes cercariae are completely arrested in the skin of humans by allergic reactions. For effective acquisition of protective immunity, cooperation of both humoral and cell-mediated effector mechanisms is necessary (Nansen, 1985; Capron *et al.*, 1977).

Studies on the role of humoral factors in *S. mansoni* infection in humans has shown that infected people had higher percentage of complement dependent IgG antibodies (63.4%) cytotoxic to schistosomules compared to sera from non-infected persons (8.7%)(Capron *et al.*, 1977). However, further studies on humoral factors have shown that during *S. haematobium* and *S. mansoni* infection in humans, there was stimulation of IgE antibodies response that was protective (Dunne *et al.*, 1992; Hagan *et al.*, 1991). The recent demonstration of the comparable protective IgE antibody responses in schistosomosis mansoni in baboon further validates the use of this animal species as model for studying human schistosomosis (Nyindo *et al.*, 1999). Despite of the production of protective humoral factors in humans, production of blocking antibodies in young children has been implicated as the major cause of inability of young children to develop effective immunity (Khalife *et al.*, 1986; Butterworth *et al.*, (1987). So far very little work has been done as regards to the role of humoral factors on the immunity of bovine schistosomosis. Bushara *et al.*, (1994), have shown that, immune sera from calves with maturing *S. bovis* infections contained factors capable of causing, in addition to worm death, suppression of worm fecundity.

Apart from humoral factors cell-mediated responses such as neutrophils, eosinophils, macrophages basophils and monocytes have been shown to play significant role in the development of protective immunity in schistosomosis in different animal species (Capron *et al.*, 1982). In attacking the juvenile parasite (schistosomulum), studies have shown that attrition *in vivo* commonly occurs at post skin sites particularly in the lungs and killing is mediated mainly through antibody dependent cellular cytotoxicity (ADCC) reactions (Capron *et al.*, 1982). Studies have shown that there was a loss of susceptibility of microphage-mediated killing during maturation of the schistosomula from the skin to the lung stage (Sher, *et al.*, 1982). Studies in murine schistosomosis have shown that regulatory cytokines control the host cell-mediated immunity. The involved cells, the CD^{+4}or CD^{+8} T-cells, can be divided into two subsets (Th1 and Th2) based on the type of cytokine they produce (Sher, 1995; Fallon *et al.*, 1998). These rodent models have shown that during early stages of infections the parasite stimulates certain T-helper type 1 cells (Th1, CD^{+4} or CD^{+8} phenotypes) to secrete macrophage-activating lymphokines (mainly interleukin-2 (IL2), tissue necrosis factor-alpha (TNF-α and interferon gamma (IFN-γ) (Sher and Coffman, 1992; Mosmann and Subash, 1996). The activated macrophages would produce toxic substances such as reactive nitric oxide that eventually kill the parasite (James and Nancy, 1993). Studies in mice infected with *S. mansoni* have shown that at the onset of egg laying there is a switch from predominantly a Th1 cytokine to a Th2 cytokine pattern (Pearce *et al.*, 1991, Fallon *et al.*, 1998). The egg antigens stimulate another type of T-cells (Th2, CD+4 or CD+8 phenotypes) to secrete Th_2 cytokines such as IL4, IL5, IL10 and TNF-α (James and Nancy, 1993). Unlike the Th2 responses, which recruit macrophages,

Th2 responses recruit eosinophil cells for killing the parasite (Mosmann and Subash, 1996). The Th2 cytokine responses are considered to be induced mainly by the eggs (Vella and Pearce, 1992). Large granulomas composed of macrophage; lymphocyte and eosinophil cells that occur during early primary schistosome infections have a damaging effect on the liver parenchyma and may block the small portal veins of the host. Therefore, sequestration and reduction in the size of the granuloma is beneficial to the infected host. It has been suggested that Th2 has a role of regulating the size of egg granulomas (immuno-modulation) in the tissues but at the same time is responsible for down regulation of Th1 responses (Sher, 1992). The Interleukin, IL-10 has been implicated as the major Th2 cytokine responsible for down regulation (Sher, 1992). Therefore, while the early Th1 responses have an effect of preventing super-infestation of the host with the parasites, the Th2 responses apart from having immuno-modulating effect on egg granulomas that is beneficial to the host, has down regulatory effect on the Th1 responses allowing re-infection and survival of worms, which is beneficial for the parasite but detrimental to the host. Recent studies have also shown that with the ageing of schistosome infections there is stimulation of another type of T-cells (Th3 CD+4 or CD+8 phenotype) which releases fibrogenic cytokines such as transforming growth factor-β (TGF-β) that are mitogenic to fibroblasts and are responsible for fibrosis formation (Meeusen, 1999). Studies in baboons have also demonstrated the role of cytokines in the regulation of granulomas and treatment with praziquantel has been shown to boost the TGF-β (Mola *et al.*, 1999). Similarly, studies *in vitro* have demonstrated that, cytokines (Il-10) could modulate granuloma formation in human schistosomosis mansoni similar to the observations in rodent and monkey models (Couissinier-Paris and Dessein, 1995;

Falcão *et al.*, 1998). At present, there is a lot of work going on regarding the role of the regulatory cytokines in controlling the host cell-mediated immunity and such details are beyond the scope of the present study.

So far, the information on the above effectors of mechanisms is derived from experiments involving laboratory rodents rather than natural ruminant hosts. However, there are indications that bovines may have unique immune responses. Existence of bovine type 1 and type 2 responses have been demonstrated and interleukins have been shown to be involved in the regulatory response of cattle to *Babesia bovis* and *Fasciola hepatica* infections (Chitko-McKown, *et al.*, 1995; Brown *et al.*, 1998). Hence, it is necessary to combine this rodent-based knowledge with parasitological observations from classical ruminant field studies and experimental approaches. Such studies have shown that chronically *S. bovis* infected cattle harbour relatively large numbers of worms that correspond with the low faecal egg excretion (Bushara *et al.*, 1980). The mechanisms involved were studied by (Bushara *et al.*, 1980). By transferring worms from chronically infected cattle into naive cattle it was observed that a relatively higher faecal egg excretion resulted in the naive animals than it did in the old animals. Studies have also shown that, goats, which have developed resistance to primary infection, could acquire additional worms upon challenge but egg excretions continue to remain low (Johansen *et al.*, 1997b). These results together with other similar studies in *S. mattheei* (Lawrence, 1973) in cattle suggest that anti-fecundity was the major mechanism of immunity in ruminant schistosomosis. Recent studies have shown that antibodies against a 28kDa glutathione S-transferase (GST) of *S. bovis* were responsible for the anti-fecundity effect as well as anti-viability of the schistosome eggs (da Costa *et al.*, 1999).

Therefore, 28 kDa *S. bovis* GST is a potential immunogen and most of the current native and synthetic vaccines against schistosomes are based on this antigen.

2.1.7 Diagnosis

Due to the chronic nature of bovine schistosomosis and fasciolosis, clinical diagnosis of these infections is difficult as they are often complicated by other concurrent debilitating diseases such as trypanosomosis and other helminth infections (Reinecke, 1970). Parasitological techniques such as filtration technique (Lawrence, 1970, Kassuku *et al.*, 1985b; Olaechea *et al.*, 1990) and miracidia hatching technique (Lawrence, 1970; Kassuku *et al.*, 1985b) that detect ova in the faeces have been recommended for use in laboratory and in field studies due to their being relatively simple and cheaper compared to other methods. However, egg detection has its limitations, as it cannot be used to estimate the worm burden because of the great variation in faecal egg counts within the sample between individuals (Yu *et al.*, 1998). Therefore, when it is necessary to accurately evaluate worm burden, such as during anthelmintic trials or determination of the development of acquired resistance, perfusion techniques are used (McCully and Kruger, 1969; Johansen *et al.*, 1996a). Parallel to the parasitological techniques, more sensitive immuno-diagnostic methods based on detection of serum antibodies were used extensively (Hussein, 1972; Preston and Duffs, 1975; Kassuku and Ng'ambi, 1988). However, due to low specificity, which results from cross-reaction between trematode parasites (Fagbemi and Obarisiagbon, 1991;) the detection of serum antibodies is of limited application. Cross-reaction in some immunodiagnostic tests also occurs between trematodes and other parasite species such as cestodes (Geerts *et*

al., 1981) and GIT-nematodes (de Noya *et al.*, 1996). For instance, in humans, the cross reactivity between schistosome species and GIT-nematodes in enzyme linked immunsorbent assay (ELISA) with soluble egg antigens (SEA) from *S. mansoni* is due to existence of common glycosylated epitopes (de Noya *et al.*, 2000). Alternatively, assays based on detection of circulating cathodic antigens (CCA) and circulating anodic antigens (CAA) are becoming more popular, not only because of their high sensitivity but also due to high specificity and good correlation with worm burden (Deelder *et al.*, 1989; Bergquist, 1992; Johansen *et al.*, 1996b). The popularity of the method has increased even more as it can be used in the assessment and monitoring of chemotherapy (de Jonge *et al.*, 1989; Shaker *et al.*, 1992). More specific serological techniques involving purification and characterization of a cysteine protease (Fagbemi and Hillyer, 1992) have also been developed. Other techniques, such as detection of serum proteins, liver enzymes and electrolytes and haematological parameters, are used indirectly to diagnose the infection (Dargie 1980; Saad *et al.*, 1980; Kassuku *et al.*, 1985a; Mbassa and Willeberg, 1991). These methods are useful as they show the electrolyte and nitrogen status of the animal. The loss of protein and electrolytes are the major causes of lowered productivity in the infected animals (Holmes and Chowdhury, 1994).)

2.1.8 Treatment and control

2.1.8.1 General control methods

In the control of human and domestic animal trematode infections, it is essential to interrupt the life cycle of the parasite. Various strategies have been employed such as snail control using molluscicides (Madsen *et al.*, 1986; Fenwick,

1987), biological methods (Madsen, 1990), provision of clean water and environmental manipulations. Other methods include farmers' education and anthelmintic treatment of the definitive hosts (Dinnik, 1967; Bushara *et al.*, 1982; Markovics *et al.*, 1985). However, snail control methods such as the use of chemical molluscicides usually have negative environmental impact due to their pollution effects. Even after successful killing of snails, the re-colonization of the water bodies by snails is fast. Apart from pollution effects, chemical molluscicides are very expensive and practically impossible to apply on the extensive communally grazed areas along riverbeds in tropical Africa. Therefore, due to costs and temporary nature of snail control methods, and taking into account the current situation of the extensive communal grazing systems in many parts of Africa, there is insufficient economic justification for using such methods at present. Similar difficulties exist in applying snail control methods against human schistosomosis, not only because of ineffectiveness but also due to the ever-declining socio-economic resources in sub-Saharan Africa (Gryseels and Polderman, 1991; Gryseels *et al.*, 1992).

Another alternative method for controlling the disease is the development of a vaccine (Bergquist and Colley, 1998). Initially, some achievements were made in the production of live attenuated vaccines (Nansen, 1975; Taylor *et al.*, 1976). The attenuated live vaccines such as irradiated *Fasciola* metacercariae (Bitakaramire, 1973; Nansen, 1975) and irradiated schistosome cercariae or schistosomula (Taylor *et al.*, 1976; Majid *et al.*, 1980b) proved to be highly efficacious. However, these vaccines have limited application for use on a large scale in the field, as they cannot be produced continuously in large quantities. Furthermore, they require sophisticated storage and transportation facilities such as the use of liquid nitrogen (James and

Farrant, 1976). Therefore, the current strategy is the development of defined antigen vaccine followed by synthesis in large quantities through the rapidly developing biotechnological methods (Pritchard, 1988; Capron *et al.*, 1994). Initial studies showed that the concealed cuticular trematode antigens such as glutathione-s-transferase (GSTs - kD28 and kD26 which are secreted transiently by the parasite), schistosome paramyosin, (sm 97 which is not exposed to the surface) and phospholipid (GP 32 and 38 which are anchored on the surface) could be potent immunogens (Pritchard 1988; Sher *et al.*, 1989). Further studies have shown that, glutathione-s-transferase of the schistosomes (a 28 kDa protein) was the major antigen, which was responsible for inducing immunity in man and ruminants (Capron *et al.*, 1994).). Suppression of *S. bovis* egg production in cattle without reduction in worm burden by vaccination with either glutathione S-transferase or keyhole limpet Haemocyanin was observed in the Sudan (Bushara *et al.*, 1993). Vaccination-using GSTs in goats (Boulanger *et al.*, 1994) and in sheep (Boulanger *et al.*, 1999*)* led to significant reduction in worm burden. Since the adult schistosome does not multiply within the body of the host and since pathology is caused mainly by the viable eggs, the observed anti-fecundity (Capron *et al.*, 1994; Liu *et al.*, 1995; Bergquist and Coley, 1998; Boulanger *et al.*, 1994, 1999) and anti-egg viability (da Costa *et al.*, 1999) in animals vaccinated with GSTs make GSTs potential vaccine candidates, even though they may not be accompanied by reduced worm burden. Although there have been enormous efforts to develop trematode vaccines, commercial production of these vaccines has not been achieved. Therefore at present chemotherapy is considered to be the most reliable method available for attacking the parasites in the field.

2.1.8.2 Chemotherapy

The purpose of treatment of schistosomosis is to cure infected animals and control the spread and/or transmission of the infection. The strategic use of anthelmintics has been recommended when based on established epidemiology of the diseases to reduce mortality and morbidity of infection in areas of high transmission (Mahlau, 1975, Boray, 1994). The history on the treatment of schistosomosis goes back to 1,700 BC where antimony was used in Egypt (Cioli, 1998). However, the first published record on successful treatment of schistosomosis was carried out by Hutchison in China in 1913 (Cioli, 1998) and in Egypt by Diamantis in 1917 (Cioli, 1998) using emetine. The antimony and its salts were highly toxic in ruminants where they caused cessation of rumination, severe diarrhoea, anorexia and death. Antimony and antimony salts (tartar emetic and stibophen) was the only drug available for treatment of schistosomosis in man and animals until early1960s, when new drugs such as metrifonate, lucanthone, niridazole, hycathone and oxamniquine were introduced into the market in 1960, 1962, 1964, 965 and 1969 respectively Cioli, 1998). In cattle, Dinnik (1967) used metrifonate (Neguvon®) against *S. bovis,* but an effective cure was only achieved after four to six treatments. Furthermore, only insufficient cure of sheep infected with *S. mattheei* using lucanthone and hycanthone was observed (Lawrence and McKenzie, 1970). Injectable trichlorphon was used to control an outbreak of *S. mattheei* in cattle, but at least 11 treatments at intervals of three to five days apart were necessary (van Wyk *et al.*, 1974). In general, all these early drugs were highly toxic and/or less effective especially for the treatment of immature worms than the present ones.

The discovery of praziquantel (PZQ) in 1972, a safe, highly efficacious and broad-spectrum cestodicide and schistosomicide was the most important development in the chemotherapy of schistosomosis (Webbe and James, 1977). Since then, the drug has been extensively used successfully as the drug of choice in the morbidity control of human schistosomosis (Brindley, 1994; Cioli, 1998). However, to date very little work has been carried out to cure cattle and small ruminants schistosomosis in general (Bushara *et al.*, 1982; Markovics *et al.*, 1985, Johansen *et al.*, 1996a,c).

Studies have shown that praziquantel acts by causing immediate flaccid paralysis (loss of sensitivity to further stimuli) of the schistosome muscles (Blair *et al.*, 1992, Day *et al.*, 1992) and extensive surface blabbing, swelling, vacuolation and disruption of the tegument and sub-tegumental tissues (Becker *et al.*, 1980; Shaw and Erasmus, 1987; Harnett, 1988). Furthermore, other workers have demonstrated that such damage on the tegument and sub-tegumental tissues exposes the concealed parasite antigens that are potent immunogens (Day *et al.*, 1992) and it also depletes glutathione from the parasite (Ribeiro *et al.*, 1998). The main mechanism, which brings all these changes, is not fully understood. However, the alteration of intracellular Calcium (Ca^{2+}) homeostasis has been considered to be the major cause. Studies have shown that praziquantel interacts with specific Ca^{2+} permeable sites on the tegument and sarcoplasmic membrane of the parasite and under elevated Mg^{2+}: Ca^{2+} ratios, these sites are blocked by Mg^{2+} leading to flaccid paralysis (Blair *et al.*, 1992).

It has also been established that the actions of praziquantel in mice depend on appropriate immunological stimulation (Doenhoff *et al.*, 1987). Praziquantel was

observed to kill fewer worms in T-cell deprived mice than in immunologically intact controls. The evidence of immune-dependent action of praziquantel has also been demonstrated in mice by combining the drug with rabbit antiserum raised against adult *S. mansoni* (Doenhoff *et al.*, 1987). Active immunization of mice with *S. mansoni* worm membrane antigens enhanced the efficacy of praziquantel (Fallon and Doenhoff, 1995).

Apart from immune dependency, it has also been shown that praziquantel action is stage-dependent and it is less effective against immature worms in comparison to mature ones (Sabah *et al.*, 1986, Shaw, 1990). Such stage dependent susceptibility of praziquantel has been proved to occur both *in vitro* and *in vivo* (Xiao *et al.*, 1985). The mechanisms involved are not very clear but it has been suggested that it could be related to the host immune response (Shaw and Erasmus, 1987; Modha *et al.*, 1990) as well as to the phospholipid composition (Shaw and Erusmus, 1987).

Unlike in human beings and in laboratory animals, very few studies have been carried out in cattle to determine the correct dose of the PZQ to be used. In the Sudan, Bushara *et al.*, (1982) used 20 mg/kg of praziquantel in cattle experimentally infected with *S. bovis* at 9 and 14 weeks post infection, and the drug was found to be highly effective and reduced EPGF to zero, and worm burden was reduced by 98.9%. In Israel, Markovics *et al.*, (1985) used praziquantel in heifers naturally infected with *S. bovis* and 20 mg/kg was found to be highly effective while 10 mg/kg reduced EPGT only for a week and the count returned to the level seen before treatment. In *S. nasale* infections in cattle, 20 mg/kg of praziquantel was found to be highly effective and caused considerable cessation of clinical signs, reduction in egg counts and

progressive regression of the nasal granulomatous growths (Rahman *et al.*, 1988; de Bont *et al.*, 1989). In goats 60 mg/kg of praziquantel was found to be highly effective against *S. bovis* (Johansen *et al.*, 1996a). Even a lower dose of 12 mg/kg (10% praziquantel) given intra-muscularly was highly effective in the treatment of cattle experimentally infected with *S. japonicum* (Hu *et al.*, 1989). So far there is no clear-cut recommended dosage for use in ruminants although the above studies suggest that morbidity control in bovine schistosomosis can be achieved with even lower doses than 40 mg kg^{-1} used in humans. However, when it is necessary to eliminate all worms for example during drug efficacy trials or in immunity studies, it may be necessary to use a much higher dose than the recommended curative doses.

Although there are several advantages in early treatment of ruminant schistosomosis, there has always been a fear regarding its consequences on the liver pathology and the acquired immunity (Reinecke, 1970, Bushara *et al.*, 1983b; Johansen *et al.*, 1996a,c). Earlier treatment of ruminant schistosomosis with trichlorphon led to severe pathological changes and even deaths as observed in sheep (Lawrence, 1976). However, the observed negative consequence of treatment with earlier drugs including antimonial compounds was due to drug toxicity rather than factors derived from the death of worms. Extensive studies on the use of praziquantel in laboratory animals and humans have shown that this drug is well tolerated and causes involution of schistosomal related pathology (Fu-yuan *et al.*, 1984, Andrade *et al.*, 1993; Boisier *et al.*, 1998; Hatz *et al.*, 1998). Studies have shown that ruminants also tolerate praziquantel treatment (Bushara *et al.*, 1982, 1983b; Markovics *et al.*, 1985, 1993; Johansen, 1996a) although treatment is accompanied by severe liver lesions associated with dead worms (Johansen *et al.*, 1996c). So far

no reports of negative clinical signs have been observed following praziquantel treatment in ruminants. Since praziquantel treatment of baboons infected with *S. mansoni* has been observed to boost production of TGF-β cytokine (Mola *et al.*, 1999), which is responsible for the formation of fibrosis, the use of antifibrotic agents during treatment of schistosomes with praziquantel is essential. The antifibrotic agents such as octreotide (Mansy *et al.*, 1998a,b), and tiaprofenic acid (Botros *et al.*, 1988; Hegazy *et al.*, 1997) and beta-aminopropionitrile (Giboda and Smith, 1997) have been shown to reduce the degree of hepatic fibrosis when used as adjuvant to praziquantel in the treatment of schistosomosis in mice. Similarly, it has been observed that anti-interleukin-4 treatment in mice infected with *S. japonicum* diminishes the secretion of Th2 cytokines leading to inhibition of hepatic fibrosis (Cheever *et al.*, 1995). Perhaps similar anti-interleukins can be searched for *S. bovis* infections in cattle and be used following praziquantel treatment.

Another fear of using highly efficacious anthelmintics such as praziquantel is the danger of interfering with development of concomitant immunity (which depends on presence of adult worms), especially when the treatment is carried out at an early patent stage of the infection. However, studies in the Sudan have shown that chemotherapy of natural *S. bovis* infection in cattle that have already acquired resistance did not break the existing resistance (Bushara *et al.*, 1983b). Similarly, studies in goats have demonstrated that the elimination of primary *S. bovis* infection by praziquantel did not hamper the development of immunity (Monrad *et al.*, 1999). Studies in mice have also shown that although immunity declines with time following treatment, significant resistance against re-infection could still be detected at 16 weeks post infection (Farghaly *et al.*, 1993). In human beings chemotherapy

has been observed to even accelerate the development of acquired immunity to *S. mansoni* (Mutapi *et al.*, 1998).

While no new drug has been introduced in the market for the treatment of schistosomosis for the past 29 years after the introduction of praziquantel, the use of other potent drugs such as oxamniquine has declined (Cioli, 1998). Furthermore, although there are several new drugs on trial such as artesunate (Li and Wu, 1998; Arujo *et al.*, 1999), artemether (Xiao *et al.*, 1994) and cylosporin-A (Bout *et al.*, 1986; Cioli, 1998), these drugs are still in the trial stage and they have not entered the market. Therefore, overuse of praziquantel alone has increased the danger of schistosomes developing resistance as reported in Senegal, where low cure rates using recommended doses of 40 mg kg^{-1} (Stelma *et al.*, 1997) and even at high doses of 60 mg/kg (Guissé *et al.*, 1997) have been reported. Further laboratory studies have also proved that *S. mansoni* isolates from Senegal had reduced susceptibility to praziquantel in comparison with isolates from Kenya and Puerto Rica (Fallon and Doenhoff, 1995). In the Senegal study, where transmission of *S. mansoni* was very high it was argued that the reduced cure rate of praziquantel was a result of the drugs' limited effect on immature worms (Sabah *et al.*, 1986). However, the danger of developing resistance is real, as it has been possible to produce resistant strains of *S. mansoni* against praziquantel after treatment of mice with sub-curative multiple doses in laboratory studies (Fallon and Doenhoff, 1994).

2.2 Bovine fasciolosis

2.2.1 Epidemiology

When studying bovine schistosomosis under field conditions in tropical Africa, inclusion of aspects of fasciolosis are inevitable. This is not only because the infection is highly prevalent in East and Central African countries, as reported in Zambia (Silangwa, 1974), Tanzania (Mahlau, 1970; Msanga, 1985; Kambarage *et al.*, 1995), Zaire (Chartier *et al.*, 1990), Kenya (Wamae, 1990) and Uganda (Magoma *et al.*, 1999), but also because fasciolosis causes possibly higher production losses and severe pathological lesions than schistosomosis (Kambarage *et al.*, 1995; Maingi and Mathenge, 1995; Pandey and Ahmadu, 1998; Wamae *et al.*, 1998). On the other hand, putting too much emphasis on fasciolosis has masked other coexisting liver disease conditions in the tropical Africa such as schistosomosis. Results from limited studies in Tanzania suggest that they are equally important (Dinnik and Dinnik, 1965; Kassuku *et al.*, 1986; Semuguruka, 1992 and Makundi *et al.*, 1998). Even the slaughterhouse reports of condemned livers due to fibrosis are associated with fasciolosis, the coexisting schistosomes and *Dicrocoelium hospes* cause similar lesions (Msanga, 1985; Makundi, 1993; Makundi *et al.*, 1995; Hussein *et al.*, 1975).

In East and Central Africa *Fasciola gigantica* is the most predominant liver fluke species that infects domestic animals. However, the little liver fluke, *Dicrocoelium hospes* may be next after *F. gigantica*, especially in Uganda (Kajubiri and Hohorst, 1977) and in the western and southern highlands of Tanzania (Mahlau, 1970; Makundi *et al.*, 1995). *Fasciola hepatica* is said to exist in some limited parts of the tropical highlands of Kenya (Preston and Castellino, 1977) and Ethiopia (Malone *et al.*, 1998). The existence of *F. hepatica* in Tanzania is debatable (Mahlau,

1970) and the reports of its occurrence (Goodman *et al.*, 1973; Akkaro and Maro, 1992; Kusiluka *et al.*, 1995) could be faultly diagnosed. On the other hand, studies in Ethiopia have shown that while *F. gigantica* is found below 1800 m above sea level, *F. hepatica* is limited to altitudes above 1200 m above sea level (Malone, *et al.* 1998; Yilma and Malone, 1998). Such reports suggest that some highland areas in Tanzania like the Usambara Mountains and the southern highlands are favourable areas for *F. hepatica* should it happen to be introduced there. Recently, studies have shown that the temperate lung nematode of cattle, *Dictyocaulus viviparus*, is prevalent among dairy cattle in northern and southern Tanzania (Thamsborg *et al.*, 1998) and was possibly introduced through the imported dairy heifers in the early 1960s or possibly earlier. It is highly possible that *F. hepatica* may have been also introduced, however further studies are needed to establish its existence.

Unlike *S. bovis*, which has a wide range of intermediate hosts, the distribution of *F. gigantica* in East and Central Africa depends exclusively on *Lymnaea natalensis*, which is its primary intermediate host (Dinnik and Dinnik, 1963; Mzembe and Chaudhry, 1981; Mutani *et al.*, 1983; Southgate *et al.*, 1985, 1989; Chartier *et al.*, 1990). However, *F. gigantica* may develop in other *Lymnaea* species such as *L. columella* that is replacing *L. natalensis* in South Africa (De Cock *et al.*, 1989), *L. auricularia* in the Indian sub content (Soulsby, 1982; Yadav and Gupta, 1988), *L. acuminata* (Keshav *et al.*, 1996) and *L. rubiginosa* in Malaysia (Soulsby, 1982). The report of it occurrence in *Biomphalaria alexandrina* in Egypt (Farag and Sayad, 1995) is an indication that its intermediate host spectrum is wider than is known at present. *F. gigantica* may infect a wide range of domestic ruminants

as well as game animals (Sachs and Sachs, 1968, Mustaffa, 1974; Sachs, 1977; Ziegler *et al.*, 1998; Oguge *et al.*, 1997).

The transmission biology of ruminant fasciolosis has been reviewed by Boray (1969) and by Chejina (1994). In most aspects of transmission resembles that of schistosomosis, although marked differences exist, in particular in the life cycle and the habitat preferences of the intermediate host snails. For instance, while schistosome eggs have a very short life span outside the definitive host, experimental studies have shown that the development of *F. gigantica* eggs and eventual hatching starts from 17 days and continues up to 111 days (Ogambo-Ongoma and Goodman, 1973). Such findings were also observed under natural conditions in the Kenya highlands, whereby miracidia that remained in the egg shells for up to 90 days were able to hatch and infect snails normally (Dinnik and Dinnik, 1959). Such behaviour ensures survival and succession of this parasite species during unfavourable climatic conditions, as it is doubtful whether *L. natalensis*, its primary intermediate host, is capable of aestivating similar to *Bulinus* snails (Hammond, 1965). Although, like *Bulinus* snails, *L. natalensis* can temporarily survive an amphibious existence (Soulsby, 1982) and some limited experimental findings have shown that *L. natalensis* can survive in hard dry mud for at least 24 weeks (Bitakaramire, 1968), permanent springs and streams with slow-moving water are the most favourable habitats for these snails (Hammond, 1965). Studies in the traditional management systems in Zambia have shown that cattle during the dry season are deliberately tracked into lowland (river basin) areas in search of pasture and water. However, they also get trematode infections (Silangwa, 1974). During the dry season snails concentrate in the few remaining water pockets and there is potentially enormous

numbers of metacercariae on the surrounding pasture which are eaten by the grazing animals. Even in a few places in Tanzania, such as Kilimanjaro, Arusha and most of the urban areas where zero grazing is practised in order to avoid major parasites, there is still a danger of animals being infected during the dry season as a result of grass cutting in areas which are used for communal grazing. In such situations the longevity of metacercariae in the pastures plays an important role and studies have shown that they can survive for more than 6 months in stored rice straw (Chejina, 1994). In general, the management of animals plays the most significant role in regard to transmission of bovine trematode infections.

2.2.2 Fasciolosis as an emerging zoonosis

Although *Fasciola* is a common parasite of domestic and wild ruminants in the last two decades it has become a public health problem in particular in the Eastern Mediterranean region (Farag, 1998a; Mas-Coma *et al.*, 1999). Previous surveys on the parasitic infections in humans in Nigeria showed that the prevalence of *Fasciola* infections was very low and suggestions were that the type of food and method of cooking in tropical Africa prevented or reduced the incidences of human fasciolosis (Obiamiwe, 1977). Therefore, most of detections of *Fasciola* eggs in human faeces in North Africa and Eastern Mediterranean Region were neglected and considered spurious infections (Farag, 1998a). However, there are indications from the recent studies that human fasciolosis is an emerging public health problem in North Africa and Middle East (Youssef and Mansour, 1991; Farag, 1998a; 1998b). Although fasciolosis in the past was limited to well-defined water shed areas, the disease has spread to new geographic areas due to the ever increasing environmental changes

and modification of human behaviour of eating uncooked and poorly washed aquatic vegetables containing metacercariae (Mas-Coma *et al.*, 1999, Savioli *et al.*, 1999). The recent outbreak of human fasciolosis in the Republic of Iran in 1989 where more than 10 000 people were affected and lasted for 18 months is a good example (Farag, 1998a; Savioli *et al.*, 1999; Mas-Coma *et al.*, 1999). Since then, human fasciolosis has been reported in Egypt, Morocco, Tunisia, Yemen, Iraq and Lebanon (Farag, 1998b; Mas-Coma *et al.*, 1999). In Egypt extensive studies on human fasciolosis has shown that in some villages in the Nile Delta prevalence rates vary between 2% and 17% and the dietary habits of eating raw aquatic vegetables has been implicated as the major cause (Farag, 1998b). So far the major cause of human fasciolosis in the Middle East has been shown to be *F. hepatica,* however, in Egypt both *F. gigantica* and *F. hepatica* prevail in humans (Farag, 1979) suggesting that *F. gigantica* is potentially a zoonotic parasite. Also human cases due *F. gigantica* have been reported in several tropical African countries such as Niger (Maiga *et al.*, 1991) and Cape Verde (Nozais *et al.*, 1998). Even the reported case of severe human fasciolosis observed in a 12-year old girl arriving in Australia from Tanzania could be due to *F. gigantica* and not *F. hepatica* (Goodman *et al.*, 1973). Furthermore the zoonotic potential of infection with *Fasciola* species through eating of raw meat was shown in recent experimental studies where feeding of pigs with livers from mice with immature flukes aged three to five days led to development of adult parasites in pigs (Taira *et al.*, 1997). Such results undoubtedly suggest that eating of raw livers with immature flukes, as is the case in some cattle keeping tribes in Tanzania, may lead to development of the disease. In summary, fasciolosis is at present considered by World Health Organisation (WHO) as an important human parasitic disease and it

can occur as epidemics in humans in endemic areas and can be exported through vegetables to areas away from the endemic areas (Mas-Coma *et al.*, 1999; Savioli *et al.*, 1999).

2.2.3 Immunity

Studies have shown that cattle can develop resistance to *Fasciola* species following single moderately high primary or continuous low number of metacercariae infections with the parasite (Chejina, 1994). The development of resistance against liver flukes is influenced by factors such as age, nutrition and existence of other diseases. In cattle it has been demonstrated that calves are more susceptible to primary infections than older animals (Howell and Boray, 1994). Detailed studies have shown that resistance to challenge infection develops roughly at 12 weeks after primary exposure and self-cure of the adult fluke occurs eight weeks later (Howell and Boray, 1994). Apart from age, studies have shown that an interrelationship exists between resistance against *Fasciola* infection and the nutritional status of the animal (Hammond, 1965; Boray, 1969; Schillhorn, 1974). Field studies by Hammond (1965) in East Africa and by Schillhorn (1974) in West Africa have shown that the punitive effect of fasciolosis was more pronounced during the dry season when feed was scarce. Results from experimental studies in sheep showed that the unfavourable effect of the fluke infection was more pronounced in ewes that were fed basal feed rations than in those given high protein diets or high protein and mineral supplements (Nour *et al.*, 1979).

Under natural conditions, infection of animals with more than one trematode species such as *Fasciola,* schistosome, *Dicrocoelium* or paramphistome is not

uncommon (Mahlau, 1970; Chartier *et al.*, 1991; Makundi *et al.*, 1998). Experimental studies have shown that heterologous immunity exists between these trematode parasite species (Monrad *et al.*, 1980; Sirag *et al.*, 1981; Yagi *et al.*, 1986). For instance, pre-infection with primary 10,000 *S. bovis* cercariae followed by challenging of calves with 900 *F. hepatica* metacercariae at 10 weeks after the primary infection led to 30% significant reduction in the *F. hepatica* flukes (Sirag *et al.*, 1981). Inversely, primary infection of calves with 1 000 *F. gigantica* metacercariae followed by challenge with 10 000 *S. bovis* cercariae led to 94% reduction in *S. bovis* establishment (Yagi *et al.*, 1986).

Unlike in cattle, studies in sheep have shown that irrespective of the age, resistance to challenge infections does not occur after primary *F. hepatica* infection or prior sensitisation with parasite antigens (Howell and Boray, 1994). So far, the development of resistance in sheep is highly debatable. While studies by Corba *et al.*, (1987) showed that sheep may develop strong resistance following anthelmintic treatment of primary infections, Howell and Boray (1994) found that moderate or heavy primary infections followed by repeated re-infections after effective chemotherapy did not generate resistance. However, contrary to the previous observations in *F. hepatica*, recent studies have showed that sheep can acquire resistance against *F. gigantica* challenge infections (Roberts *et al.*, 1996; Taira *et al.*, 2000). Experimental studies in West Africa have shown that *F. gigantica* is more infective for goats and that the disease was more severe in goats than in sheep (Ogunrinade, 1984).

The mechanism associated with the observed resistance especially in cattle is poorly understood. It has been hypothesized that the major factor is the observed

liver fibrosis in the infected animals, which is accompanied by biliary hyperplasia and calcification of the bile ducts. The fibrosis, hyperplasia and calcification of the bile ducts provide physical barrier that prevent the immature flukes from reaching the predilection site and the adult flukes from movement and possibly feeding within bile ducts (Boray, 1969). In cattle calcification of the fibrotic bile ducts and encrustations of calcium, form complete casts that may completely block the bile ducts and the cut surface of the calcified walls gives the appearance of the stem of clay pipe (Soulsby, 1982).

In summary, as far as susceptibility and response to infection of domestic and laboratory animals are concerned, sheep, goats, rabbits, rats and mice are considered to belong to the low resistance group (does not develop enough immunological response to immobilize and eliminate the parasite), while buffaloes, bovines and equines are considered to belong to the delayed resistance group (there is delayed host reaction towards the late phase of infection that lead to imposing of mechanical barrier in form of portal fibrosis (Boray, 1969). Pigs, dogs and cats have strong natural resistance that may easily eliminate *Fasciola* infections without developing serious disease and are regarded as early resistance groups (Boray, 1969; Chejina, 1994).

2.2.4 Treatment and control

Unlike ruminant schistosomosis, fasciolosis is a disease well known by both farmers and veterinarians in tropical Africa and in the southern highlands of Tanzania; it is among the major diseases hindering livestock productivity (Mahlau, 1970, 1975; Ecmovic and Mahlau, 1973, Akkaro and Maro, 1992). Unlike in the

developed countries where fasciolosis has been effectively controlled through the control of snails by draining wet pastures, strategic use of anthelmintics and improved grazing management, very little has been done in tropical Africa. The applications of these methods are impracticable in animals managed under communal grazing systems. Most often, the farmers will treat the most severely affected herds or animals selectively and the economics behind treatment of a majority of animals that are asymptomatic is rather unrealistic in such situations (Hammond, 1965; Silangwa, 1974). There are some few farmers in tropical Africa who have embarked on semi-intensive and intensive farming coupled with an introduction of pure and cross breeds where fasciolosis is also a big problem (Mahlau, 1975; Maingi and Mathenge, 1995). In such situations strategic use of anthelmintics together with improved grazing management based on thorough understanding the epidemiology of the disease in the area may result in the control or even eradication of the problem.

As was the case with schistosomicides, the search for non-toxic and potent fasciolicides followed the same trend. At the beginning of this century, less effective drugs such as carbon tetrachloride, hexachloroethane and hexachlorophene were used for more than 50 years (Boray, 1969). These drugs were highly toxic as they caused liver damage and kidney dysfunction. However, with time more potent and less toxic drugs such as bilevon, rafoxanide (Ranide®) oxyclozanide (Zanil®), and diamphenethide (Coriban®)(Amour and Corba, 1972; Okao, 1975) were introduced into the market. These were still less effective against immature stages of flukes and re-treatment with drugs such as oxyclozanide is necessary (Kassuku *et al.*, 1991). However, the introduction of triclabendazole in the early 1980s (Boray *et al.*, 1983;

Rapic *et al.*, 1984) was a step forward in the war against *Fasciola* since it was effective against immature flukes that were the major limitation of the early drugs (Mahlau, 1975; Kassuku *et al.*, 1991).

Triclabendazole has a broad application for most of the trematodes such as *Paragonimus* species (Calvopina *et al.*, 1998; Liu *et al.*, 1999), *Fascioloides* species (Craig and Huey, 1984), *Clonorchis* species (Shiramizu *et al.*, 1989) and *Eurytrema* species (Kono *et al.*, 1996). However, it has been shown that it has little effect against *Schistosoma, Dicrocoelium* and *Paramphistomum* species (Guralp and Tinar, 1984; Buescher and Richards, 1988; Rolfe and Boray, 1988; Islam and Samad, 1989; Upatoom, 1989; Upatoom *et al.*, 1993,). Like praziquantel, triclabendazole has been observed to cause swelling, loss of spines and finally destruction of the tegument as well as disruption of the vitelline cells of the fluke (Stitt and Fairweather, 1993, 1996). Due to overuse of triclabendazole in the United Kingdom and Australia there are indications that resistance against the drug may develop (Overend and Bowen, 1995; Fairweather and Boray, 1999; Mitchell *et al.*, 1998). To overcome resistance to the drug, Boray, (1997) used a combination of more than one drug (triclabendazole and clorsulon) and due to their synergistic effects he could cure strains that were resistant against triclabendazole. The use of more than one drug is recommended to control the development of resistance since there are very few drugs that have entered the market over the past 20 years (Cioli, 1998).

In summary, the control of ruminant fasciolosis and schistosomosis is possible through improvement of grazing management based on the understanding of their epidemiology of the diseases under local conditions. The education of farmers, especially in the developing countries, is crucial as most of the farmers know the

disease but are not aware of the involvement of the intermediate host snails. Also, improvement of watering facilities as carried out in the Masai range project in northern Tanzania was a step towards improvement of grazing management in the communal systems (Masanja, 1986). However, such creation of small dams for watering cattle must go hand-in-hand with educating the farmers on the knowledge of possible risks of creating more favourable habitats for snails as occurred in Senegal (Diaw *et al.*, 1998). Therefore, the effective control strategy lies in the integration of various available methods with emphasis on educating the community and ensuring full participation in the planning, implementation and monitoring of the control strategy.

2.3 Bovine paramphistomosis

2.3.1 Aetiology, distribution, intermediate snail and definitive host spectra

The parasites of the family paramphistomatidae (stomach or conical flukes) are pear-shaped and are found mainly in the rumen and less often in other parts of gastrointestinal tracts of domestic and wild ruminants. The life cycle resembles closely that of *Fasciola* species except for the predilection site, primarily the fore-stomachs, and sometimes small intestine and caecum instead of the liver. Few species occur in pigs, warthogs and equines. Although large numbers of these species are very common parasites of domestic and wild ruminants worldwide and some species may be of economic importance especially in the tropics, very little attention has been paid to them compared to other trematodes such as *Fasciola* and schistosomes. At least 20 species of the stomach fluke have been reported to occur in domestic and wild ruminants in Africa (Dinnik, 1964a). In Tanzania, limited studies

have reported more than 12 species of conical flukes in domestic and wild ruminants, which includes *Paramphistomum* (*microbothrium, phillerouxi, sukari, sukumum*, and *daubneyi*), *Cotylophoron* (*cotylophorum, fullerborni* and *jacksoni*), *Calicophoron*(*C. raja*), *Bothriophoron* (*B. bothriophoron*), *Carmyerius* (*C. mancupatus*) and *Stephanopharynx* (*S. secundus*) (Dinnik, 1964a,b; Sachs and Sachs, 1968; Nyundo, 1994).

Studies in Tanzania on conical flukes of wildlife and domestic ruminants showed that some of the species such as *C. raja* and *P. phillerouxi* do not show specificity towards their definitive hosts, while *S. secundus* was not found in domestic herbivores (Sachs and Sachs, 1968). Also, in West Africa Ogunrinade and Adetunji (1983) have reported a failure of a bovine strain of *P. microbothrium* to survive in rumen of sheep.

Unlike *Fasciola* species that are transmitted mainly by *Lymnaea* and bovine schistosomes by *Bulinus* snails, the paramphistomes have a much wider number of intermediate host snail species (Nyundo, 1994). The intermediate host snail varies with the species of the parasite and the locality. While in Europe *Lymnaea truncatula* and *Biomphalaria glabrata* have been observed to serve as intermediate host for *P. daubneyi* (Abrous *et al.*, 1999), in Australia *Gyraulus scottianus* and *Helicorbis australiensis* are the intermediate hosts for the *Paramphistomum ichikawai* and *Calicophoron calicophorum* respectively (Rolfe *et al.*, 1991). In Africa several pulmonate snails of the subfamilies *Planorbinae, Bulininae* and *Lymnaea* serve as intermediate hosts. However, the diploid *Bulinus tropicus/truncatus* snail species, which are refractory to schistosomes (Mutani *et al.*, 1983) are the major intermediate hosts for paramphistomes.

2.3.2 Pathogenicity, immunity and chemotherapy

The pathogenic effect of paramphistomes is due to the trauma caused by the immature stages on the proximal part of the small intestines and the severity of the changes depends on the number of the flukes. Studies in sheep experimentally infected with *P. ichikawai* showed that in light infections the lesions were mainly found on the duodenum, primarily as localized enteritis and villous atrophy, while in heavy infections there was severe destruction of the mucosa which extended to the jejunum (Rolfe *et al.*, 1994). Very few studies have been carried out in cattle in Tanzania. However, post mortem examination of a ten-year-old cow that was extremely weak showed that apart from having heavy schistosome, liver fluke and lungworm burdens, the whole of the duodenal mucosa was covered by immature paramphistomes (Makundi, 1993). Also, reports from the Ministry of Agriculture and Livestock in Tanzania show that outbreaks due to paramphistomosis in cattle are not uncommon (Nyundo, 1994). Therefore, treatment of these infections should be considered. Studies in Australia have shown that anthelmintic treatment of fasciolosis, gastro-intestinal nematodes and paramphistomosis with levamisole and oxyclozanide in cattle led to significant increase in milk production (Spence *et al.*, 1996). Unfortunately, only very few drugs among the commonly used anti-nematodes and anti-trematodes have an effect on paramphistomes, except drugs such as oxyclozanide and niclosamide (Rolfe and Boray, 1988; Spence *et al.*, 1996).

As with *Fasciola* and *Schistosoma* parasites, limited studies have shown that cattle and sheep can develop resistance following primary exposure to paramphistome infections. For instance, studies in Russia showed that exposure of cattle to primary *Liorchis scotiae* infections led to development of resistance against

challenge infections which was expressed as death of large numbers of parasites after migration, inhibition of growth and migration, and passing out of dead immature worms (Meremins, 1975). Similarly, studies in India have shown that primary exposure of sheep to 500 γ-irradiated *P. epiclitum* metacercariae (3-krads) led to effective resistance against challenge with 10,000 normal *P. epiclitum* metacercariae and no worms were recovered 45 to 60 days later (Hafeez and Rao, 1984). Although there are limited studies, which have been carried out on the African species infecting cattle, these results suggest that cattle and small ruminants in Africa are capable of developing resistance against primary paramphistome infections.

Although paramphistome infection co-exists in the same animal with other trematode parasite species such as *Fasciola* and schistosomes (Makundi, 1993; Nyundo, 1994), very few investigations have been carried on the possibility that pre-infection with paramphistome may induce protective cross-resistance against incoming *Fasciola* or schistosome infections. Limited studies in mice have shown that immunization with somatic extracts of *F. hepatica* may stimulate antibody responses to heterologous *Dicrocoelium lanceolutum* and *Paramphistomum* (Sadzkowski, 1988). The understanding of such interaction of parasites, which have closely related life cycles but different pathogenic effect on the same animal where they co-infect, is essential as this will provide additional information required for the planning of a control strategy.

In summary, studies of various aspects of paramphistomosis in Tanzania are yet to be done since this parasite seems to be widely distributed and co-exists in the same animals together with *Schistosoma, Fasciola and Dicrocoelium* parasite species. Furthermore, unlike other trematode parasites of domestic ruminants, this

parasite has a wide range of snail species, serving as intermediate hosts. As many of these snail species also act as hosts for other parasite species, the compatibility between parasite and snail becomes very complex. A good example is the enabling of the diploid snails of the *tropicus/truncatus* group to serve as intermediate hosts for *S. bovis* in East Africa when concurrently infected with amphistomes (Southgate *et al.*, 1985, 1989; Makundi, 1993).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Field studies at Lulanzi dairy farm

3.1.1 Study area

The field studies were carried out at Lulanzi dairy farm in Iringa District in the southern highlands of Tanzania. The village is 40km South East of Iringa town and at latitude of 07° 57'S, longitude of 35° 51' E and altitude of 6200 feet above sea level (a.s.l.). The southern highlands of Tanzania has undulated topography with high hills and valleys with many springs, ponds, swamps, streams and river tributaries that joins up to form big rivers such as Little Ruaha river (Fig. 1). The area has one rainy season, which is from November to May and the average annual rainfall is 700-1000 mm. The area has a cool climate with the coolest months being between June and August. During the rainy season these big rivers flood the plains through which they cross and after the rains, there are numerous pools of water that are left behind which are suitable habitat for freshwater snail breeding. During the dry season these flood plains also serve as the major sources of green pasture and water for the livestock as well as trematode infections. Lulanzi dairy farm like most parts of the southern highlands of Tanzania has the same climate and this study was undertaken at this farm since our results from the previous studies have shown that it has the highest prevalence of trematode infection in cattle especially in *S. bovis* (Makundi *et al.*, 1998). A site at Kilima permanent pond (Fig.2) in the Lulanzi dairy

Figure 1. A view of the Little Ruaha River in Iringa district at the end of annual rains. The flood plain along the river valley has numerous water bodies that are favourable breeding sites for the freshwater snails.

where cattle were mostly watered was considered to be the most probable area where transmission intensity of the infection was highest. Major activities that are carried out at the farm include dairy cattle farming and cultivation of beans, cereal crops (maize and wheat), Irish and sweet potatoes, vegetables (tomatoes and cabbages in the valleys), forest for timber (mainly Cyprus, eucalyptus and wattle trees) at the hilltops as well as sub-tropical fruit trees (apples, pears and peaches). Improvement of animal health and productivity at this farm is crucial since dairy farming is one of the major sources of income for the village. Most of the villages in the southern tropical highlands of Tanzania in Iringa, Mbeya and parts of Ruvuma regions have similar climate and production systems like that of Lulanzi village, especially the small-holder dairy cattle keeping. Therefore, results from the field studies on snails and natural *Schistosoma* and *Fasciola* infections in cattle at Lulanzi were aimed at providing information that may be used in the planning of a control strategy for these infections in the rest of the southern tropical highlands.

3.1.2 Longitudinal snail studies

Freshwater snails were collected once every month for a period of a year from one site where cattle were watered at Kilima permanent pond (Fig.2) in Lulanzi village. In each monthly visit to the site, snails were collected using round scoop made from a kitchen sieve supported by an iron frame and mounted on a handle, 1.5m long. The scoop had a diameter of 17 cm and mesh size of 1mm average pore diameter. The area was searched for snails for fifteen minutes and all snails were empted on a white polyethylene tray. The snails were picked from the tray by using a polyethylene teaspoon and packed into a 5 litre plastic bucket together with little

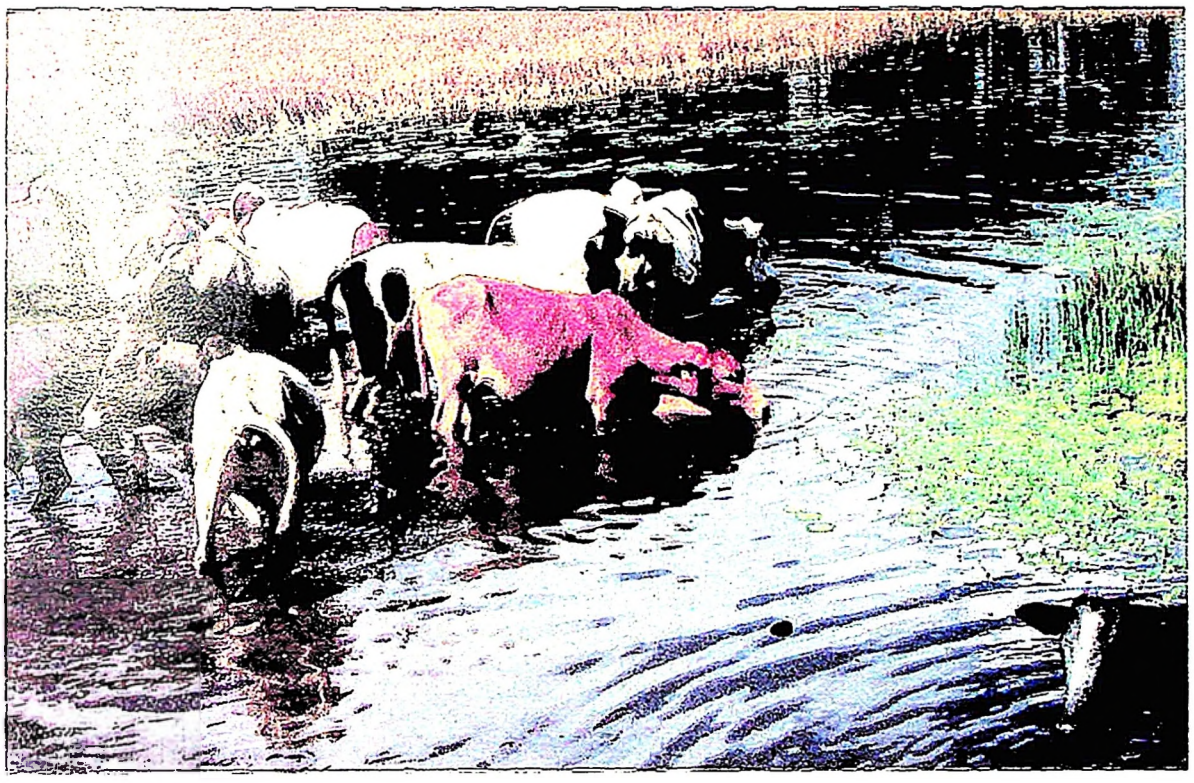

Figure 2. A focal site at Kilima permanent pond in Lulanzi village, Iringa district, where cattle were mostly watered. The clear water and the floating water lilies plants are good indicators that the site was favourable habitat for freshwater snail breeding

moist soil and aquatic plants collected from the site. The bucket was covered with a lid that has holes to allow aeration. The snails were transported under moist and cool conditions to SUA, Morogoro for subsequent laboratory diagnosis.

At the laboratory, all snails were enumerated and species differentiation was carried out by morphological techniques. The size and shape of the shell were useful features for differentiating the snails. Internal soft tissue structures such as the arrangement and shape of the teeth on the radula and the size and shape of the penis and its preputium were also useful for species identification of the snails. Snails with operculum belong to prosobranch family and are not considered to be of medical or veterinary importance. Fresh water snails without operculum (the pulmonates) are of medical and veterinary importance as some serve as intermediate hosts for most of the trematode parasites of medical and veterinary importance that infect humans and his livestock. Pulmonate snails with tall shells, sharp spire and aperture opening to the right belong to the *Lymnaea* species, the major intermediate host for *Fasciola* species. Pulmonate snails with tall or globose shells and aperture opening to the left belong to the *Bulinus* species the intermediate hosts for schistosome with terminal spined eggs and paramphistomes. Pulmonate snail with discoid shells more than 2 mm but less than 6 mm high belong to *Biomphalaria* snails species the intermediate hosts for schistosomes with lateral spined eggs such as *Schistosoma mansoni*. Other characters such as truncation on the columella was used to differentiate snails of the *Bulinus africanus* group (with truncation) from those of *Bulinus tropicus/truncatus* group (without truncation). The snails of the *B. africanus* group are the major intermediate host for the terminal spined schistosome species of man (*S. haematobium*) and of domestic ruminants (*S. bovis, S. mattheei*, and *S. leiperi*).

Furthermore, the size ratio of the penis sheath to that of the preputium was used to differentiate *Bulinus globosus* which are refractory to *S. bovis* infections from *Bulinus africanus* (with penis sheath longer and larger than the preputium) that are the primary intermediate host for *S. bovis* in Tanzania. Although the diploid snails of *B. tropicus/truncatus* group are refractory to schistosome infections they are major intermediate host for paramphistomes in East and South African countries. For detailed differentiation of the snail species the field guide manual for East African fresh water snails described previously by Kristensen (1987) was used. For the confirmation of the species, of the *Bulinus* snails, samples were sent to a World Health Organization (WHO) malacology collaboration centre at the Danish Bilharziasis Laboratory (DBL) where electrophoresis studies were carried out by comparing them with *B. tropicus* snails from Mozambique, Zimbabwe and Senegal.

For species identification of the digenean trematodes cercariae shed from the snails the morphological structures were used. Each snail was cleaned and placed into a 10 ml glass beaker with 5 mls of distilled water and placed under strong artificial light for at least half an hour or an overnight to stimulate them to shed cercariae. The swimming cercariae were viewed under stereomicroscope. The type of the tail whether forked or single and presence of stylet near the oral sucker were the most useful diagnostic features. In addition presence or absence and position of the ventral sucker was useful in differentiation of monostomes from amphistomes (with posterior placed ventral sucker). For the forked tailed cercariae (Furcocercus cercariae), the length of the furcae and the presence or absence of pharynx was used to differentiate blood flukes (Brevifurcate-apharyngaete cercariae - without pharynx and with short furcae) from strigeids cercariae (Longfurcae-pharyngeate cercariae –

with long furcae and pharynx). Among the brevifurcate-apharyngaete cercariae, the cercariae which belong to the mammalian *Schistosoma* species differ from those of avian and *Spirorchid* species by not having eye spots. For detailed identification of the cercariae the field key for identification of cercariae from African fresh water described previously by Frandsen and Christensen (1985) was used.

Monthly data on rainfall was collected from the nearby Dabaga seed farm meteorological station. The temperature data could not be obtained due to breakdown of the equipment at that time of the study.

3.1.3 Animal studies

3.1.3.1 Management of animals

Thirty-five 6 – 9 months old castrated male (Zebu X Boran) calves were purchased from Dakawa national ranch where previous studies (Kassuku-personal communication) has shown that it was free from *Fasciola* and *Schistosoma* parasite species. Before introducing the calves into the study area the calves were screened for haemoparasites and helminth infections. Determination of haemoparasite infection in the calves was carried out by screening under a compound microscope of blood smears made from blood collected from a punctured ear vein of each calf and stained with . Helminths infections were determined by the methods described below in section, 3.1.3.3. Among the calves, a few had very low GIT-nematode eggs in their faeces. Out of the 35 calves, 23, 5, 2 had 3 had 0, 100, 200 and 300 EPGF respectively. Two calves were not examined. Also, all calves were free from trematodes and haemoparasites infections. All animals were treated against GIT nematodes and trypanosomes using the manufactures recommended dose of 0.5 mls

/kg of body weight of Milsan® (1.5% w/v levamisole and 3% w/v oxyclozanide mixture) and 7 mg/kg of body weight of Berenil®, (diminazine-aceturate) respectively. Treatment against trypanosomosis was essential since Dakawa ranch where the calves were bought is an endemic area for trypanosomosis. Milsan® is additionally effective against *Fasciola* and paramphistomes.

At the Lulanzi dairy farm, two groups of cattle, a dairy and a beef herd, were kept. The beef herd comprised mainly the indigenous zebu cattle and bull calves, which were weaned from the dairy herd. The grazing management was such that the beef herd grazed mainly on an unimproved pasture while the dairy herd grazed the same areas, but in addition could graze in a few small paddocks with improved pastures. The grazing conditions were monitored throughout the study period without actually quantifying the amount of the feed intake and utilisation by the individual animal.

The watering sources for the animals were mainly the natural water bodies, but for the dairy cattle there were also piped water facilities such as water pumps, pipes and troughs. The experimental calves were placed together with the indigenous (beef) herd at the farm and were managed in the same manner as indigenous cattle in the neighbouring villages. The animals were protected against tick-borne diseases by dipping them in a dip tank with Superdip®, which is an organophosphorus compound, once a week during the dry period and twice a week during the rainy season. All animals were monitored for occurrence of haemoparasites and the level of GIT nematode infection.

3.1.3.2 Experimental design

The summary of the experimental design for the field study is given in Table 1. The thirty-five calves were ear-tagged and were correctly weighed on a weighing bridge connected to a spring balance (Salter-England- one division can weigh 5 kg) and five weight-matched groups (A, B, C, D and E) of seven calves each were formed. The calves were arranged in an descending order according to their individual live body weights and the first calf (heaviest one) was allocated to group A, the second was allocated to group B, the third to group C, the fourth to group D and the fifth to group E. The exercise was repeated until all calves were allocated into the five groups. Therefore, the initial arithmetic means for the body weights of the five groups were not statistically different ($P \geq 0.05$). Groups A, B, C and E calves were introduced into the transmission area in mid October 1993, a month before the seasonal rains started and were allowed to graze with the indigenous beef herd. Group D was kept in a safe paddock to prevent them from being infected with *Fasciola* and schistosomes. Weighing and collection of faecal and blood samples were carried out monthly for all 35 calves. The samples were subsequently taken to the laboratory for assessments of worm infections and clinical pathological changes. At the end of June 1994, all calves in groups A, B, C and E had acquired both *Schistosoma* and *Fasciola* infections, and calves in groups A and B were treated with both praziquantel (60 mg/kg) and triclabendazole (Fasinex®) (12 mg/kg). For the praziquantel, pure powder drug from manufacturing company Bayer was used. A bit higher dose of praziquantel, 60-mg/kg of body weight was used to ensure killing of most the worms since very few treatment trials have been carried out in cattle. The calves were drenched after the drug powder sample for each animal

was dissolved in 10mls of propylene glycol. For triclabendazole, the calves were drenched with a drug bought from the market and the manufactures recommended dose of 12-mg/kg-body weight was used. Both drugs were administered simultaneously. Immediately after treatment, groups B (treated) and E (untreated) were removed from the transmission site and sacrificed six weeks later. At post mortem, worm burdens for *Fasciola* and *Schistosoma* and schistosome tissue egg enumeration were determined according to the parasitological techniques described below. Groups A (treated) and C (untreated) calves continued grazing and group D calves, which served as post-treatment challenge control, were introduced into the transmission area.

Table 1. Design for the field study at Lulanzi dairy farm. Determination of the effect of continuous natural exposure to *Schistosoma bovis* and *Fasciola gigantica* with or without interruption of the primary infection by anthelmintic treatment (Group size: seven animals).

Group	Exposure to *S. bovis* and *F. gigantica*	Praziquantel and triclabendazole treatment	Post treatment exposure	Necropsied 6 weeks after treatment	Termination of the field study
A	+	+	+	-	+
B	+	+	-	+	
C	+	-	+	-	+
D	-	-	+	-	+
E	+	-	-	+	
Months post infection	0	7	7	9	16

A = exposed, treated and challenged; B = exposed and treated; C = exposed, untreated and challenged; D =Challenge control; E = exposed and not treated.

Blood and faecal sampling and weighing of the calves was continued on a monthly basis and when all calves in group D had acquired both *Fasciola* and *Schistosoma* infections in February 1995, they were removed from the transmission site and killed eight weeks later. At post mortem, *Fasciola* and schistosome worm burdens and schistosome tissue egg enumeration were determined as during the early infections.

3.1.3.3 Faecal egg enumeration

Handful fresh faecal samples were collected from the rectum of each calf using long disposable plastic gloves. The gloves were tightened as close to the faeces as possible to exclude air and thus preventing fast development of the eggs. The

samples were correctly labelled using waterproof marker pen and were immediately transported under cool conditions in a cool box with ice packs to the laboratory. For determination GIT-nematode infection, three grams of faeces were correctly weighed on an electronic scale (Ohaus, LS-200, Buch & Holm A/S). Detection and enumeration of the gastro-intestinal eggs was carried out by using Modified McMaster technique described previously by Hansen and Perry (1994).

Similarly, three grams of faeces were correctly weighed on the same electronic scale and a Modified Bell filtration ninhydrin staining method described previously by Kassuku *et al.* (1985b) was used for detection and enumeration of *S. bovis* eggs. The correctly weighed faecal sample was mixed with 50 mls of physiological saline (0.85 g of sodium chloride in 100 mls of water) in a 100 ml plastic container with a screw cap and homogenized by hand shaking for three minutes. The contents were poured into a 500 ml urinary flask through a coffee strainer and 250 μm sieve and a wash bottle was used washed the contents into the flask using physiological saline. After filtration, more physiological saline was added to fill the flask to the brim and the filtrate was allowed to sediment in dark place for 30 minutes. The supernatant was poured off and the flask was refilled with physiological saline and sedimentation repeated for another 30 minutes. The exercise was repeated until the supernatant was clear. Physiological saline was added to the flask and the sediment mixed thoroughly and a vacuum pump (Vaclif®, Bie & Berntsen, A/S, Jyland, Denmark) was used to filter the mixture through Whatman's filter paper No.40. The filter papers had ruled lines and were placed on a drop of saturated ninhydrin solution on a large glass slide. The Whatman's filter papers were left for overnight under bright light to dry. The dry filters could be stored for long

period without losing their quality. Eggs were enumerated by viewing the moist filter papers under stereomicroscope. The spindle shape and purple staining of the miracidium inside the unstained eggshells of *S. bovis* eggs were differentiated them from those of paramphistome and *Fasciola*, which are oval and were completely stained purple with the ninhydrin.

For *Fasciola* and *Paramphistomum* species, only detection for presence or absence of the eggs was carried out. Five grams from each faecal sample were correctly measured on an electronic scale and put into a 100 ml plastic container. The container was half filled with clean tap water, closed and mixed thoroughly by shaking. The sample was filtered into a 500 ml urinary flask through a coffee strainer. The sample was allowed to stand for 20 minutes and the supernatant was carefully decanted without disturbing the remaining sediment. The conical flask was refilled with water and allowed to stand for another 20 minutes before the supernatant was decanted. The exercise was repeated until the supernatant was clear. At the end, three drops of malachite green solution were added and allowed to stand for at least three minutes. The sediment was poured on a plastic petri dish with ruled parallel lines and with the aid of a stereomicroscope, the eggs were detected. The fresh *Fasciola* eggs were differentiated from those of *Paramphistomum* as they appeared yellow and had eccentrically placed nucleus towards the operculated and had small yolk cells, while *Paramphistomum* eggs were colourless with centrally placed embryo and large yolk cells. The eggs of schistosome were not detected by this sedimentation malachite staining technique as they hatched during exposure to light and sedimentation using fresh water.

3.1.3.4 Clinical pathology

Monthly body weight for each animal was measured using a weighing bridge connected to a spring balance (Satter-England). In order to avoid fluctuations due to intake of water and fodder, animals were routinely weighed early in the morning before they were released for grazing.

During each monthly sampling, blood was collected from the jugular vein of each calf in heparinized tubes and was immediately transported under cool condition to the laboratory. For estimation of the packed cell volume (PVC), the blood was gently mixed well and heparinized capillary tubes (Supe & Rior, Marienfeld-Germany) were filled with blood and one end of each tube were sealed with a plasticized sealer (Sigillum –Modulohm I/S, Vasekaer. Copenhagen). The capillary tubes were placed into a Hawksley micro-haematocrit centrifuge and after spinning at speed of 10 000 rpm for five minutes, the height of red blood cells was read as a percentage on specially designed scale on Hawksley micro-haematocrit reader.

For the estimation of the haemoglobin concentration, a method based on the oxidation of haemoglobin by ferricyanide and its subsequent conversion to cyanmethaemoglobin by potassium cyanide was used. 20 µl of well-mixed blood was mixed with 5mls of buffered Drabkins solution (Boehringer-Mannheim, Germany) and allowed to stand for at least three minutes. The absorbance of the diluted blood sample was read on a calorimeter at 540nm wavelength against the buffered Drabkins solution. Also, the absorbance of the same volume of standard cyamethamoglobin (Boehringer-Mannheim, Germany) was read against the buffered Drabkins solution. The Haemoglobin concentration of each blood sample was calculated using the following equation.

$$\text{Haemoglobin concentration (g/100ml)} = \frac{\text{Absorbance of the blood sample}}{\text{Absorbance of the standard}} \times St \times D$$

Where: St = Concentration of Hb in g/ml in the standard.

D = The dilution factor (250) of the diluent.

3.1.3.5 *Schistosoma* worm enumeration

In order to remove the adult schistosome worms from the mesenteric veins of the calves, perfusion technique was carried out by modifying the techniques used previously by McCully and Kruger (1969). The bull calves weighing between 100 and 200 kg were each injected intravenously with a total of 75 000 IU heparin (Leo®) and after three minutes, the animal was stunned using a captive bolt. Bleeding was carried out immediately by cutting the throat with a sharp knife. The whole of the right fore limb was removed followed by removal of the underlying ribs while leaving the diaphragm and all organs in the thoracic cavity intact. The skin on the right flank was carefully dissected out. The abdomen was opened through the Para-lumbar fossa and care was taken not to puncture the liver. In order to locate the cranial mesenteric artery, the diaphragm pillar was dissected out exposing the underlying two blood vessels of the same diameter (celiac and cranial mesenteric arteries) branching from the abdominal aorta (Fig. 3). In cattle, the cranial mesenteric artery lies closely and posterior to the celiac artery. The latter supplies the stomachs liver and spleen with blood. In addition, it supplies the duodenum and pancreas through the cranial pancreato-duodenal artery, which branches from the hepatic artery, one of the three major branches of the celiac artery. The cranial pancreato-duodenal artery from the celiac artery forms anastomoses with the caudal pancreato-

Figure 3. Presentation of the cranial mesenteric artery *in situ* of calf at left lateral recumbency with the ribs on the right side been removed. The posterior muscular part of diaphragm (f) was opened and pulled out to expose the abdominal aorta (a-b) and the cranial mesenteric artery (d) lying close but posterior to celiac artery (c). The cranial mesenteric artery supplies the small intestines and large intestines while the celiac artery supplies, stomachs, spleen, liver and part of pancreas and anterior duodenum. The spinal column (s) and part of the lung (e) are also shown

duodenal artery, which originates from the cranial mesenteric artery. In small ruminants such as sheep, the cranial mesenteric and celiac arteries often have a common origin from the abdominal aorta (Smallwood, 1992). A small incision was made in the cranial mesenteric artery as close as possible to the abdominal aorta and an intravenous catheter was introduced and fixed in position with a nylon suture. The portal vein was located at the liver hilum that is normally under the gall bladder and pancreas and covered by a lymph node. A blind dissection was carried out to expose the portal vein, and it involved loosening the liver attachments to the serosa but without puncturing the Glison's membrane. A small incision close to the liver was made in the portal vein and an intravenous catheter was introduced into the portal vein caudally directed towards the intestines and fixed like the former one. To avoid losing some of the worms, the open end of the catheter was plugged by the finger during fixing of the catheter. A peristaltic pump (Easy-load, Master Flex Model 7518-00, Cole Parmer Instruments, USA) with inlet and outlet nozzles was used to pump warm physiological saline (0.85 g of sodium chloride in 100mls of distilled water) from a bucket into the cranial mesenteric artery so as to flush out the adult worms residing in the mesenteric veins. In order to pump the fluid, a polyethylene tube with open end was placed in the bucket with the physiological saline while the other end was connected to the inlet nozzle of the pump. Then another polyethylene tube was connected between the outlet nozzle and the catheter at the cranial mesenteric artery. In order to collect the worms, a third polyethylene tube was connected to the catheter at the portal vein and was directed on to a metal sieve with 40 μm average pore diameter (APD) placed on top of a plastic bucket. The pump was switched on and with a moderate speed the worms were flushed out of the blood

vessels. Meanwhile, the small intestines were gently manipulated manually by hand and this increased the expulsion of the worms. At least 15 litres of physiological saline were used to remove the worms. Efficient flushing of the worms was judged by ensuring that no mesenteric vessel remained with blood. However, some worms could still remain by clinging on the walls of the vessels by means of their suckers. When most of the worms were removed, the pump was stopped and a second sieve of the same average pore diameter (40 μm) was introduced to replace the original one and 1% formal-saline (1ml of 40% formaldehyde + 0.85 g of sodium chloride in 100 mls of distilled water) was used to remove the redundant worms instead of plain physiological saline. This was carried out during the first part of the field studies at Lulanzi dairy farm for groups B and E. During the termination of the field studies 10% ethyl alcohol in physiological saline was used to remove the worms, which remained in the mesenteric veins after perfusing with physiological saline, to avoid the toxic effects of formaldehyde. The worms on the two sieves were put separately into universal bottles and were preserved in 70% ethyl alcohol for subsequent enumeration.

For the perfusion of the liver, the anterior vena cava in the thoracic cavity was located and the inlet catheter was transferred from the cranial mesenteric artery and introduced caudally towards the liver. The posterior vena cava was located and was clumped just caudal to the liver but anterior to the renal vein using a large artery forceps. The outlet catheter in the portal vein was loosened and its direction changed to point cranially into the liver, and it was fixed in position. The liver was first perfused using physiological saline followed by 1% formaldehyde in physiological

saline or 10% ethyl alcohol in physiological saline as in the intestines. Gentle squeezing of the liver increased the speed of perfusion and expulsion of worms.

In order to enumerate the schistosomes, the worms were placed on a petri dish with ruled lines at the bottom and were enumerated *in situ* under stereomicroscope without mechanically separating the females from the males. The slender female within the gynaecophoric groove of the male could be seen protruding outside. Occasionally, the females were completely hidden inside of the gynaecophoric groove and it was necessary to open it with the help of small thumb forceps.

3.1.3.6 *Fasciola* worm enumeration

For the recovery of most of the adult *Fasciola* flukes, immediately after opening of the viscera, the main bile duct was ligated to prevent worms leaving the liver. After perfusion of liver to remove schistosomes, all large bile ducts were opened as far as possible with a small pair of scissors and the liver flukes were collected using thumb forceps. Furthermore, the liver was sliced into very thin pieces (roughly about 1 cm thick) and put in warm saline for at least an hour to allow immature flukes to come out. The liver was squeezed to facilitate efficiency of worm recovery. In the case of incomplete worms (when only part of worm was available due being cut into pieces) the ventral sucker was used as a criterion for enumeration of the flukes. Paramphistome fluke were not enumerated but gross assessment of whether present or absent was carried out after opening the rumen, omasum, reticullum, abomasum and duodenum of the calves.

3.1.3.7 Schistosome tissue egg enumeration

In order to determine the amount of eggs in the tissues, fresh tissues were collected mainly from the liver, different parts of small and large intestines, gall bladder, urinary bladder, spleen and pancreas and enumeration of schistosome eggs was carried out after the tissue digestion method used previously by Makundi (1993). Five grams of the tissue was correctly weighed on an electronic scale (Ohaus, LS-200, Buch & Holm A/S) and by using a pair of scissors the tissues were cut in tiny pieces within a 5 ml plastic container. The homogenized tissues were washed into a 100 ml conical flask with 50mls of 3% potassium hydroxide and mixed thoroughly. The conical flask was tightly closed with a piece of aluminium foil and incubated at 37°C for 18 hours. After incubation 50mls of distilled water were added to stop the reaction. By using a 1ml syringe the contents in the flask were thoroughly mixed and 1ml of the mixture was withdrawn and placed Sedgewick-Rafter® chamber (Graticules Ltd. Kent, England) enumeration chambers. The eggs were enumerated by viewing then under compound microscope.

3.2 Experimental *S. bovis* infections in calves

3.2.1 Management of the animals

The experimental studies were carried out at Mazimbu dairy farm in SUA Morogoro. The experimental design resembled the field design at Lulanzi dairy farm rather closely but with a few alterations. Forty-two, six-to-nine-month old castrated (Zebu X Boran) bull calves were purchased from Dakawa ranch in Morogoro region where previous studies has shown that it was free from trematode infections (Kassuku-personal communication). Treatment for haemoparasites and GIT

helminths was carried out in the same way as in the field study at Lubanzi dairy. The calves were kept indoors in a large house that was previously used for pig keeping. Seven rooms that could accommodate six animals each were utilised, but the big doors were left open so that the calves could mix freely and share water and feeds. Initially, for the first four weeks, *Chloris gayana* hay alone was given to the animals. However, due to poor performance, each animal was thereafter given daily supplements of two kilos of sunflower seed cake/maize bran mix (1:3) and one litre of molasses. From January to April, hay was in short supply and was replaced by fresh elephant grass. From April to the end of June the animals were fed fresh *Chloris gayana* and supplementary feed in the same amount as before. Onwards to the end of the experiment the animals were fed hay only. The main problem that was encountered during the study was that of obtaining enough feed for the animals as the study coincided with the pre-El Nino severe drought. Although water was supplied at *ad libtum* occasionally especially during dry season, July-November, animals had to stay for hours waiting for water to be fetched by a tractor from a long distance. Although there was an outbreak of three days' sickness and one animal had trypanosomosis, such problems were relatively minor compared to the scarcity of the feed. In addition, two animals escaped and disappeared in the bush on the first day that the animals were introduced in the farm, while a third animal got strangled in the manger.

3.2.2 Snail breeding and cercariae production

Based on information from previous studies (Mutani *et al.*, 1983; Kassuku *et al.*, 1986; Makundi, 1993) *Bulinus africanus* was observed as the major

intermediate host for *S. bovis* (Iringa strain). Therefore, *B. africanus* snails were collected from Little Ruaha River near Iringa town and breeding of the snails was carried out by establishing self-maintaining balanced aquaria. A glass aquaria of the size (20 cm x 25 cm x 30 cm with a lid) containing aquatic plants *Sagittaria natans* for oxidation of the water, a *Daphnia pulex* populations for reducing bacteria growth and a sea sand substratum for releasing calcium to the water was used. The snails were fed boiled dried lettuce and tetramin fish food. However, due to difficulties of obtaining tetramin fish food in our local shops, layers mash was used instead and was quite suitable especially for feeding juvenile snails. The breeding of the snails was carried out by the balanced aquaria method described previously by Madsen (1985).

Infection of the snails was carried out by placing each snail in a 10 ml glass beaker with 5 mls of distilled water and were infected with three *S. bovis* miracidia hatched from the faeces of cattle from Lulanzi village, Iringa district. The miracidia hatching was carried out by sedimentation technique describe previously by Makundi (1993). The miracidia were picked one by one from a petri dish illuminated by a side lamp under stereomicroscope using a pipette. Initially, there were 28 calves to be infected at once with 8 000 *S. bovis* cercariae. Since one snail can produce at least 600 cercariae in one day, the production of 22 4000 cercariae required at least 400 infected snails. The production of cercariae was good for the primary infection and some large snails could produce up to 2 000 cercariae per day. Therefore, the primary infection doses of 8 000 cercariae were met. After the primary infections were carried out in November, there was a rapid decline in the number of breeding snails from December on wards. By January and February even egg laying had stopped. The deaths were possibly due to high room temperatures, which were

between 28° C and 32° C. Snail breeding started to become physiological again from April onwards and by July more than 300 snails were shading cercariae. Unexpectedly, while preparations for infection of the animals were going on, a rat (*Rattus rattus*), which entered the laboratory by chance, ate more than 250 infected snails. The rat had entered the laboratory following a one-week exhibition on rodents in one of the neighbouring laboratory rooms three weeks before, and it was surviving mainly on paper. Therefore, cercariae were harvested from the remaining snails at least once a week and after three weeks all animals had received a total dose of 6 000 cercariae.

3.2.3 Experimental design

The summary of the design for the experimental *S. bovis* infections is shown in Table 2. The calves were weight-matched into six groups (A, B, C, D, E & F) by the same methods that were used in the field studies at Lulanzi village. The infection of the calves with cercariae was carried out by the modification of the tail immersion technique described previously by Christensen *et al.*, (1984). Cercariae were harvested from the infected snails by placing each snail in 10 ml glass beakers with 5 ml of distilled water and expose them to artificial bright light for one hour. The cercariae shed from all snails were mixed in 500 ml beaker and the volume recorded. The contents in the beaker were gently mixed and concentration of the cercariae was estimated. The estimation of the total number of cercariae in the mixture was carried out by enumerating of Lugol's iodine stained cercariae in one millilitre of the mixture.

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Before infection, the hairs on tails of the calves were shaved and the tails were thoroughly cleaned with water. The calves were infected through the tail by immersing the tail into a plastic bag with a known volume of the mixture containing 8 000 *S. bovis* cercariae. For carrying out smooth infection, each calf was properly restrained by tying all four legs with a piece of rope and the calves were laid down at right lateral recumbency. The infection lasted for half an hour.

Body weight (bwt), schistosome egg per gm of faeces (EPGF), packed cell volume (PCV) and blood haemoglobin (Hb) was determined at two-week intervals at the beginning (during the acute phase) and later at monthly intervals. At necropsy schistosome worm burdens and tissue egg enumeration were determined. All parameters were determined by the same techniques that were used during the field studies at Lulanzi dairy farm.

Treatment of the calves with praziquantel 60 mg per kilogram of body weight was carried out at week 13 post infection (p.i.). Due to difficulties in obtaining cercariae at the planned time, challenge of the calves was delayed from the 13^{th} week to the 35^{th} week. Also due to the fewer number of snails, which were shedding, challenge was carried out over a period of three weeks, each time infecting equal numbers of calves from each group. The first week nine calves with three animals from each of group A, C and D were infected. In the two weeks that followed, two calves from each group were infected during each week. Harvesting of cercariae from the infected snails was carried out once a week to minimize stress and possible killing of the snails. The infection, treatment and challenge of the groups are as shown in Table 2.

Table 2. Design for the experimental *Schistosoma bovis* infection in calves. Determination of the effect of challenge *S. bovis* infection with or without interruption of the early patent primary infection by praziquantel.

Group	Number of animals	Primary infection	Praziquantel treatment	Perfusion (1)	Re-infection	Perfusion (2)
A	7	+	+	-	+	-
B	6	+	+	+		
C	7	+	-	-	+	-
D	7				+	-
E	5	+	-	+		
F	7	-	-	-	-	-
Weeks post infection		0	13	26	35-38	52

3.2.4 Pathological observations

At post mortem gross pathological changes were recorded and fresh tissue samples for histopathology were collected in specimen bottles with 10% buffered formalin. At the laboratory, the tissues were processed for subsequent staining. A cut section of each tissue sample was placed in a mould and correctly labelled. The tissues within the moulds were placed in cassettes and placed in automatic machine where washing out of the extra fixative, increase of dehydration by passing them through increased strengths of ethyl alcohol, clearing in xylene and embedment in paraffin wax to provide them with rigid support occured. Thin, 5 µm thick sections were sliced by using microtome blades mounted on microtome machine. The sliced tissue sections were placed on glass slides and were stained with different stains. Haematoxylin and Eosin (H and E) was used to demonstrate the general architecture of the tissues while for demonstration of collagen, reticulin and elastic fibres, van Gieson's (VG), Gomori's and Verhoeff's stains were used respectively as described by Bancroft and Allan (1990). Although van Gieson's stain is remarkably the most

successful single histological technique specific stain of collagen (Bancroft and Cook, 1984) it has disadvantage of not staining young collagen. Still it was a method of choice since the Mason's trichrome blue which can stain young collagen fibres is known to lead to indifferent results in particular when formalin is used as fixative (Bancroft and Cook, 1984) as in the present study.

3.3 Studies on distribution of schistosome eggs in the small intestine

3.3.1 Infection of the calves

Eight 6-9 month old Friesian calves were infected with 5 000 *S. bovis* (Iringa strain) cercariae. The same modified tail immersion technique Christensen *et al.,* (1984) which was used in the experimental studies at Mazimbu. The animals were kept indoors and given water and hay at *ad libtum*. Barley and mineral licks supplements were supplied throughout the experimental period.

3.3.2 Tissue egg enumeration

The animals were sacrificed by stunning them using a captive bolt and bled by cutting the throat with a sharp knife. The sacrificing of the calves was carried out at different stages of infection. Two animals were sacrificed at seven weeks post infection, another two at 18 weeks post infection, while the remaining four were sacrificed at 32 weeks post infection. At post mortem the small intestine was collected soon after slaughter and the serosal/peritoneal fat was removed as much as possible. The small intestine was subdivided into three equal parts in length, *i.e.* anterior (A) middle (B) and posterior (C). Each of the three parts was further subdivided into three equal parts. From the middle of each part, a 15 cm long portion

was sampled. The sampled part was opened longitudinally using a pair of scissors and was washed in saline to remove the intestinal contents. The mucosa was scraped out using a blunt knife (butter knife) leaving the submucosa, muscular and serosa layers. Representative samples of 2.5 g each were collected from the mucosa and the remaining muscular-serosa layer for each sample. Egg detection and enumeration was carried out by the digestion method that was used during the field studies.

3.4 Statistical analysis

The data was entered, validated, cleaned and analysed using Statistix and SAS statistical software. For body weight (BWT), packed cell volume (PCV) and haemoglobin (HB) parameters, parametric statistics were employed directly as these variables were normally distributed. The faecal and tissue egg enumeration were skewed to the left and their data were log transformed (LogEPG = Log_{10} (EPG+1) before employment of parametric statistics. For the variables, which were examined on a monthly basis such as BWT, PCV, Hb and EPGF, repeated measurements for general linear model (GLM) were used to test the differences between the animal groups. At slaughter, least significant difference (LSD) procedure for the comparison of the geometric means of EPGT for the different groups in one-way ANOVA test was used. The worm enumeration data did not follow physiological distribution and could not be transformed to physiological distributed data and non-parametric, Kruskal-Wallis test was used to measure the difference between the group mean ranks.

The efficacy of the drugs was calculated as shown in the following formula:

$$\text{Efficacy} = (1 - X_t / X_c) \times 100$$

Where,

X_t = Number of mean worms or eggs in treated group

X_c = Number of mean worms or eggs in untreated group

CHAPTER FOUR

RESULTS

4.1 The field study

4.1.1 Snail study

Morphological studies of 483 pulmonate snails collected from the site where cattle were watered showed that 420 (86.9%) were *Bulinus natalensis,* 48 (9.9%) *Lymnaea natalensis* and 15(3.1%) *Biomphalaria* species indicating that *B. natalensis* was the most abundant snail species. Of the 420 *B. natalensis* that were collected during the study period, 23(5.47%) were found shedding mammalian schistosome cercariae, 70(16.7%) amphistome cercariae and 5(1.2%) both mammalian schistosome and amphistome cercariae, while 50(11.9%) were shedding other cercariae, mainly *Xiphidiocercariae* and *Strigea* species. The results of the seasonality of transmission of *S. bovis* by *B. natalensis* are shown in Fig. 4. Schistosome transmission was observed mainly in April, May and June, which is the period towards the end of the rainy season. Eggs of *S. bovis* were observed six weeks later in the faeces of sheep exposed to the mammalian cercariae shed from the *B. natalensis* snails that were naturally infected.

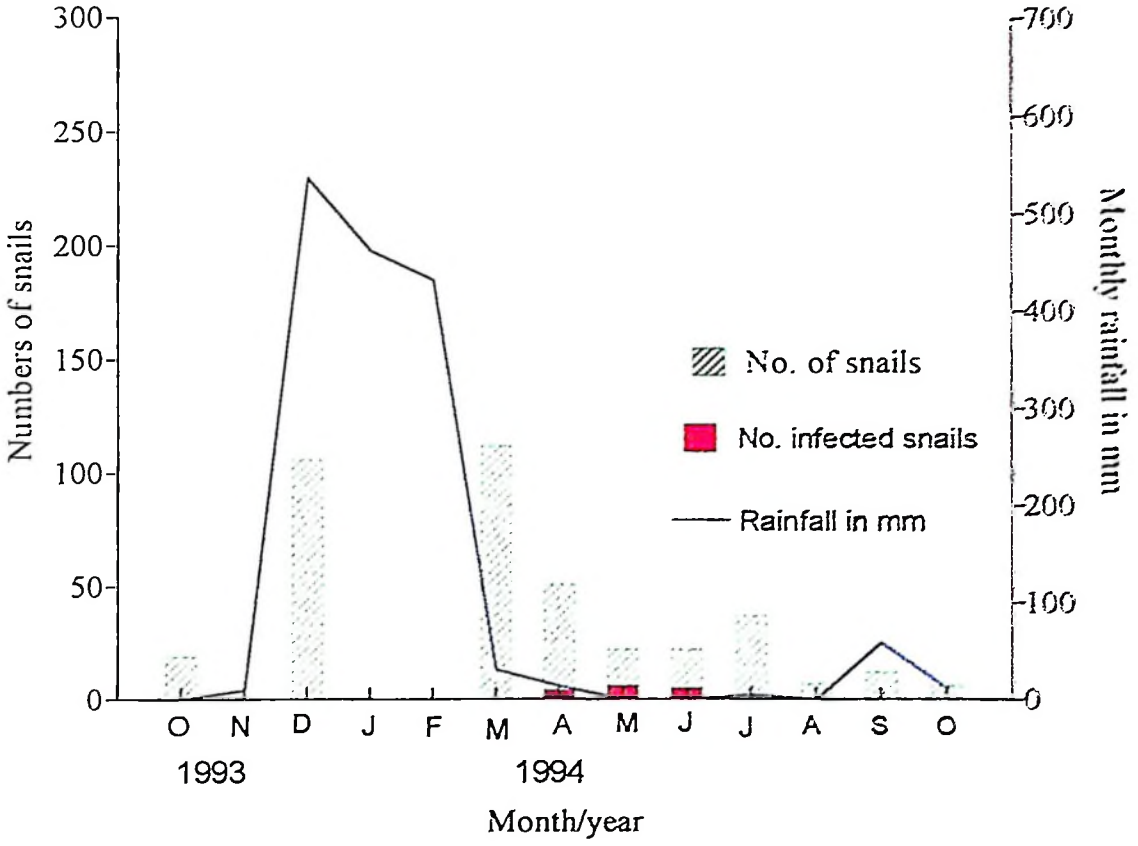

Figure 4. Seasonality of transmission of *S. bovis* by *B. natalensis* at Kilima permanent pond in Lulanzi village, Iringa District, Tanzania. Snails infected with *S. bovis* were observed mainly towards the end of the rainy season, i.e. from April to June.

4.1.2 Animal study

4.1.2.1 *Schistosoma* egg excretion

Faecal egg excretion of *S. bovis* in all groups of the calves grazing natural pastures are is shown in Fig. 5. The schistosome eggs first appeared in the faeces of the exposed groups (A, B, C and E) in early January and the group mean egg per gram of faeces (EPGF) gradually increased with time and by June it was approaching peak. Following treatment of group A at the end of June, there was a sharp decline in the number of EPGF to zero in the treated group. In the untreated group (C), the egg excretion continued increasing and reached peak in July. Thereafter it gradually declined to a very low level and by the time the experiment was terminated in February 1995, the mean number of EPGF was less than 10. Upon natural challenging of the treated group (A), the eggs reappeared again in the faeces in August, exactly two months after treatment. However, it was not until October that most of the calves in that group had eggs in their faeces four months after anthelmintic treatment. Similarly, in-group D that was introduced to check for the post treatment transmission, the appearance of eggs in the faeces resembled that of group A. A low peak (EPGF<15) was reached in January in the treated group (A) and by the time the experiment was terminated the egg excretion was declining and was not significantly different from that of the untreated challenged group (C). However, the mean number of EPGF in the challenge control group (D) was still increasing at the time when the experiment was terminated.

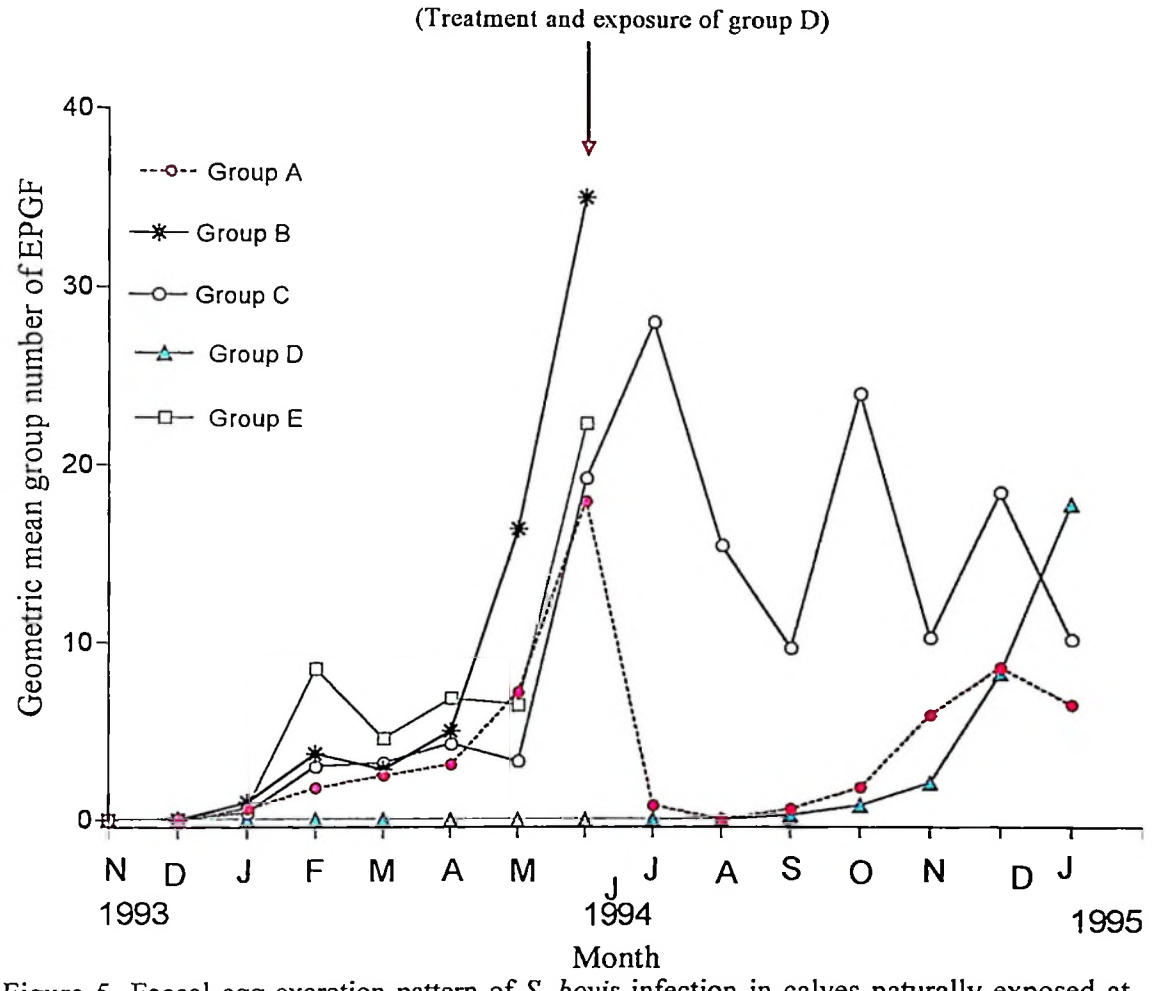

Figure 5. Faecal egg excretion pattern of *S. bovis* infection in calves naturally exposed at Lulanzi dairy farm. A = exposed, treated and challenged; B = exposed and treated; C = exposed, untreated and challenged; D =Challenge control; E = exposed and not treated Treatment = (Praziquantel 60 mg/kg, triclabendazole 12mg/kg given per oral simultaneously).

4.1.2.2 *Fasciola* and paramphistome egg excretion

The patterns of faecal egg excretion for paramphistome and *Fasciola* flukes are shown in Figs. 6 and 7 respectively. Paramphistome eggs first appeared in the faeces in January while those of *Fasciola* were seen in February. While most of the calves were positive for paramphistome by March, it was not until May for *Fasciola*. Following treatment, *Fasciola* eggs declined to zero in the faeces of the treated animals (group A).

On the contrary, paramphistome eggs continue detected in the faeces of the treated calves. Upon natural re-infection *Fasciola* eggs appeared in faeces of the challenge control group (D) in August and all calves were infected by October. In the treated group (A) this was delayed until December 1994 and not all calves had eggs in their faeces when they were removed from the transmission area (Fig. 7).

4.1.2.3 Live body weight

The results of the mean BWT of the calves are shown in Fig, 8. The calves were introduced into the farm in October 1993 at the end of the dry season. During this period there was no weight gain and some animals lost weight as a result of the drought that prevailed. Between February and June there was rapid increase in the body weight in all groups as a result of the abundance of fodder. However, the weights in the unexposed group (D) were significantly higher ($P \leq 0.05$) than that of the exposed calves (groups A, B, C and E). Following treatment with both praziquantel and triclabendazole and re-exposure in June at the beginning of the dry season, all animals started losing body weight. The untreated re-exposed group (C) of calves was the most affected. While the mean body weight of the treated group

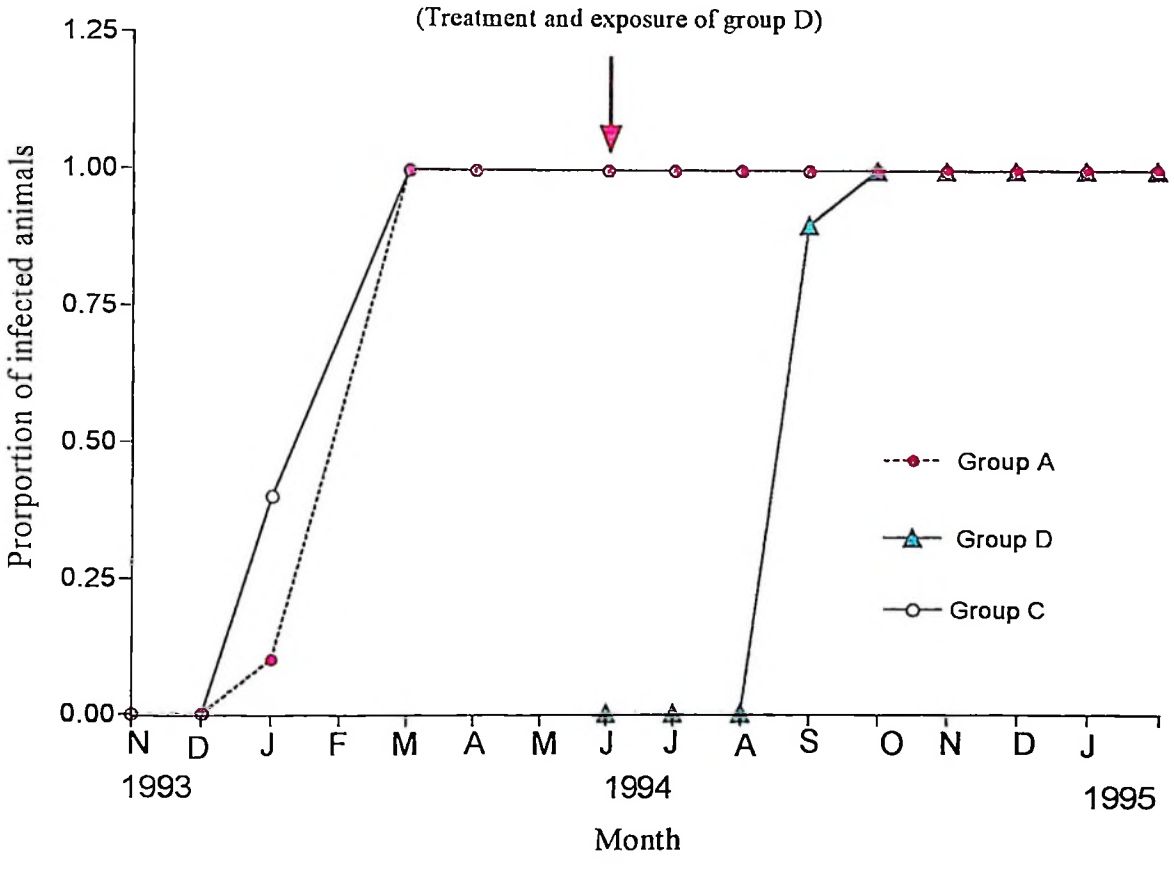

Figure 6. Proportion of calves infected with paramphistome species based on detection of eggs in the faeces. Groups A = exposed, treated and challenged; B = exposed and treated; C = exposed, untreated and challenged; D =Challenge control; E = exposed and not treated Treatment = (Praziquantel 60 mg/kg, triclabendazole 12mg/kg given per oral simultaneously).

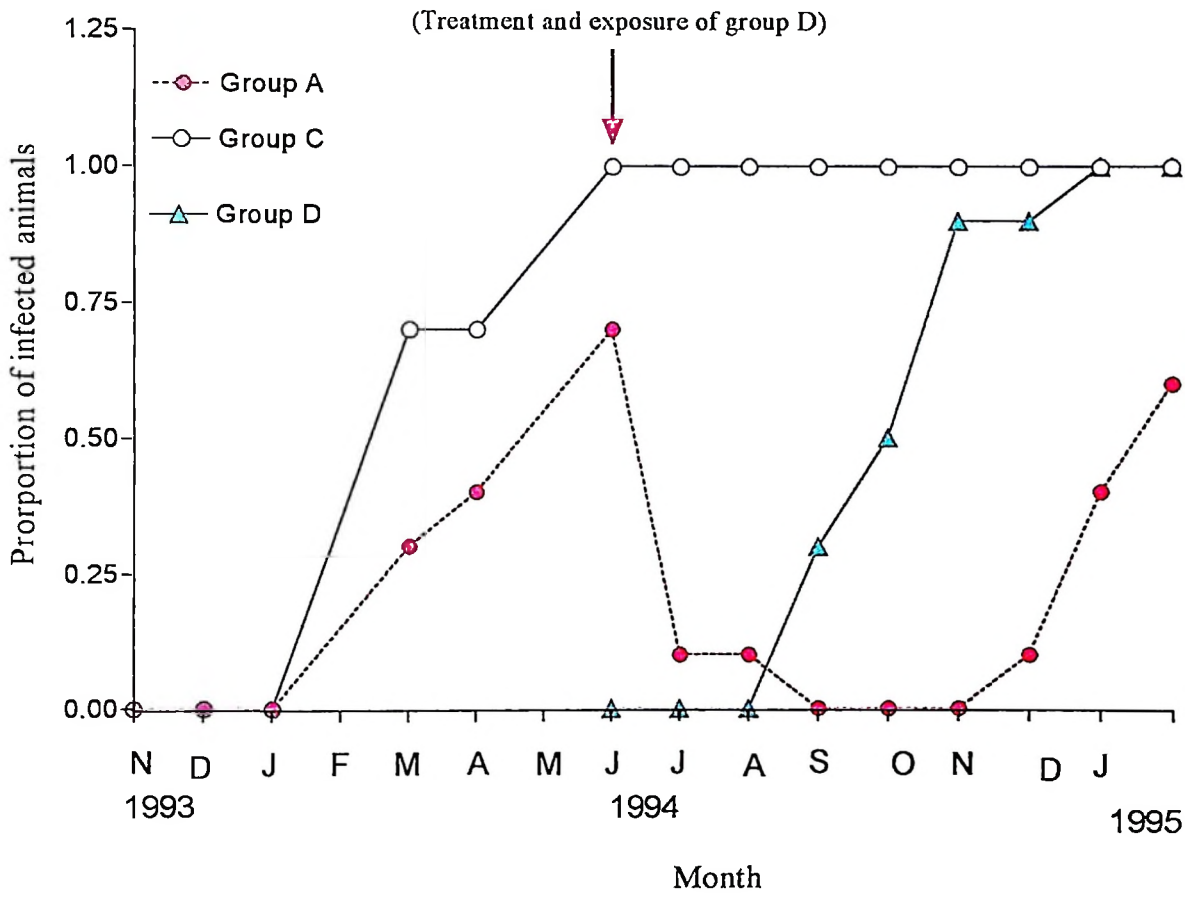

Figure 7. Proportion of calves infected with *Fasciola gigantica* based on detection of eggs in the faeces. Groups A = exposed, treated and challenged; B = exposed and treated; C = exposed, untreated and challenged; D =Challenge control; E = exposed and not treated Treatment = (Praziquantel 60 mg/kg, triclabendazole 12mg/kg given per oral simultaneously).

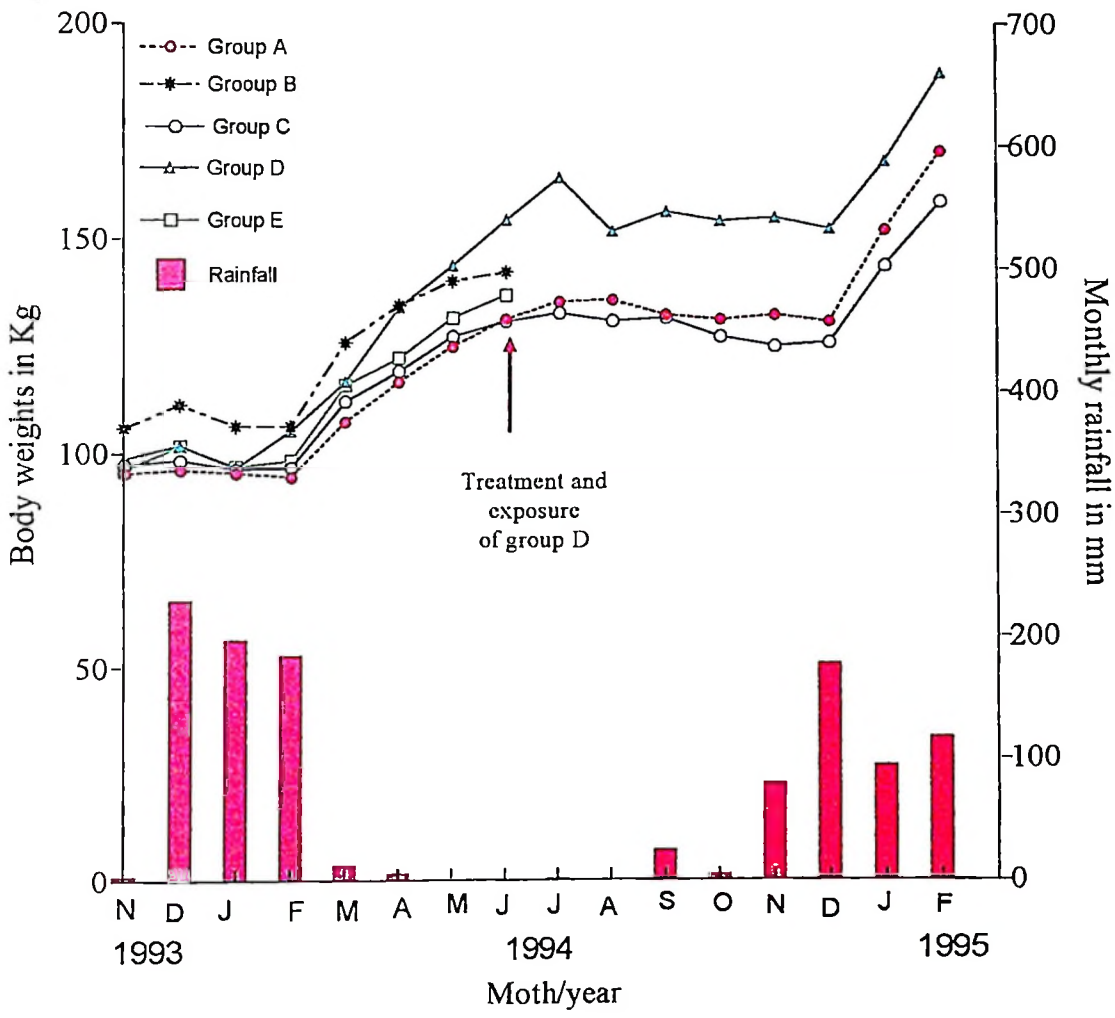

Figure 8. Body weight gains in calves exposed at a natural transmission site for *S. bovis* and *F. gigantica*. (A = exposed, treated and continued grazing to the end; B = exposed, treated and removed after treatment; C = exposed, not treated and continued grazing to the end; D = introduced as post-treatment challenge control; E = introduced as pre-treatment exposure control). Rainfall = average monthly rainfall; treatment = praziquantel 60 mg/kg, triclabendazole 12 mg/kg given per oral simultaneously).

(A) remained unchanged throughout this period and was not different from that of the control group (D), that of the untreated re-exposed group (C) declined progressively. At the peak of the dry season, August to November, the untreated re-exposed group (C) had significantly lower ($P \leq 0.05$) body weight than the groups A and D. Following the appearance of the grass in December 1994 as a result of the short rains in October and November, there was a rapid compensatory growth in all groups and their weight gains were not significantly different ($P \geq 0.05$).

4.1.2.4 Haematological parameters

The results of the PCV and Hb are shown in Figs. 9 and 10 respectively, their patterns resembling that of the body weight. Before patency of the trematode infections, the PCV and Hb of all animals were not significantly different ($P \geq 0.05$). At the beginning the two parameters were low and similar in all groups due to scarcity of feed. However, reappearance of enough feed from February and onwards was followed by a rapid increase in the values of both parameters. The improvement of Hb and PCV was significantly higher in the unexposed group (D) than the other groups (A, B, C and E). Following onset of the dry period in June and onwards, both parameters declined. However, the decline was more statistically significant ($P \leq 0.05$) in the untreated re-exposed group (C) than in the treated re-exposed group (A) and the challenge control group (D). However, when feed was available, both parameters improved in all groups, in particular those of the treated group (A).

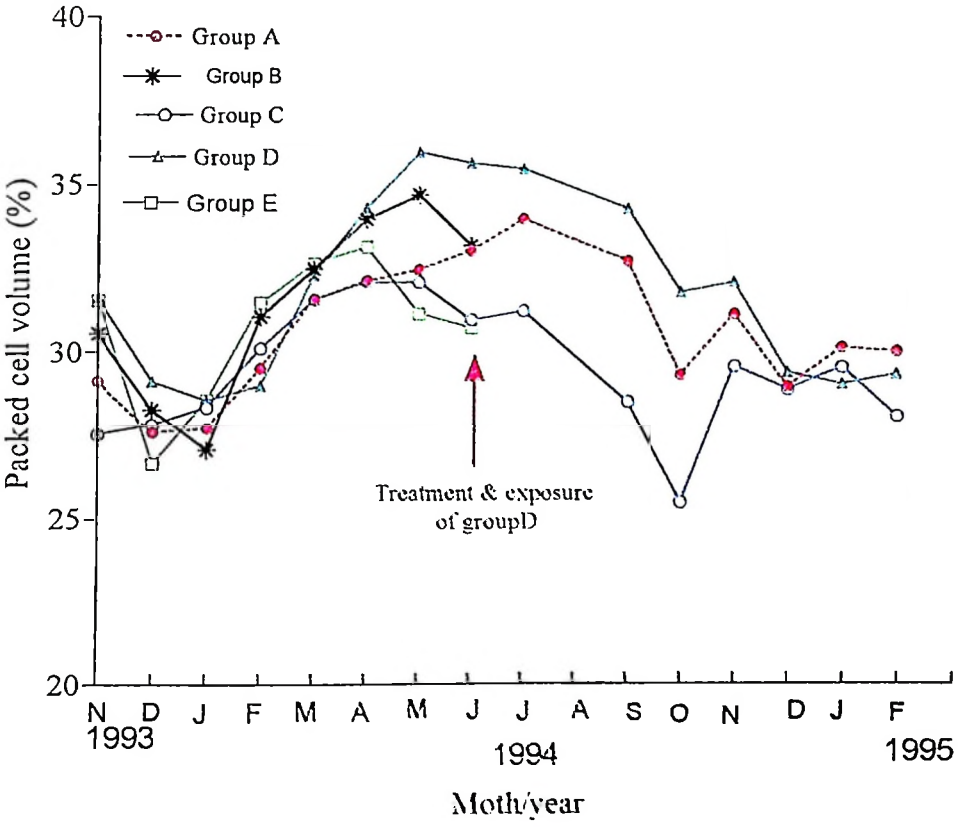

Figure 9. Packed cell volume changes in calves exposed at a natural transmission site *for S. bovis* and *F. gigantica*. Groups, A = exposed, treated and challenged; B = exposed and treated; C = exposed, untreated and challenged; D = Challenge control; E = exposed and not treated. Treatment = (praziquantel, 60mg kg and triclabendazole, 12 mg/kg given per oral simultaneously)

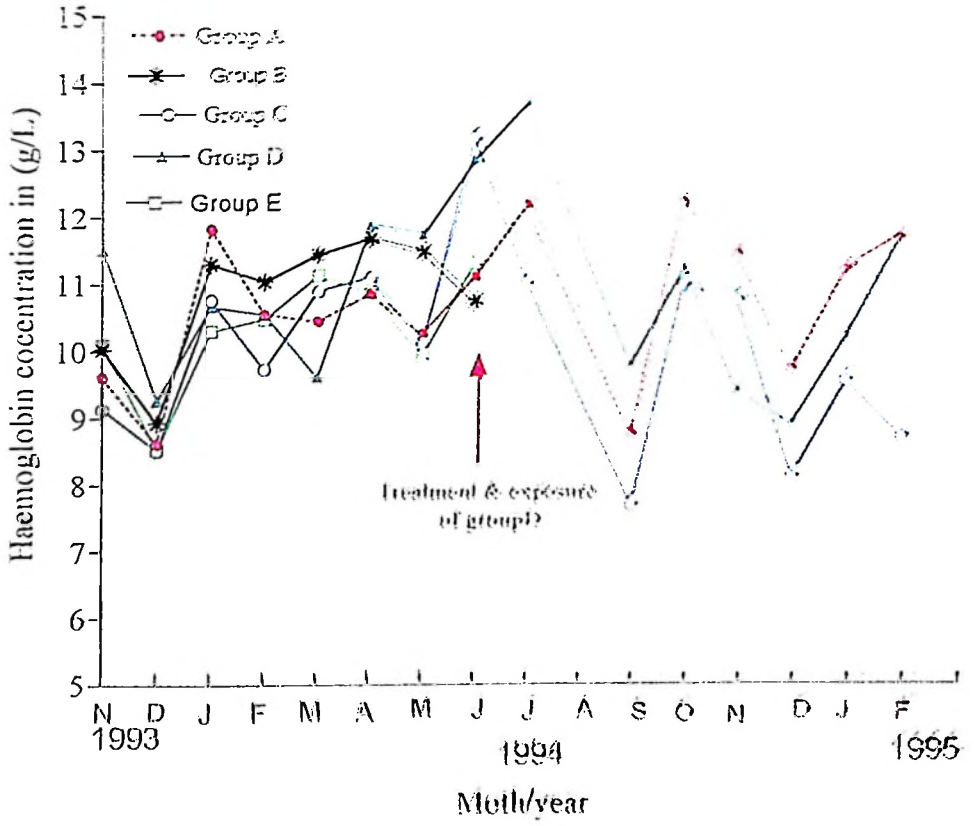

Figure 10. Blood haemoglobin concentration changes in calves exposed at a natural transmission site for *S. bovis* and *F. gigantica*. Groups. A = exposed, treated and challenged; B = exposed and treated; C = exposed, untreated and challenged: D = Challenge control; E = exposed and not treated. Treatment = (praziquantel, 60mg/kg and triclabendazole, 12 mg/kg given per oral simultaneously).

4.1.2.5 Worm burden

The mean number of *F. gigantica* and *S. bovis* worms in the different groups is shown in Table 3. The combined triclabendazole and praziquantel treatment reduced *F. gigantica* and *S. bovis* worms by 100% and 95.82 %, respectively, based on the comparison between the treated group (B) and untreated group (E) sacrificed six weeks after treatment. In the calves that were sacrificed at the end of the study following treatment and re-exposure, the treated and re-exposed group (A) had significantly ($P \leq 0.05$) fewer *Fasciola* and *Schistosoma* worms than the untreated re-exposed group (C). However, the mean number of *Schistosoma* worms in the treated group (A) was not significantly different ($P \geq 0.05$) from that of the challenge control group (D). For *Fasciola* worms, group D had significantly more flukes ($P \leq 0.05$) than the treated re-exposed group (A). By comparing the number of worms in the untreated groups during early (group E) and late stages of infection (group C), there was significantly ($P \leq 0.05$) more schistosome worms in calves in group C than in group E. For *Fasciola* flukes, this was *vice versa* showing that there was a reduction in worm burden even without anthelmintic treatment, as the infection progressed. It is also of interest to note that the few *S. bovis* worms that remained in the livers of the group B calves that were treated at an earlier stage of infection were mainly males. These males were found primarily in the liver and although they were not measured, they appeared very small in size compared to those recovered from the untreated calves. While the ratio of male (557) to female (281) in group E, examined at the early stage of the infection, was 1.98:1 that of group C examined at the late stage of infection (male = 935, females = 415) was a bit higher, 2.25:1.

Table 3. The arithmetic mean number of *F. gigantica* and *S. bovis* worms recovered from the different groups of calves exposed to natural infections for different durations. Praziquantel and triclabendazole treatment was carried out 8 months after exposure of groups A and B.

Group of calves	Length of natural exposure	Number of Worms			
		Fasciola ± S.E*	*Schistosoma*		
			Males ± S.E	Females ± S.E	Total ± S.E
Group A)	8 months, treated & re-exposed 8 months	6 ± 3	356 ± 50	183 ± 30	539 ± 77
Group (B)	8 months & treated	0	30 ± 13	5 ± 2	35 ± 14
Group (C)	16 months	17.0 ± 6	935 ± 283	415 ± 114	1350 ± 283
Group (D)	8 months as a post treatment control	10.0 ± 3	379 ± 58	231 ± 164	610 ± 120
Group (E)	8 months not (treated)	20 ± 12	557 ± 41	281 ± 39	837 ± 67

* S.E = Standard error of the mean

4.1.2.6 *Schistosoma* tissue egg counts

The mean number of eggs per gram of tissue (EPGT) for the different groups of calves is shown in Table 4. In the animals of the treated group, which were sacrificed six weeks after treatment (groups B and E), there were significant reductions ($P \leq 0.05$) in the number of EPGT as compared to the untreated group. The reduction varied between the different organs, *i.e.* liver 80%, small intestine 91-96%, large intestine 80-88% (details not shown). In the calves that were sacrificed at the later stage of infection following re-exposure after treatment, the untreated re-exposed group (C) had an overall higher number of EPGT than the other groups (A and D). Significantly higher ($P \leq 0.05$) proportions of eggs were found in the livers of

the untreated animals (group C) that were sacrificed at the late stage of the infection as compared to untreated ones (Group E) sacrificed at the early stages of infection.

Table 4. The geometric mean number of *S. bovis* tissue eggs per gram recovered from the different groups of calves exposed to natural infections for different durations. Praziquantel and triclabendazole treatment was carried out after 8 months of exposure of groups A and B.

Group of calves	Length of natural exposure	*Schistosoma* eggs	
		Intestines ± S.E*	Liver ± S.E
Group (A)	8 months, treated & re-exposed 8 months	190 ± 70	28 ± 8
Group (B)	8 months + treated	67 ± 40	8 ± 5
Group (C)	16 months	153 ± 80	290 ± 129
Group (D)	8 months as a post treatment control	188 ± 71	16 ± 6
Group (E)	8 months not treated	271 ± 69	40 ± 26

* S.E = Standard error of the mean

On the contrary, the animals that were sacrificed at the early stages of infection (group E) had an overall higher number of EPGT in the intestines than group C. No significant difference was found in the number of EPGT between the control group (D) and the treated re-exposed group (A).

4.2 Experimental *S. bovis* infections in calves

4.2.1 *Schistosoma* faecal egg excretion

The results from egg excretion in different groups are shown in Fig. 11. The pre-patent period was 6 weeks and peak egg excretion in the faeces was observed between 8 to 10 weeks post infection. Following treatment, the EPGF in the treated group (A) declined sharply to zero. In the untreated group (C), the EPGF continued to rise and after reaching a peak at 10-14 weeks after infection it started declining sharply and by the 14^{th} week post infection it had reached a very low level (EPGF <20). This low level EPGF was maintained and was not changed by the challenge infection at the 35^{th} week post infection. Challenging of the treated group (A) led to an egg excretion pattern similar to that of the challenge control group (D). However, a low peak (25-30 EPGF) was reached by both groups and there was no significance difference ($P \geq 0.05$).

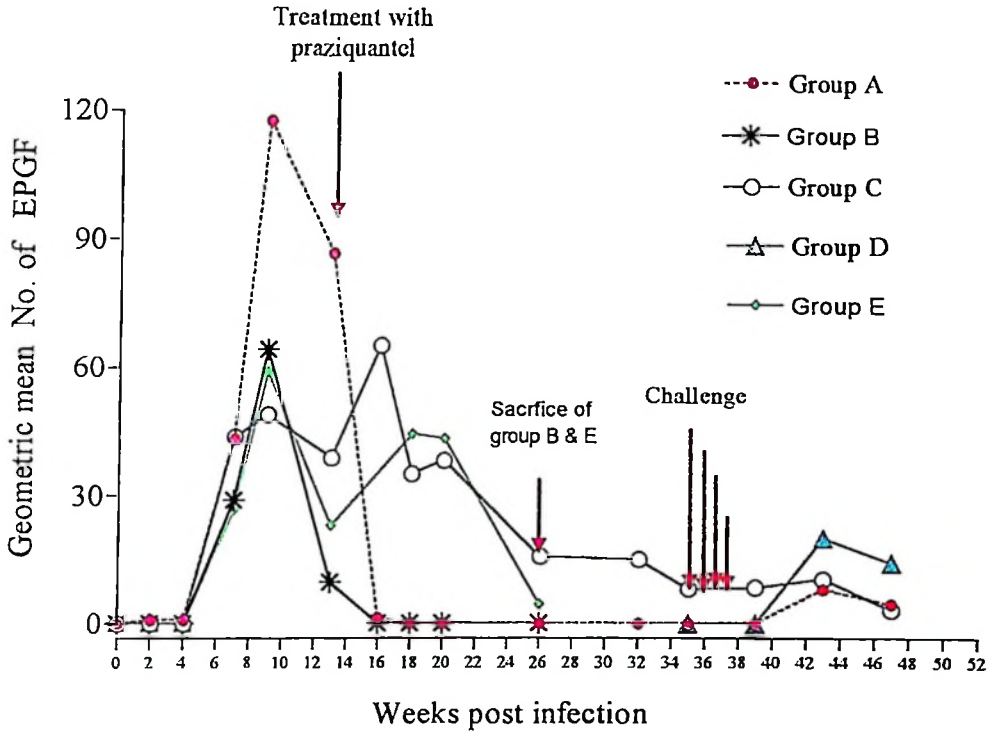

Figure 11. Faecal egg excretion in calves experimentally infected with S. bovis. Groups (A, B, C and E) were infected with 8 000 *S. bovis* cercariae. At week 13 post infection (p.i.) groups (A and B) were treated with praziquantel (60 mg/kg). At week 26 groups (B-treated and E-exposed and untreated) were sacrificed to asses worm and tissue egg burden and pathological changes during primary infection. Between week 34-37 groups (A-treated and D-untreated) were challenged with 6 000 *S. bovis* cercariae and at the same time group D calves were infected to serve as challenge control. Group (F) was not infected (not shown).

4.2.2 Body weight gains and haematology

The results of the body weight gains are shown in Fig. 12. Before patent infection the weight gains were similar in infected and uninfected groups. However, after eggs started to appear in the faeces the weight gains of the infected groups (A and C) were lower than that of uninfected group (F) but there was no statistical difference between the groups ($P \leq 0.05$). Following treatment, the weight gains of the untreated group continued to remain slightly lower than that of the treated group (A). The weight of the treated group (A) improved and was not different from that of the control group (F). Upon challenge the weight gain of the untreated group continued to be lower than that of the other groups although not significantly different. The decline in body weights from week 30 onwards was due to the difficulties in obtaining the proper feed due to drought. The trend of the haematological parameters resembled that of body weight gains. In most part of study period, the PCV (Fig. 13) and Hb (Fig. 14) values of the uninfected control group (F) remained high. No significant difference ($P \geq 0.05$) was observed in the PCV and Hb values of the treated (A) and untreated group (C).

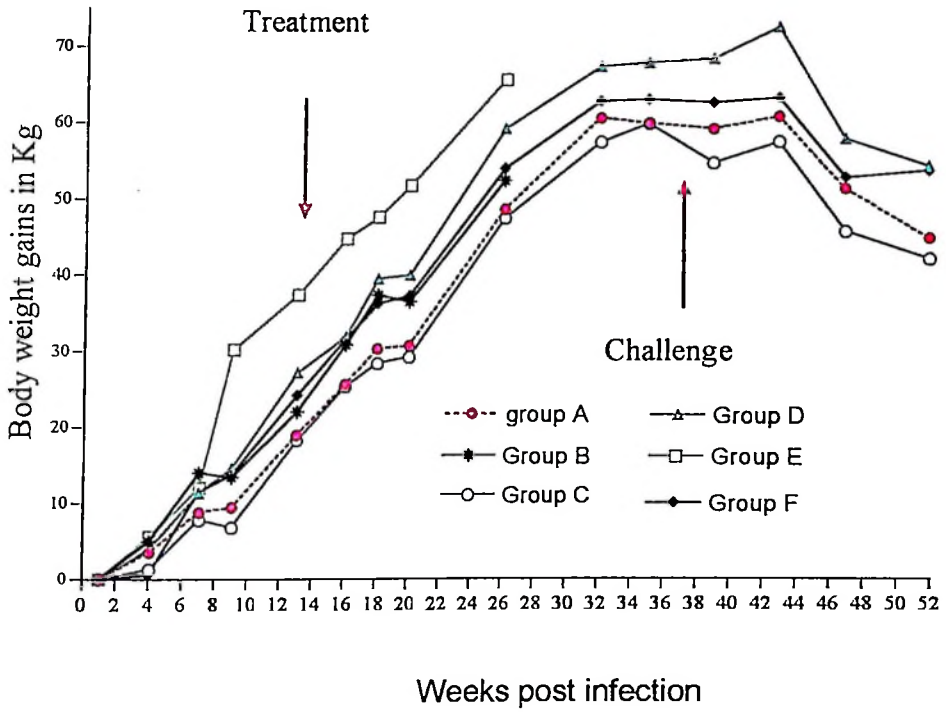

Figure 12. Cumulative body weight gains in calves experimentally infected with S. bovis. Groups (A, B, C and E) were infected with 8 000 *S. bovis* cercariae. At week 13 post infection (p.i.) groups (A and B) were treated with praziquantel (60 mg/kg). At week 26 groups (B-treated and E-untreated) were sacrificed to asses worm and tissue egg burden and pathological changes during primary infection. Between week 34-37 groups (A-treated and D-untreated) were challenged with 6 000 S. bovis cercariae and at the same time group D calves were infected as a challenge control. Group (F) was not infected.

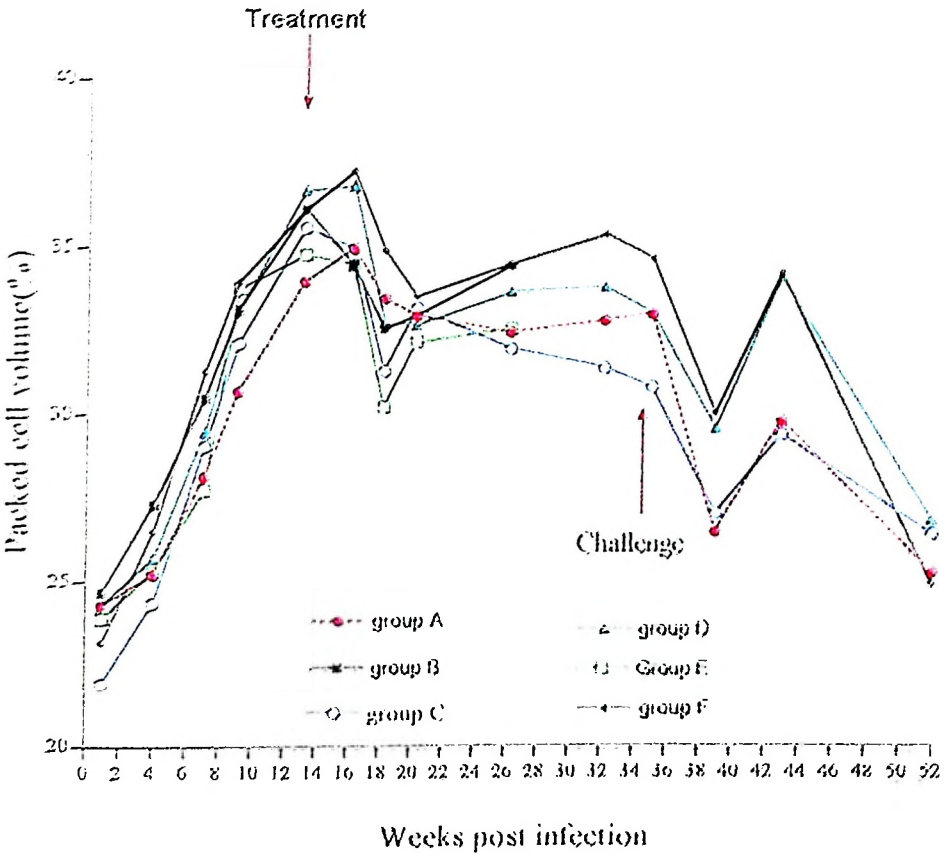

Figure 13. Packed cell volume changes in calves experimentally infected with S. bovis. Groups (A, B, C and E) were infected with 8 000 *S. bovis* cercariae. At week 13 post infection (p.i.), groups (A and B) were treated with praziquantel (60 mg/kg). At week 26 groups (B-treated and E-untreated) were sacrificed to asses worm and tissue egg burden and pathological changes during primary infection. Between week 34-37 groups (A-treated and D-untreated) were challenged with 6 000 *S. bovis* cercariae and at the same time, group D calves were infected as a challenge control. Group (F) was not infected.

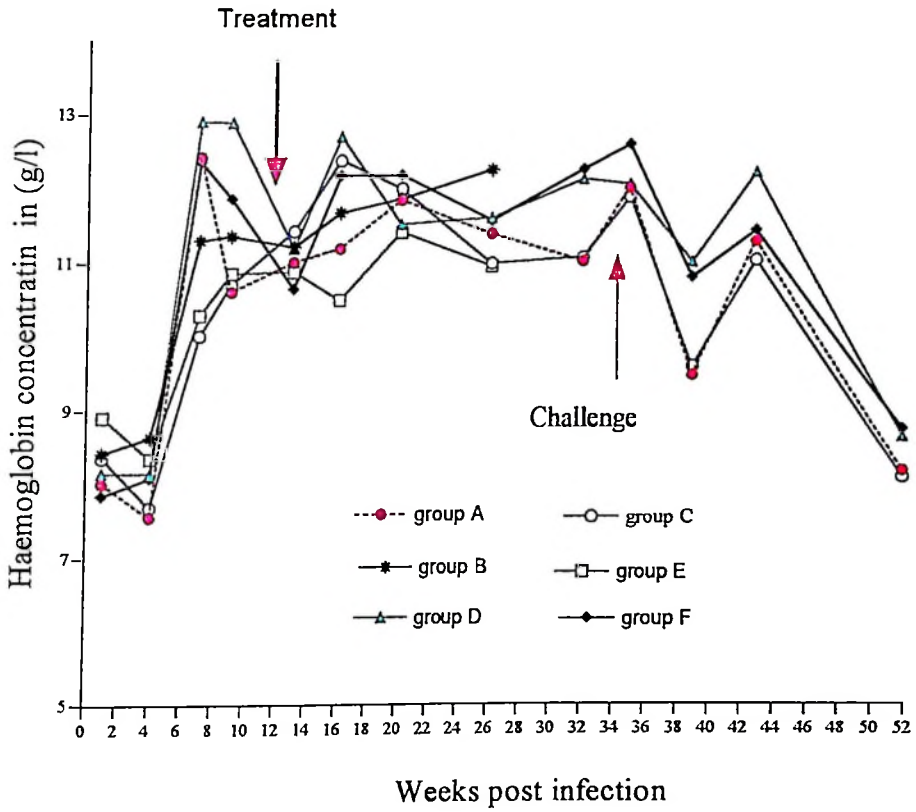

Figure 14. Haemoglobin concentration levels in calves experimentally infected with *S. bovis*. Groups (A, B, C and E) were infected with 8 000 S. bovis cercariae. At week 13 post infection (p.i.), groups (A and B) were treated with praziquantel (60 mg/kg). At week 26 groups (B-treated and E-untreated) were sacrificed to asses worm and tissue egg burden and pathological changes during primary infection. Between week 34-37 groups (A-treated and D-untreated) were challenged with 6 000 *S. bovis* cercariae and at the same time, group D calves were infected as post treatment challenge control. Group (F) was not infected.

4.2.3 Worm burden

The results of *S. bovis* mean worm burdens for the different groups are shown in Fig. 15. No worms were recovered from the treated group (B) animals, which were sacrificed after 13 weeks following praziquantel treatment. The untreated re-exposed group (C) had more *S. bovis* worms than other groups but the difference was not statistically significant ($P \geq 0.05$). The ratio of female to male worms was higher in the calves that were sacrificed at 26 weeks post infection (group E) as compared to those sacrificed at 52 weeks post infection (groups A and C). There was no significant difference in the number of worms between the treated group (A) and the challenge control group (D).

4.2.4 *Schistosoma* tissue egg enumeration

The results of the *S. bovis* tissue egg count per gram (EPGT) for the experimental calves are shown in Fig. 16. No eggs were detected in the treated calves (group B) that were sacrificed 13 weeks following treatment. During early infection there was significantly ($P \leq 0.05$) more eggs in the small intestine than in the liver of the untreated group (E). Inversely, in the old infections the proportion of eggs deposited in the liver of untreated re-exposed group (C) calves was significantly ($P \leq 0.05$) higher than in the small intestines. The mean number of EPGT in the liver and intestines of treated re-exposed group (A) and the control group (D) were not significantly different ($P \geq 0.05$).

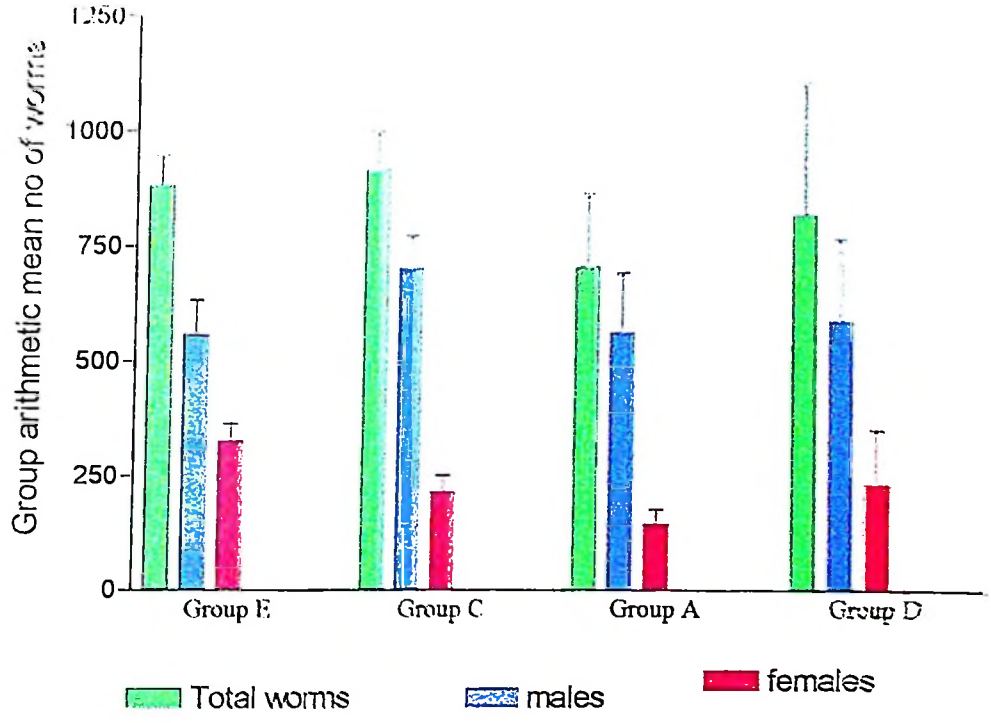

Figure 15. Arithmetic group mean number of worms (with standard error bars) in calves experimentally infected with *S. bovis*. Groups (A, B, C and E) were infected with 8 000 S. bovis cercariae. At week 13 post infection (p.i.), groups (A and B) were treated with praziquantel (60 mg/kg). At week 26 groups (B-treated and E-untreated) were sacrificed to asses worm and tissue egg burden and pathological changes during primary infection. Between week 34-37 groups (A-treated and D-untreated) were challenged with 6 000 *S. bovis* cercariae and at the same time, group D) calves were infected as a challenge control. Group (F) was not infected

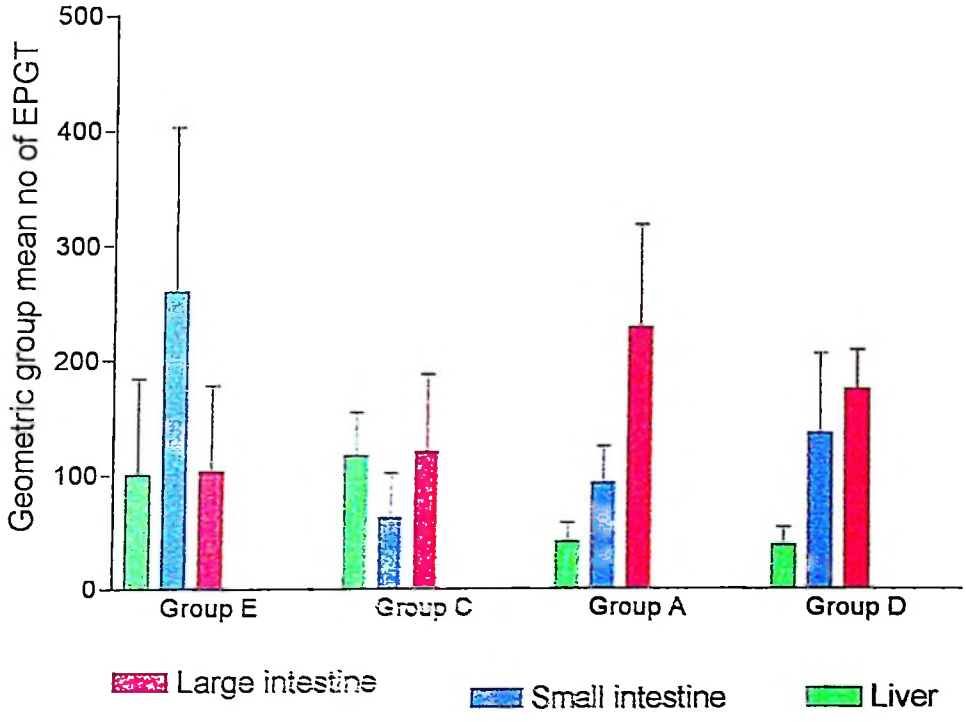

Figure 16 Geometric group mean (with standard errors) number of tissue eggs per gram in calves experimentally infected with *S. bovis*. Groups (A, B, C and E) were infected with 8 000 *S. bovis* cercariae. At week 13 post infection (p.i.), groups (A and B) were treated with praziquantel (60 mg/kg). At week 26 groups (B-treated and E-untreated) were sacrificed to asses worm and tissue egg burden and pathological changes during primary infection. Between week 34-37 groups (A-treated and D-untreated) were challenged with 6 000 *S. bovis* cercariae and at the same time, group D calves were infected as a challenge control. Group (F) was not infected.

4.2.5 Pathological observations

4.2.5.1 Animals sacrificed at 26 weeks post infection

4.2.5.1.1 Gross pathology

Following treatment of calves at week 13 and necropsy at week 26 post exposure for group B and E, there was a marked difference between the gross pathological lesions observed in the treated group (B) in comparison with the untreated group (E). Grossly, the treated calves (group B) had relatively more severe pathological lesions. These were characterised by scattered white-yellowish nodules reaching up to 2 cm in diameter (Fig. 17a, black arrow), adhesions of the liver to the diaphragm (adhesive peritonitis) (Fig. 17a, black arrow), and thickening of the intra-hepatic portal veins that were quite conspicuous under the capsule in the liver parenchyma. The liver was firm and on the cut surface, some of the observed nodules proved to be necrotic and abscessed areas (Fig.17b, black arrow) while in the other portal areas they were fibrosed. No worms were observed in the mesenteric veins of the treated (group B) calves and the only detectable lesions were mild thickening on the mesenteric vein at a short distance from the point of attachment of mesentery to the intestinal wall. The gross pathological liver lesions of the untreated group (E) (Fig. 18a) that were sacrificed at 26-week post exposure were less severe in comparison with the treated animals (group B). The only noted feature was a slight thickening of large portal veins (Fig. 18a). Unlike in the intestines of the treated group (B), the untreated group (E) had numerous adult schistosomes in their mesenteric veins that were accompanied with few vascular thickenings (intravascular granulomas) and marked petechial and echymotic haemorrhages in the intestinal mucosa.

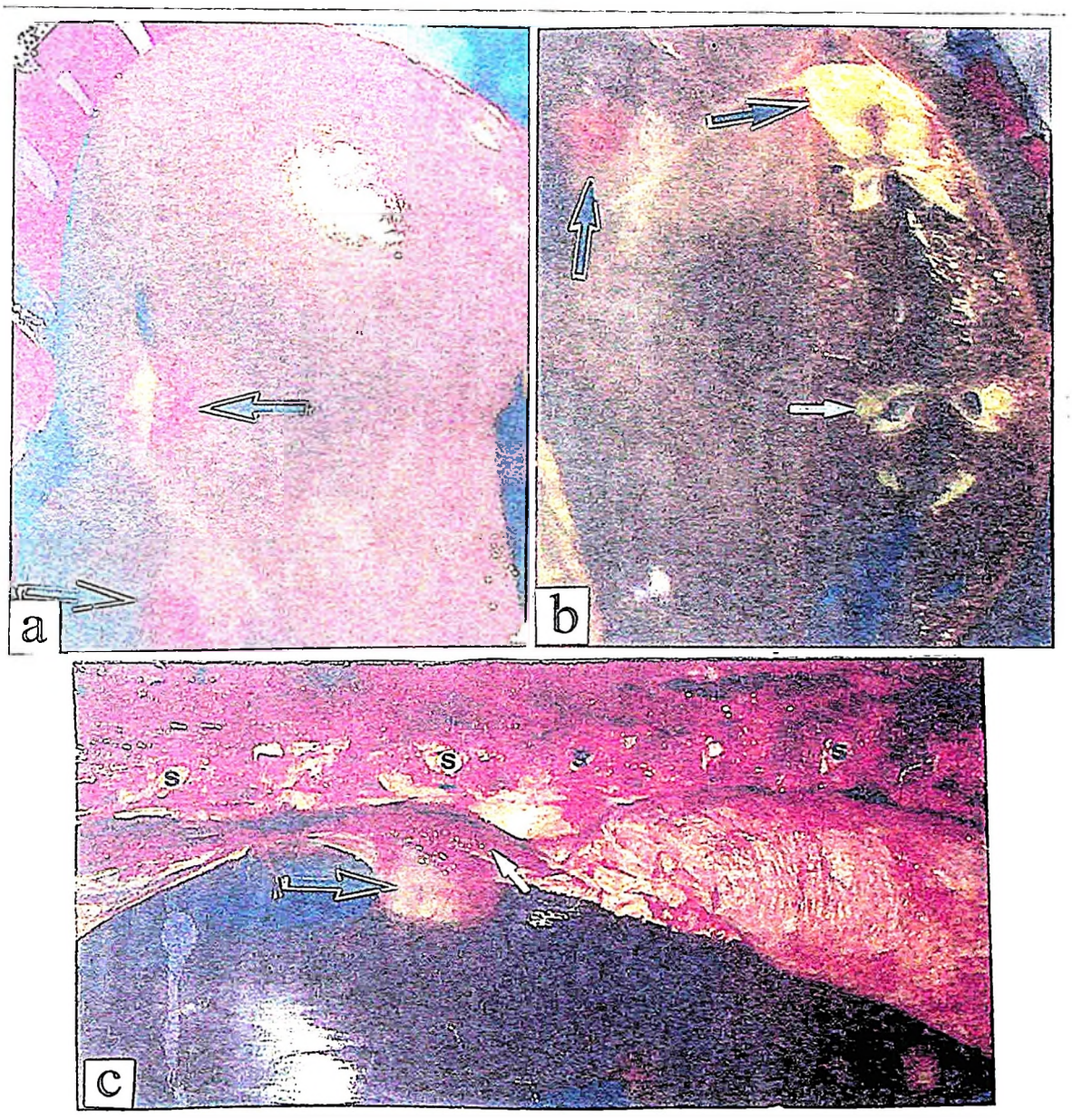

Figure 17. Gross pathological lesions in the liver of calves (group B) treated against *S. bovis* infection at week 13 p.i. and sacrificed at week 26 p.i. (a) Liver with yellowish white large nodules (black arrows), (b) Cut liver surface showing abscess (black arrow) and thickened portal areas due to fibrosis (white arrow). (c) Liver capsule adhesions to the diaphragm (black arrow), diaphragm (white arrow) and the vertebral column (s).

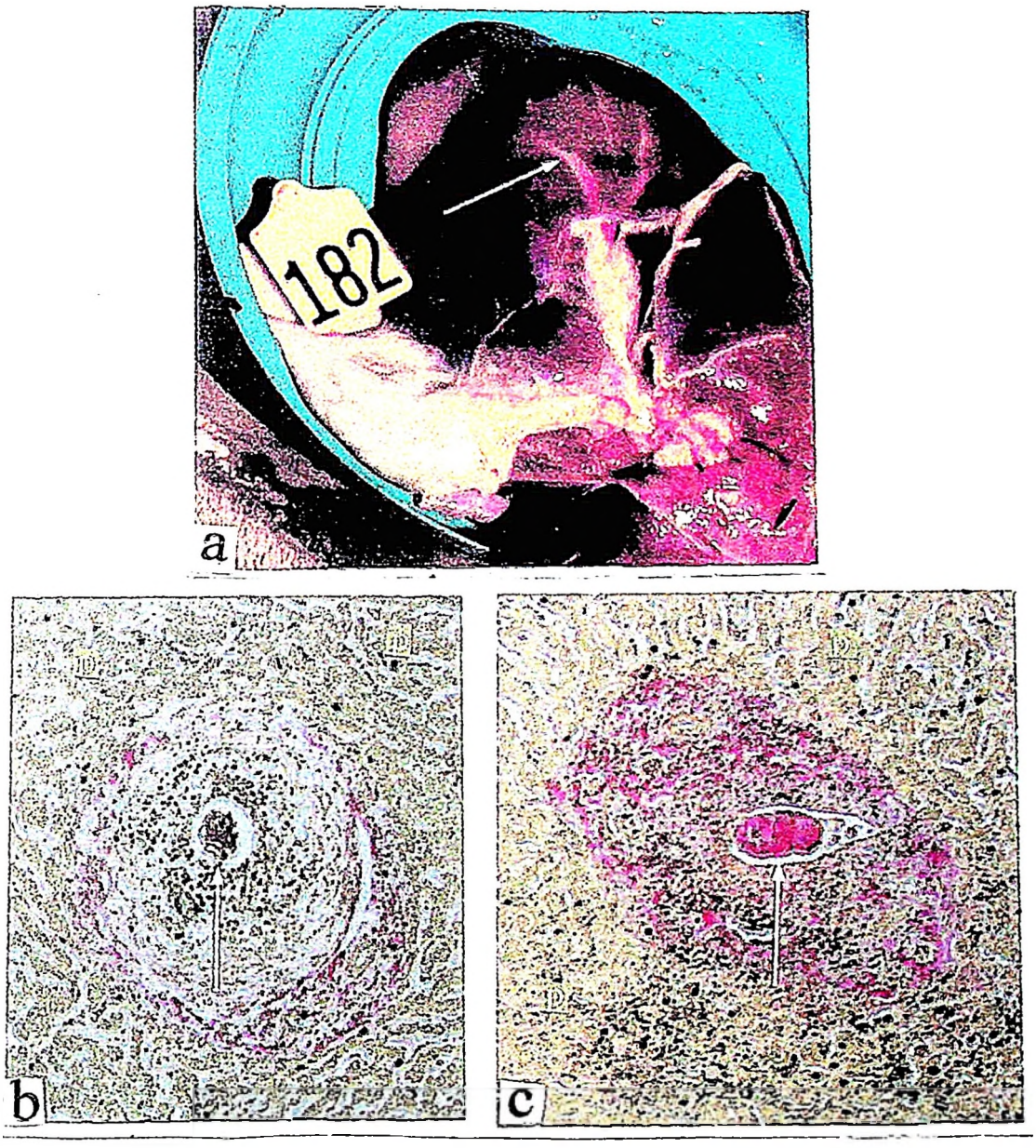

Figure 18. Gross (a) and histopathological (b and c) lesions in the liver of calves, which were not, treated (group E) against early patent experimental *S. bovis* infections. (a) The liver with mild gross pathological lesions such as the thickening of the portal tracts (white arrow). (b and c) Dominance of large egg granulomas with little mature collagen fibres (red) at the periphery. *Schistosoma* egg (white arrow) surrounded by inflammatory cells and normal liver parenchyma (P). Van Gieson's stain. X 200.

4.2.5.1.2 Histopathology

In the livers of the untreated calves (group E), there was marked inflammatory cell infiltration along the portal tracts and most of the *Schistosoma* egg granulomas were located mainly in the portal and peri-portal areas. These consisted mainly of a wide zone of eosinophils, lymphocytes and macrophages around the schistosome egg in the middle (Fig.18b and 18c) and had minimal deposition of mature collagen fibres (Fig.18b and 18c, red colour). Along the sinusoids some of the Kupffer cells were laden with dark-blackish *Schistosoma* pigment. In the portal veins, there was intimal proliferation; medial hypertrophy and perivascular cuffing with inflammatory cells predominated by eosinophils. In some areas few adult schistosomes were observed in the portal veins but without provoking any marked inflammatory reaction. In general, there were minimal collagen fibres accompanying the observed portal and peri-portal inflammatory reactions in comparison to the severe fibrosis observed in the treated group (B).

In the intestines of the untreated group (E), *Schistosoma* eggs, some of which were surrounded by inflammatory cells, were observed mainly in the intestinal mucosa. Most of the well-developed egg granulomas were located in the submucosa and in some areas with numerous egg (egg masses) granulomas that distended the submucosa. There was infiltration of eosinophils, lymphocyte and macrophages especially in the lamina propria and to a lesser extent in the sub-mucosa. The livers of the treated calves (group B) had extensive portal and peri-portal fibrosis, which extended and replaced a large amount of the adjacent parenchyma (Fig. 19a). Using van Gieson's stain it was evident that the fibrous tissue was predominantly composed of mature collagen fibres (Fig.19b and c). There was minimal inflammatory cell

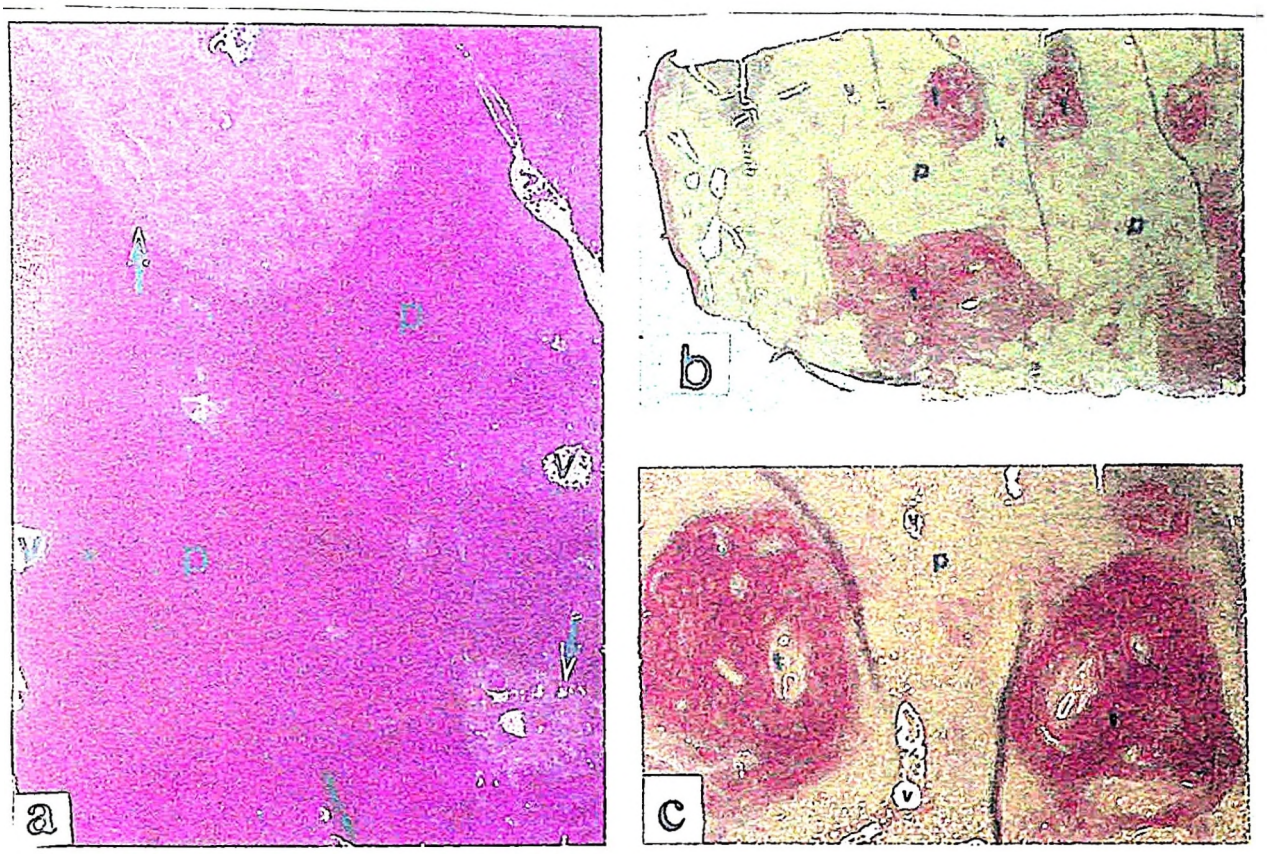

Figure 19. Histopathological lesions in the liver of calves that were treated (group against early patent *S. bovis* infections at Mazimbu farm. (a) Marked extensive fibrosis at the portal areas (black arrows) and accompanied formation of numerous blood vessels and bile ducts. The rest of the liver parenchyma (P) and the central vein (V) appear normal. H&E stain. (b) An over view of the liver under stereomicroscope showing collagen fibres (red colour) as the major composition of deposited fibrous tissue. Van Gieson's stain. X 25. (c) Higher magnification of Fig. 19b.

reaction and most of the normal blood vessels and bile duct architecture was destroyed and was replaced by several small regenerating vessels (Fig.19a). In the intestines, a few egg granulomas were noted in the rectal submucosa.

4.2.5.2 Animals sacrificed at 52 weeks post infection

4.2.5.2.1 Gross pathology

Unlike in animals that were sacrificed 26 weeks post exposure, the gross pathological lesions were more conspicuous and severe in the untreated-challenged cattle (group C) in comparison with the treated-challenged group (A) and the challenged control (group D). In the untreated-challenged animals (group C), there was marked liver fibrosis for most of the calves (4 out of 6), adhesive peritonitis and small yellow-white nodules (not exceeding 0.2 cm). The livers of these were dark-bluish-black in colour and had irregular surfaces (Fig. 20). Liver fibrosis was more marked especially on the left lobe and on the cut surface the portal areas presented a pipe stem fibrosis-like appearance (Fig. 20b). The livers of the treated-challenged group A (Fig. 20c) had less pronounced portal thickening.

In the intestines of the untreated-challenged calves (group C) there was marked thickening of mesenteric veins due to the granulomas a short distance from the attachment of the mesentery to the intestinal wall (Fig. 21a). Also there was enlargement of intestinal lymph nodes, numerous worms and haemorrhages on the intestinal mucosa especially at the ileo-caecal-colon region of the gut. The distribution of the gross lesions in the liver and small intestine in the treated challenged group (A) resembled that of the untreated challenged group (C).

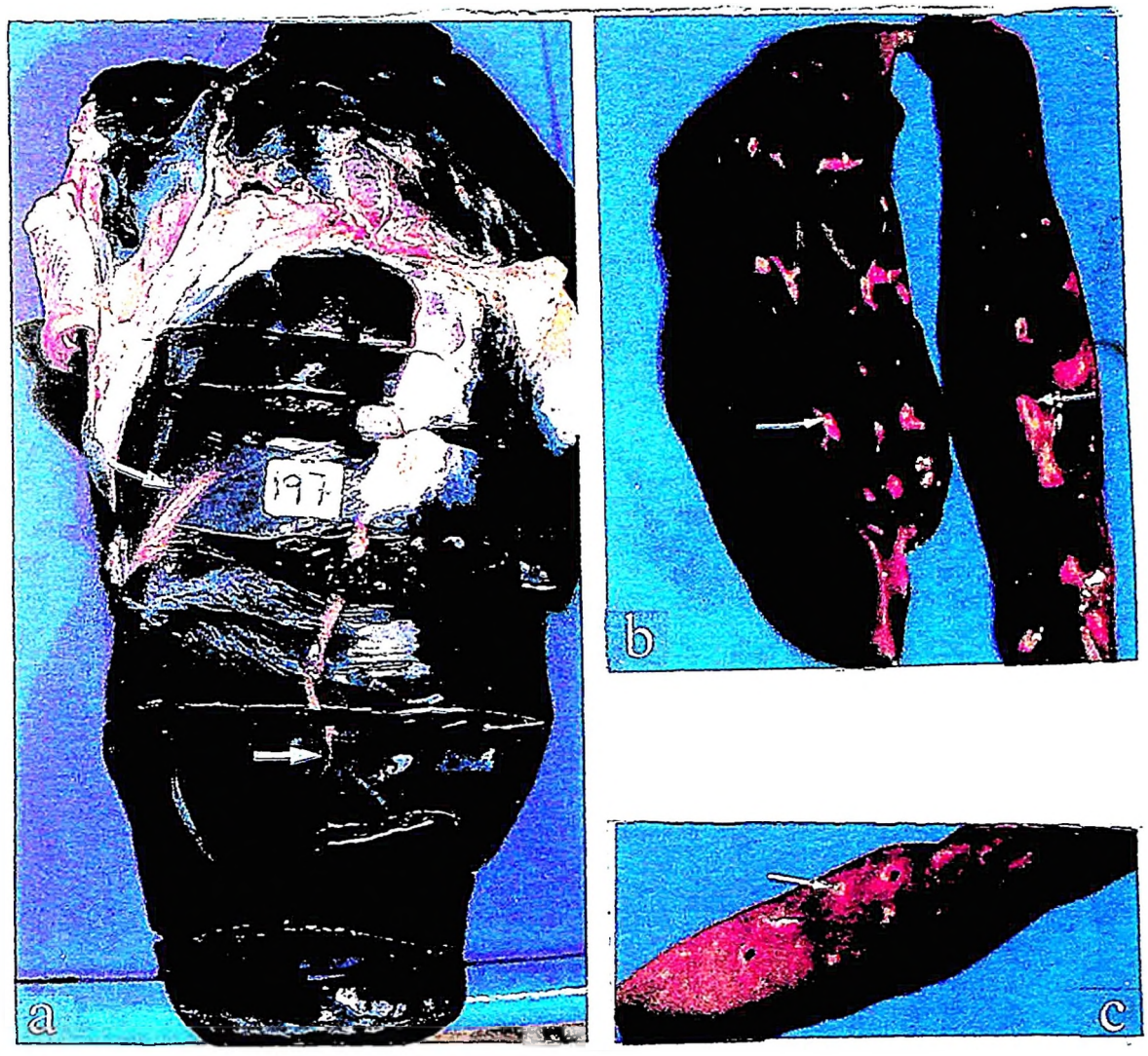

Figure 20. Gross pathological liver lesions in the untreated-challenged calves (group C) that were sacrificed at late stage (week 52 p.i.) of the *S. bovis* experimental infections. (a) Darkening of Liver with marked thickening of the portal areas (white arrow). (b) Cut liver surface showing thickened portal areas (white arrow) giving pipe stem like appearance. (c) Liver of the treated and challenged group (A) with mild lesions.

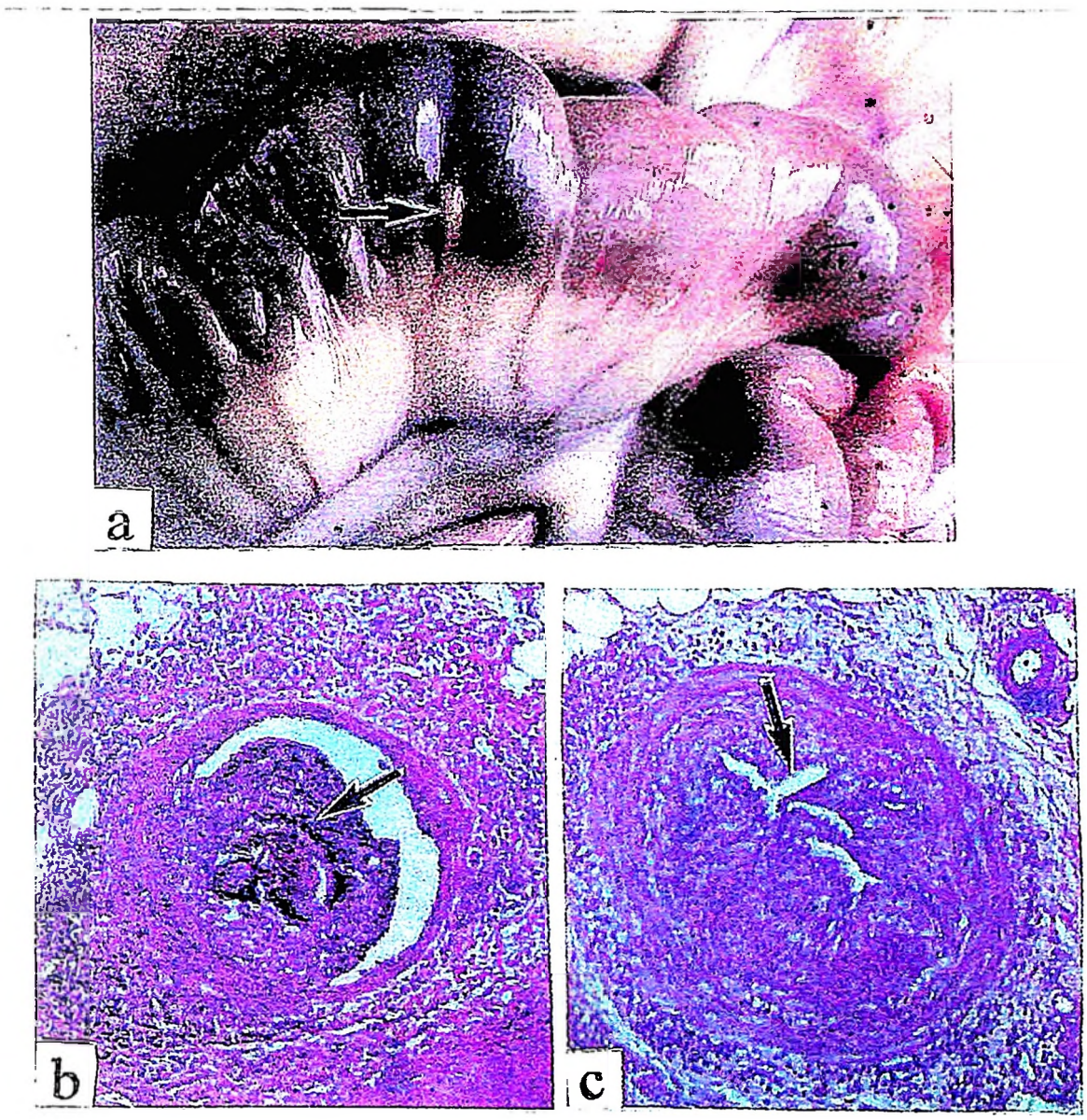

Figure 21. Gross and histopathological intestinal lesions in the untreated-challenged calves (group C) which were sacrificed at week 52 post *S. bovis* experimental infections at Mazimbu farm. (a) Gross view of an intra vascular granuloma in a mesenteric vein (black arrow). (b & c) Cross-sectional histopathological view of partially blocked vessels. (b) Partially blocked vessel with intimal granuloma thrombus accompanied by a peri-vascular cuff of inflammatory cells predominantly eosinophils. (c) Almost blocked veins due to hyperplastic proliferation of the intima with a lesser amount of mononuclear cell infiltration. Only narrow openings remained to allow blood flow (arrow). H and E stain. X100.

However, the lesions were milder. Similarly, the intestinal lesions were mild in the treated group except for the two calves that had marked haemorrhages on the intestinal mucosa. In the untreated challenge control animals (group D) there were relatively mild pathological lesions, which were not different from those observed in treated group (A).

4.2.5.2.2Histopathology .

In the intestines of the untreated-challenged calves (group C), the most dramatic histopathological lesions were the involvement of the mesenteric veins where some of them were partially or almost occluded by granulomas thrombi (Fig. 21b) or excessive hyperplastic intimal proliferation (Fig. 21c). In the less severely affected vessels (Fig. 21b), there were early intravascular egg granulomas thrombi that partially occluded the passage and was accompanied by a perivascular cuff of inflammatory mononuclear cells predominantly eosinophils. In the severely affected vessels (Fig. 21c) the hyperplastic proliferation of the intima completely blocked the outlet and only few narrow openings remained with a blood flow. Furthermore, on the intestinal epithelial mucosa of these animals there were relatively few schistosomal lesions compared to animals that were sacrificed at 26 weeks post infection. In the liver there was marked deposition of fibrous tissue (mature collagen fibres) tissue at the portal areas and in the adjacent parenchyma of the untreated-challenged calves in (group C) (Fig. 22b). Similarly, the vascular changes and hyperplastic intimal proliferation and the biliary ductular hyperplasia were more extensive. In the treated-challenged animals (group A) the liver lesions were comparably less severe (Fig. 22c).

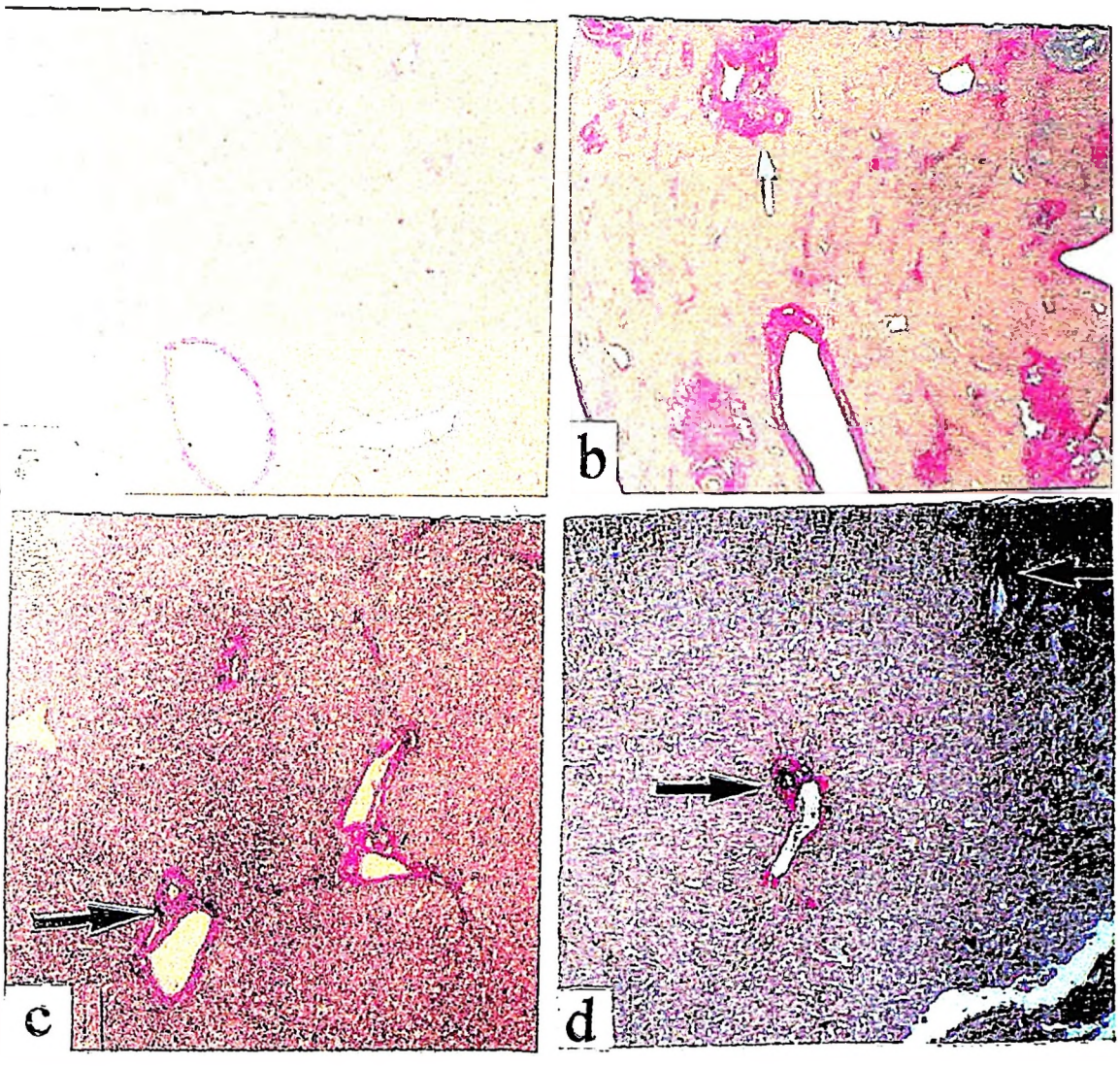

Figure 22. Histopathological liver lesions in calves sacrificed at week 52 p.i. of *S. bovis* experimental infection. (a) Uninfected control calves with negligible mature collagen deposition in the portal areas (the liver capsule stains red - white arrow). (b) The untreated-challenged calves (group C) with severe portal fibrosis extending into the liver parenchyma (red colour). X25.Van Gieson's stain. (c) The treated challenged group (A). X 40 Van Gieson's stain.

4.3 Distribution of S. *bovis* eggs in the small intestines of cattle

4.3.1 Calves sacrificed at 7 weeks post infection

The results of the relative distribution of tissue eggs in the small intestine of animals, which were sacrificed at seven weeks post infection, are shown in (Fig. 23a). There was significantly ($P \leq 0.05$) higher mean number of eggs in the anterior part of the small intestine than the other parts. Although the posterior part had lowest mean number of eggs, this was not statistically different ($P \geq 0.1$) from that of the middle portion.

4.3.2 Calves sacrificed at 18 weeks post infection

The relative distribution of *S. bovis* eggs across and along the mucosa for the animals sacrificed at 18 weeks post infection is shown in Figure 23b. Significantly more eggs ($P \leq 0.05$) were deposited in the mucosa than in the submucosa and the underlying tissues. The central part had statistically ($P \leq 0.05$) higher mean number of eggs than the anterior and posterior parts. Compared to the calves sacrificed at 7 weeks of infection, the eggs in the anterior part remained fairly unchanged while they significantly ($P \leq 0.05$) in the central and posterior parts.

4.3.3 Calves sacrificed at 32 weeks post infection

The relative distribution of *S. bovis* eggs across and along the mucosa for the animals sacrificed at 32 weeks post infection is shown in Figure 23c. Eggs were primarily deposited in the mucosa and only few eggs were deposited in the submucosa. Although the central part had more eggs than other segments and the proportion of the eggs deposited in the posterior part had increased while those

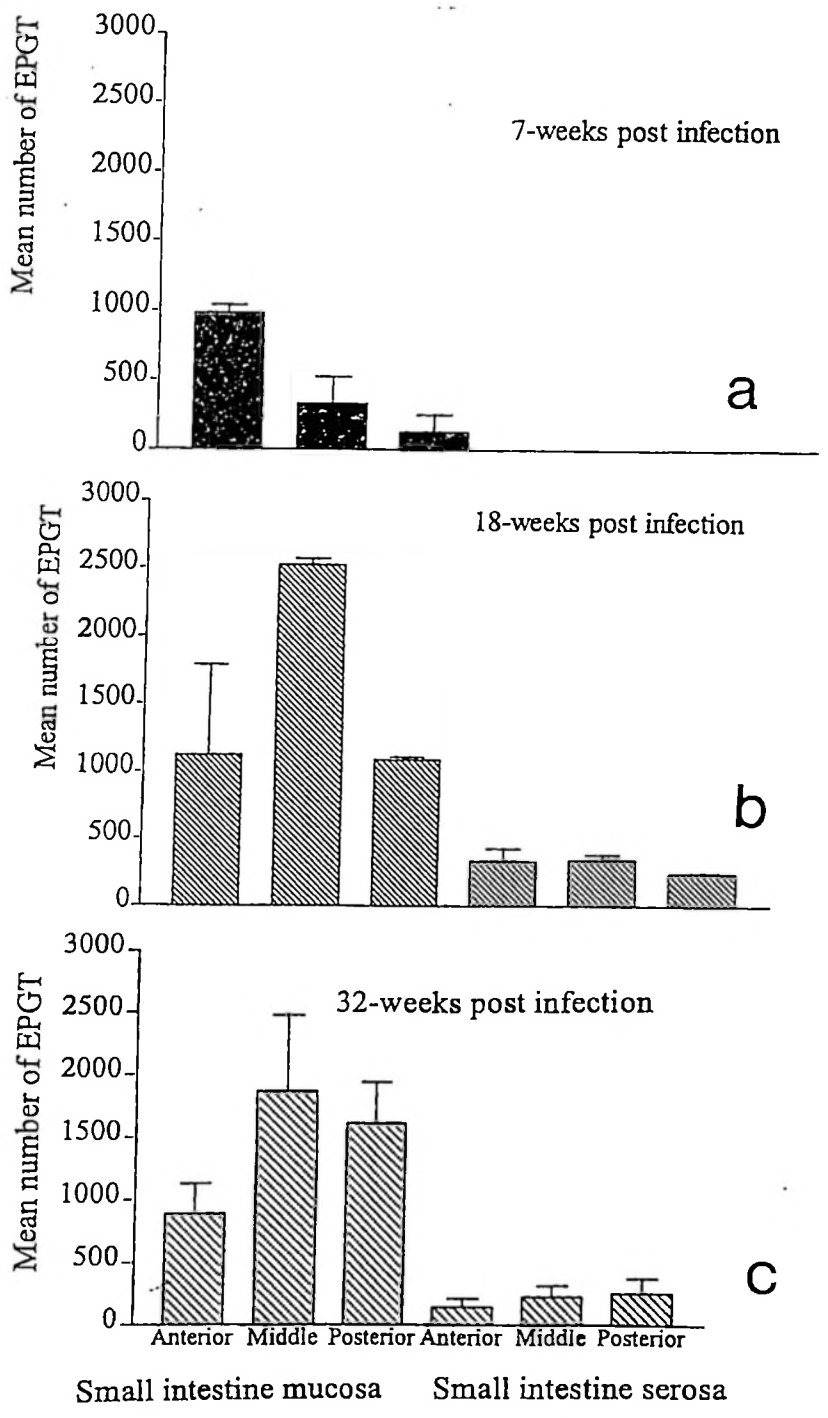

Figure 23. Geometric mean number of *Schistosoma bovis* eggs per gram of tissue (EPGT) along and across different parts of small intestine of calves sacrificed at different stages of infection. (Error bars = standard error of the means).

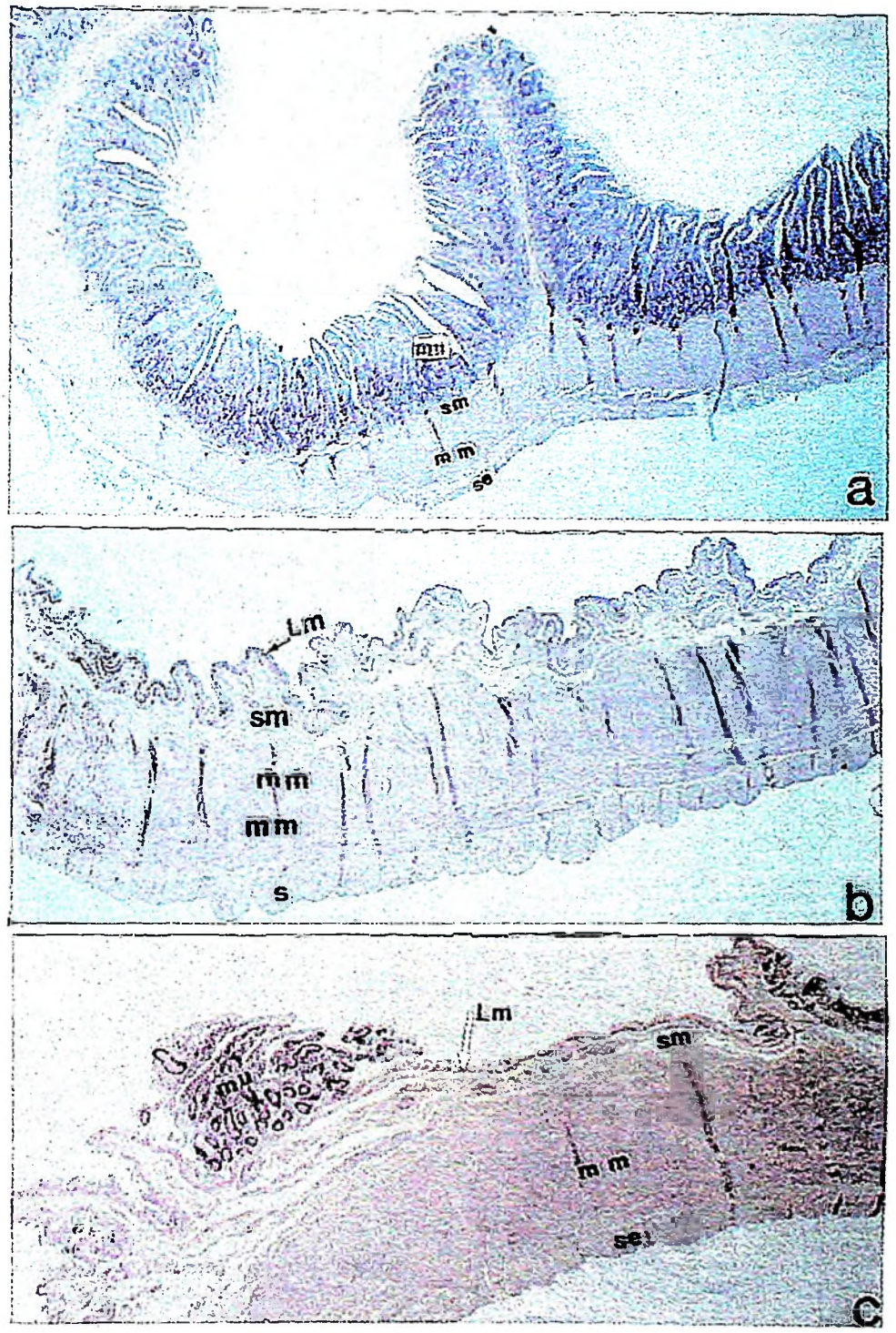

Figure 24. Histological section of scraped and normal small intestinal mucosa of cattle. (a) Un-scraped small intestinal (jejunum) of a calf (mu = mucosa, sm = submucosa, mm = muscular layers and se = serosa). (b) Scraped mucosa leaving the laminar muscularis (Lm), submucosa(sm), muscular (mm) and serosa layers (se) intact. (c) Very small parts of mucosa (mu) remained in very few areas.

deposited in the anterior part had slightly decreased, such differences were not statistically significant ($P \geq 0.1$). Therefore, at the 32 weeks post infection the eggs were more or less evenly distributed along the small intestine.

4.3.4 Histological assessment of the mucosa scraping

The results of the assessment of the scraping of the intestinal mucosa are shown in Figure 24. Before scraping, all the different parts across the intestinal wall were intact as shown in Figure 24a. The scraping removed the intestinal mucosa exclusively, leaving the lamina muscularis intact as well as the underlying submucosa, the longitudinal and circular muscle layers and the serosa. However, in a few places, insignificant parts of the intestinal mucosa were left, while in some other few areas, the laminar muscularis was broken and exposed the sub-mucosa (Fig. 24c).

CHAPTER FIVE

DISCUSSION

5.1 Snail studies at Lulanzi dairy farm

Identification of potential transmission sites and finding naturally infected snail species is the best method of determining the snail species serving as vectors for a particular schistosome species in a particular habitat (Teesdale and Nelson, 1958). Previous cross-sectional studies at Lulanzi dairy farm have shown that *B. natalensis* was the only bulinid snail species which was present in the pond where cattle were regularly watered (Makundi, 1993). So far, this snail species has not previously been found to be naturally infected with *S. bovis*. However, there are reports in Kenya that the closely related diploid *B. tropicus* snails could be naturally infected with *S. bovis* (Ouma and Waithaka, 1984; Southgate *et al.*, 1985). The results from the present longitudinal snail studies at Lulanzi dairy farm confirm that this snail can naturally transmit *S. bovis* and maintain natural transmission of this infection in cattle at the area in the absence of other suitable bulinid snails. So far, in our previous limited studies, it was shown that the first generation of laboratory bred *B. natalensis* could not be successfully infected with *S. bovis* from the same farm (Makundi, 1993). Such laboratory findings compared well with those obtained by Southgate *et al.*, (1985) who noted that only those snails which had pre-existing paramphistome infections could be naturally infected with *S. bovis*. Further laboratory studies showed that it was possible to infect *B. tropicus* with *S. bovis* if the snails had previously been exposed to miracidia of *Calicophoron microbothrium*

(Southgate *et al.*, 1989). It was suggested that amphistome infection suppressed the immune system of the snails, thus allowing *S. bovis* to develop. Similarly, studies have shown that pre-existing *Echinostoma paraensei* interferes with the ability of *Biomphalaria glabrata* to develop resistance against *S. mansoni* by releasing factors targeted on the snail haemocytes (Loker *et al.*, 1986). Paramphistomosis is highly endemic at Lulanzi farm as was observed in the present and previous studies (Makundi *et al.*, 1998). Therefore, as observed by Southgate *et al.* (1989) the concurrent paramphistome infection might have influenced the present snail to serve as the intermediate host for *S. bovis.* The observed very low mixed *Schistosoma* and *Paramphistomum* infection of *B. natalensis* snails in the present study could possibly be due to the fact that the snails were not crashed as they were kept for further confirmation of their species. Therefore, it is quite possible that some of the intra-molluscan larval stages were missed. However, it has been established that the existence of double trematode larvae infection in snails is very rare (Loker *et al.*, 1981; Kigadye, 1998), presumably because it is associated with higher mortalities than in single infected or uninfected snails (Moravec *et al.*, 1974, Walker, 1979). Also, it has been observed that in dual infections one parasite normally becomes dominant and will be shed by the snail while others become inhibited (Walker, 1979). Therefore, the additional unshed *S. bovis* larval stages within the un-crashed snails would not significantly have changed the observed dual low infection rates. Similarly, very few (3.5%) of the surviving snails infected with both *S. bovis* and *Calicophoron microbothrium* were found shedding both parasites (Southgate *et al.*, 1989). It is also quite possible that the development period for *S. bovis* in *B.*

natalensis is much longer than 30 days, which is observed in *B. africanus* and *B. forskalii*, a fact that was overlooked in the present study.

So far, most of observations in which the diploid *B. truncatus/tropicus* has been involved as an intermediate host for schistosomes seem to be in favour of *B. natalensis* rather than *B. tropicus.* Earlier observations in Ethiopia by Graber (1974) and Graber and Dynes (1974) showed that the morphology of the diploid *B. truncatus/tropicus* snails, which were transmitting *S. bovis*, resembled that of *B. natalensis*. Similarly, the diploid *B. truncatus/tropicus* which were observed to be naturally and experimentally capable of transmitting *S. bovis* after pre-existing paramphistome infection, had angular mesocones, a character which is common in *B. natalensis* species (Southgate *et al.*, 1985, 1989). However, the fact that they were not aphallic associated them with *B. tropicus* and their species identification was inconclusive. Clear-cut species identification of the diploid *B. truncatus/tropicus* based on morphological features alone is rather complex. So far all 40 populations of diploid *B. truncatus/tropicus* complex studied in Kenya by Brown *et al.*, (1991) were considered to be *B. tropicus* as all samples appeared to belong to the same species regarding their morphology, geographical pattern and their ability to interbreed. Only two out of the 40 populations of diploid *B. tropicus* snails examined were infected with *S. bovis* (Southgate *et al.*, 1985, 1989; Brown *et al.*, 1991). Although primary infection with *C. microbothrium* has been observed to facilitate the establishment of *S. bovis* in *B. tropicus*, the existence of the enzyme acid phosphotase only in the two populations that were infected with *S. bovis* (Southgate *et al.*, 1985, 1989; Brown *et al.*, 1991) suggests that genetic constitution may play a role. Detailed malacological species identification studies were not the scope of the present study. However, there

is a need in the future to carry out further genetic studies based on molecular biology techniques (Strahan *et al.*, 1990; Stothard and Rollinson, 1996) in order to gain a clear understanding of this snail taxa. Nevertheless, the present study, together with previous observations in Ethiopia and Kenya, suggests that the diploid *B. tropicus/truncatus* may under certain natural conditions such as pre-existing amphistome infections transmit *S. bovis*.

Well-defined seasonal transmission of schistosome infection in snails as seen in the present study has previously been reported in other parts of Tanzania (Loker *et al.*, 1981; Kassuku *et al.*, 1986; Marti, 1986; Lwambo, 1988). The observed high transmission mainly towards the end of the rainy season implies that high morbidity, especially in primarily exposed cattle, will occur in the middle and towards the end of the dry season. It is well accepted that the effect of the parasite is highly influenced by the nutritional status of the host (Hammond, 1965; Lawrence, 1977c; Johansen *et al.*, 1997a). Therefore, such occurrence of high morbidity during the dry season would further aggravate the poor health condition of the animals, which even in the absence of the parasite infection is severely affected by the scarcity of feed. Hence, these findings explain why most of the deaths due to schistosomosis in this farm were observed during the dry season (Makundi, *et al.*, 1998).

As observed in other farms in the Iringa district by Kassuku *et al.* (1986), the transmission of the trematode infections was intensified by providing water to large numbers of cattle at a limited water resource. Lack of watering facilities or repair of the existing ones forced the herdsmen to water the animals at a natural

water body where intermediate host snails were thriving. Therefore, the improvement of watering facilities is one of several ways of reducing the transmission intensity.

In summary, the present study has demonstrated that *B. natalensis* was the most abundant freshwater snail in this farm, and that certain local conditions, such as pre-existing of paramphistome infection, may predispose it to serve as an intermediate host for *S. bovis*. The study also showed that the high intensity of the transmission of *S. bovis* infection occurs mainly towards the end of the rainy season and is intensified by the traditional management system of watering many cattle on a limited water source.

5.2 *Schistosoma* faecal egg excretion, worm and tissue egg counts

As observed in other previous studies in cattle and goats (Majid *et al.*, 1980a; Saad *et al.*, 1980; Monrad *et al.*, 1990) the natural and experimentally *S. bovis* infected untreated calves in the present study were capable of developing resistance to challenge infections. As reported in other experimental studies (Saad *et al.*, 1980; Bushara *et al.*, 1983b), faecal egg excretion reached a peak rapidly at eight to nine weeks post infection followed by rapid drop in faecal egg excretion in the calves that were experimentally infected with *S. bovis* at Mazimbu. On the contrary, although the naturally exposed calves at Lulanzi dairy farm were positive for *S. bovis* as early as two months post exposure, attainment of peak faecal egg excretion was not reached until four months later. Also, the drop in faecal egg excretion in the naturally exposed calves was slow and gradual compared to the results obtained from the experimental studies. The observed slow development of resistance based on the faecal egg excretion in the naturally exposed calves in the present study, supports the

previous suggestion that animals under field conditions in endemic areas receive continuously low numbers of cercariae which are unable to induce full protection until threshold number of worms has been reached (Christensen *et al.*, 1983). Similarly, low primary dose of *S. bovis* experimental infection in goats could not fully provide protection against moderate challenge infection (Monrad *et al.*, 1995). So far the documented severe clinical signs of acute disease such as diarrhoea and anaemia as reported in natural outbreaks (Reinecke, 1970; van Wyk *et al.*, 1974; Markovics *et al.*, 1985) were not evident either in the field or in the experimental infections, presumably due to the relatively low primary infection to which the animals were exposed.

For a long time, presence of adult schistosomes has been considered to be essential for maintenance of the acquired resistance in animals (Smithers and Terry, 1967). Therefore, it has been feared that treatment of animals residing in endemic areas may interfere with the development of immunity or deplete the already acquired immunity. Results from the present study show that anthelmintic treatment of the early patent natural *S. bovis* infections did not alter the course of the acquisition of resistance in comparison to the untreated control calves (group C). Both untreated control calves (group C) and the treated calves (group A) had decreasing faecal egg excretion at the end, while that of the challenge control calves (group D) was still increasing. The present results agree with studies in the Sudan in which elimination of adult worms in cattle naturally infected with *S. bovis* using praziquantel did not abrogate the already acquired resistance (Bushara *et al.*, 1983c). It is interesting to note that treatment kept schistosome faecal egg excretion at very low level for a long period suggesting that treatment of calves at the time when they

are producing large numbers of eggs in their faeces has an epidemiological impact of lowering the transmission of the infection in the endemic areas.

Although praziquantel was used in the present study at a two and a half times higher dose rate compared to the recommended one for cattle, the drug was not 100% effective in the naturally exposed calves at Lulanzi dairy farm. The observed 95.81% *S. bovis* worm reduction in the naturally infected calves at Lulanzi farm supports the previous findings that praziquantel has little ability to kill the immature worms (Sabah *et al.*, 1986; Flisser and McLaren, 1989; Shaw, 1990). Such facts are further supported by observations in the present experimental animal studies where the praziquantel was 100% effective against the mature worms, which were 13 weeks old. By comparing the mean group number of *S. bovis* worms in the untreated calves (group E) sacrificed at the early stage and (group C) calves sacrificed at later stage of the infection at Lulanzi, the latter had significantly more worms showing that there was an accumulation of worms as the infection progressed. However, comparing these results to their corresponding faecal egg counts, the observed high number of worms in the old infections were producing relatively fewer eggs compared to the worms during early stage of infection. This anti-fecundity effect of the host against the worms has been described as the major mechanism of the acquired resistance in cattle and goats rather than worm reduction (Lawrence, 1973; Bushara *et al.*, 1983b; Monrad *et al.*, 1995, 1999).

Unlike in the field studies, clear-cut accumulation of schistosomes was not observed in the challenged untreated experimentally infected calves (group C) at Mazimbu farm. The possible explanation could be related to significant deaths of original worms that might have occurred in the untreated challenged calves (group

C), as seen in other studies (Bushara *et al.*, 1980; Coyne and Smith, 1991). Alternatively, it is possible that the untreated challenged calves (group C) had developed a strong protective resistance against establishment of new worms as observed in challenged natural resistant cattle in the Sudan (Bushara *et al.*, 1980). Studies have shown that the immunity that follows patent infections is correlated to egg induced pathology and is induced by the presence of the adult worms (Smithers and Terry, 1967; Harrison *et al.*, 1982). In this type of immunity, 'concomitant immunity', (Smithers and Terry, 1969), the effectors responses are directed against the schistosomula rather than the adult worms, which have already developed several ways of escaping the immune response of the host (McLaren and Terry, 1982). Hence, it is quite likely that the majority of challenging cercariae were killed. Furthermore, information on the regulation of mortality and fecundity in single *S. mattheei* infections in sheep has shown that the magnitude of the mortality was increasing with time and fecundity was decreasing with the age of the infection (Coyne and Smith, 1991). Therefore, It is quite possible that during the long time that elapsed between infection and challenge (35 weeks) some of the original adult schistosomes died. Therefore, it appears that continuous challenge, which occurs under natural conditions, is necessary for the observed worm accumulation in old infections. The significance of continuous re-infection on worm accumulation can also be drawn from other studies. Studies in the Sudan has shown that removing cattle from an enzootic area that had relatively high mean numbers of worms into a safe paddock for 14 weeks, led to significant reduction in the worm burden indicating that there was death of worms with time (Bushara *et al.*, 1980). Similarly, reduction in the worm burden as infection progressed was observed in goats that

were sacrificed at 16, 22 and 32 weeks following exposure to single primary *S. bovis* infection (Johansen *et al.*, 1997b). In addition, studies in mice have also shown that, in primary infections between eight and twelve weeks there was a net loss of more than 19% of *S. mansoni* worm pairs (Cheever *et al.*, 1994). It appears, therefore, that constant re-infection in the endemic area is essential for the observed accumulation of schistosome worms in old infection. So far, based on the set-up of the present study, there were no possibilities of distinguishing the worm cohorts at primary and challenge infections. Such studies are required, as they will provide more information regarding to the dynamics of schistosome worm populations in cattle, which continuously graze high potential transmission areas.

Apart from reducing faecal egg excretion and worm burden in both natural and experimental infections, praziquantel significantly reduced schistosome eggs in all parts of the intestine and the liver. These results are in accordance with those obtained in studies in goats and cattle (Bushara *et al.*, 1983c; Johansen *et al.*, 1996a). The pathogenic effect of schistosome infection in the definitive host is mainly due to the immunological response of the host against the eggs deposited in the tissues and the trauma they cause as they cross through the tissues (Semuguruka, 1992). Such significant reductions of the number of EPGT within six weeks after anthelmintic treatment and the EPGT remaining low even after eight months when the study was terminated, suggest that praziquantel could significantly reduce the pathogenic effect of this infection. The reduced tissue egg counts observed in the treated-challenged calves (group A) as compared to the untreated-challenged calves (group C) in the present study even after eight months following treatment correlates well with the

observed mild pathological lesions found in the calves in the present study. These results provide a clue of how often re-treatments may be carried out in cattle.

5.3 *Fasciola* and paramphistome faecal egg excretion and worm burden

While all calves had acquired paramphistome infections just three months after they were introduced into the study area, similar to *S. bovis,* it took six months for all calves to be infected with *Fasciola* worms. Such slow rate of acquisition of *Fasciola* by the calves could be expected since the calves were introduced into the area at the beginning of the rainy season during which most of the infected pasture had already been eaten. Another possible explanation could also be related to the type of grazing which was being practised in this farm. The favourable habitats for *Lymnaea natalensis* snails, the intermediate host for *F. gigantica*, are permanent streams with slow moving fresh water. Such habitats were not accessible to the animals. These areas were used for crops, fruits and vegetable production and the permanent pond was the main place where the animals were watered. In the neighbouring farms where farming is not carried out, transmission of fasciolosis is very high. The results from the snail studies also show that the Kilima permanent pond seems to favour the transmission of paramphistosomosis and schistosomosis but not fasciolosis since only few *Lymnaea* snails (9.9%) were collected from the pond compared to *B. natalensis* (86.9%).

The observed 100% reduction in *Fasciola* faecal egg excretion and worm burden following treatment with triclabendazole concurs with other studies that this drug is highly efficacious against *Fasciola* (Boray *et al.,* 1983; Rapic *et al.*, 1984; Guralp and Tinar, 1984). However, the persistence of paramphistome eggs in the

treated calves supports previous studies that triclabendazole and praziquantel had little effect against paramphistomes (Guralp and Tinar, 1984; Buescher and Richards; 1988; Rolfe and Boray; 1988). Lack of effect on paramphistome by most of the commonly used anti-trematode drugs is of serious concern to cattle and small ruminant owners since this parasite is very common in Tanzania (Nyundo, 1994). Paramphistomosis may also be a disease of economic importance in particular in the southern highlands of Tanzania, where outbreaks with mortalities ranging from 4.6 to 96 % have been observed (Dinnik, 1964a,b; Dinnik and Dinnik, 1965; Nyundo, 1994).

Contrary to the observations during primary exposure of the calves, the acquisition of both *Fasciola* and paramphistome by the post treatment control calves (group D) was fast (within two months). Such results indicate that transmission of *Fasciola* and paramphistome in this farm was more favourable at the beginning of the dry season. This is related to the type of grazing management, since during this period animals spent more time eating green and lush pastures around the pond as most of the grass in the hill areas had become scarce and dry. Silangwa (1973, 1974) has shown that the Barotse cattle in Zambia acquire *Fasciola* infection mainly during the dry season when they are deliberately grazed in the flood plains of the upper Zambezi river basin. It is of interest to note that while the naive control calves (group D) acquired *Fasciola* infection within two months of exposure, the treated calves (group A) did not acquire infection until after five months. Furthermore, not all calves had *Fasciola* eggs in their faeces eight months later when the study was terminated. This shows that the treated calves had developed resistance, which hindered re-establishment of new flukes. Also the observations of a lower number of

Fasciola flukes in the untreated calves in group C that were exposed for 16 months than in calves in group E that were exposed for seven months further shows that cattle develop resistance against *Fasciola* infections. These findings support the previous studies that cattle can eliminate primary *Fasciola* infections and become resistant to further challenge infections even if the primary infection has been eliminated by drugs (Boray, 1969; Hammond and Sewell, 1974). The present results further support previous studies in Kenya which showed that the proportion of uninfected cattle or those with light *F. gigantica* infection increased with age of the infection (Castelino and Prestone, 1979). Also the delay in the development of *Fasciola* in the treated group (A) could also be related to the observed severe liver fibrosis, which followed the anthelmintic treatment. The portal and peri-portal fibrosis of the liver may delay passage of immature flukes through the liver parenchyma and final settlement in the fibrosed bile ducts. Even if they succeed in reaching the bile ducts, such fibrosis and calcification of the bile duct will prevent the flukes from getting food. Such resistance to re-infection as shown in the treated animals in the present study are comparable to results obtained from studies on *F. hepatica* in cattle (Boray, 1969).

5.4 Clinical pathology

The assessment of the impact of helminths on productivity of livestock under natural conditions of extensive animal management systems in tropical Africa is difficult, since the regulation of the helminth population by the host is influenced by a complex number of factors, notably age, immunity and nutritional status (Holmes and Chowdhury, 1994). In the present field study, the growth of the calves

resembled the common cyclic growth pattern of extensively managed cattle under tropical conditions that is related to rainfall pattern, which determines the availability of fodder (Touchberry, 1967). Animals only show an increase in weight during the rainy season and the weights become stagnant or decrease during the dry season. Although the worm infections in the naturally exposed calves seem to be relatively sub-clinical, still the use of anthelmintic treatment prevented further weight loss during the dry period, when treated and untreated controls are compared. These results confirm the earlier suggestions by Hammond (1965) that, the punitive effects of sub-clinical natural trematode infections in the tropics are felt mainly during the dry season. Furthermore, these results explain why most animals with poor body conditions died mainly during the dry period in this farm (Makundi *et al.*, 1998). Compared to other previous field studies, the intensity of *F. gigantica* infection observed in the present farm seemed to be so low that they had no any serious consequences on the health of the well-fed cattle. Studies by Hammond and Sewell (1974) have shown that worm burdens less than 500 could neither produce clinical disease nor changes in the body weight and haematological parameters in well-fed cattle. Therefore, the observed low body weight gains and haematological parameters in the untreated re-exposed calves is probably a reflection of an enhanced pathogenicity of the parasites as a result of restricted fodder intake during the dry season. Such effect was nullified during the next rainy season, as there was availability of sufficient feed. These results help to explain some of the influences of nutrition on host-parasite interactions where a high plane of nutrition may counterpoise the effects of the parasite and *vice versa.* It also emphasises the importance of improving the ongoing animal management systems in this farm and

the southern highlands of Tanzania as a whole. The storage of animal feed during periods of feed abundance for use during the dry season together with supplementary feeding to alleviate the impact of these sub-clinical infections could be a starting point. However, improvement of the traditional livestock management system will mean incurring more expenses which cannot be appreciated at present since such production is not market oriented (Silangwa, 1974). Among the major obstacles which hinder improvement of the traditional livestock is the existence of notifiable diseases such as Rinderpest, Bovine Pleuro-pneumonia and foot and mouth diseases, which prevents marketing of animal products elsewhere. Other problems that prevent the improvement of livestock productivity such as the control of worms, is the poor infrastructure. During the rainy season roads are impassable and there is no access to the market to sell the surplus milk that is produced in the farms. In such situations advising the farmers to treat the sub-clinical infections in order to increase productivity will not be adopted, as it will not be cost effective. A need exists to validate the cost effectiveness of anthelmintic treatment of sub-clinical helminth infections. Furthermore, in order to improve livestock productivity the solution lies in the multi-sectoral approach in solving farmer's problems rather than control of worms alone.

5.5 Pathology of experimental *S. bovis* infection in calves

An attempt was made to elucidate the consequences of treatment of an early patent *S. bovis* infection in cattle with praziquantel followed by challenge infections on the resulting pathological changes. The observed more severe pathological lesions in the liver of the calves which were treated at week 13 post infection compared to

the untreated calves with the same stage of infection, concur with similar previous findings in goats (Johansen *et al.*, 1996a). Also, such results are in agreement with suggestions by Reinecke (1970) that treatment may lead to more severe pathological lesions than in the untreated *Schistosoma* infections. Unlike in the liver, the absence of worm or egg induced lesions in the intestines of the treated calves shows that praziquantel treatment can reverse the pathological lesions induced by *S. bovis* infections. The reversal of intestinal lesions and return to the normal function of the intestine is crucial, since the intestinal lesions are responsible for the observed diarrhoea, anaemia, weight loss and deaths as a result of blood, protein and electrolyte loss during the acute phase of the infection (Dargie, 1980; Saad *et al.*, 1984a; Mbassa and Willeberg, 1991, Balemba *et al.*, 2 000).

So far, no deaths or marked clinical signs were observed in the untreated and treated calves despite the observed extensive liver fibrosis in the treated calves in the present study. This could be related to the moderately low dose (8 000) of *S. bovis* cercariae that was used. Studies by Van Wyk *et al.* (1997) using massive doses of *S. mattheei* (more than 25 000 cercariae per calf) showed that some of the animals died during acute infections even before full immunity had established. It is also still not clear as to what would have happened if treatment had been carried out in massively infected calves. Johansen *et al.* (1996c) observed sudden death of hamsters a few hours following praziquantel treatment. Such deaths in hamsters could be expected, considering the more often seen blockage of portal veins by the schistosomes in rodents (Cheever *et al.*, 1994). Although cattle develop severe pathological changes during schistosomosis, the symptoms such as ascites and oesophageal varices seen in humans have not been reported in cattle (Hussein *et al.*,

1975) presumably due to the anatomical differences. Drainage of the posterior part of the abdomen in cattle may also occur via the left azygos vein in case there is stasis in the blood flow through the liver. This also occurs if the vena cava is obliterated e.g. when the rumen expands and compress the vena cava on the liver temporarily. It has also been demonstrated in rabbits that glass beads that were several times bigger than the sinusoid spaces can pass through the liver indicating that shunts also exist in the liver but this requires further clarification in cattle.

The calves, which were sacrificed at the late stages of infection (52 weeks) following challenge but without treatment, had severe schistosomal lesions in the liver which is well documented in both chronic *S. bovis* and *S. mattheei* infections in cattle (McCully and Kruger, 1969; Hussein, 1971; Massoud, 1973; Van Wyk *et al.*, 1974). The pathological lesions in the untreated challenge group were more severe than in the treated challenge group or the challenge control group. The severity of the pathological lesions in the untreated challenge group is a result of the tendency of *Schistosoma* eggs to accumulate in the liver of goats (Monrad *et al.*, 1995; Johansen *et al.*, 1996a) and cattle (Lawrence, 1977b; Saad *et al.*, 1980) as the infection progresses. It is of interest to note that the severe pathological lesions which were seen in the calves which were treated at week 13 and sacrificed at week 26 were not observed in the treated challenged group sacrificed at week 52 following challenge at week 35. Such results suggest that the pathological changes may be reversed by the anthelmintic treatment. So far, treatment neither prevented worm establishment nor deposition of eggs in the tissue when worm burden and EPGT of the treated group and challenge control are compared in both field and experimental infections. These results suggest that the difference in the extent of liver fibrosis between the untreated

and treated group was temporary and as the animals continue grazing in high transmission areas all the animals will end up developing the chronic hepatic syndrome. The main benefit of the treatment is to cure the acute infections, accelerate development of immunity and control transmission by reducing number of eggs reaching the environment.

The increase in the severity of liver fibrosis following treatment is the result of dead schistosome worms and eggs, which were shifted into the liver. The mechanisms underlying the development of liver fibrosis in old *Schistosoma* infections in cattle is poorly understood. Although live adult worms have very little pathologic effect, it has been observed that dead worms may cause more severe lesions than the eggs (McCully and Kruger, 1969; Hussein *et al.*, 1975).

In order to minimise the severe liver pathology which results from using highly effective anti-schistosomal drugs, various options exist such as pre-vaccination of the animals with Th2 cytokines before treatment or combination of anti-fibrotic agents with anti-schistosomal drugs. Studies in murine *S. mansoni* infections have shown that excessive liver fibrosis can be reduced if the animals were vaccinated with IFN-γ, IL-12 and TNFα before they were infected (Hoffmann *et al.*, 1998). Similarly, the vaccination of mice with *S. mansoni* serum egg antigens (SEA) before infection and treatment with praziquantel has shown that the SEA can serve as anti-pathology vaccine as it leads to more reduced liver pathology and even efficient worm killing (Botros *et al.*, 1988). Furthermore, it has been demonstrated in mice that IL-12 can act as an adjuvant in suppressing both granuloma formation and fibrosis induced during *Schistosoma* infections (Wynn *et al.*, 1994). Anti-fibrotic agents such as beta-amino propionitrile (Giboda and Smith, 1997) and drugs such as

octreotide (Mansy *et al.*, 1998b) in combination with praziquantel treatment have also been observed to reduce liver pathology by reducing the granuloma size, cellularity and fibrosis.

However, the interpretation of the rodent model mechanisms of dynamics of schistosome egg granulomas in early infections and formation of liver fibrosis should be taken with caution, as different mechanisms may exist in cattle and possibly in humans. For instance it has been shown in human schistosomosis that, there is no clear-cut switch from the Th1 to Th2 responses with the onset of egg laying as in mouse and both responses are seen during egg laying (Mosmann and Subash, 1996).

From the pathological observations it may be concluded that treatment of a moderately low early *S. bovis* infection in the long run cures animals of the acute intestinal lesions. However, initially it causes more additional pathology in the treated animals than in the untreated ones. Cattle tolerate these pathological changes and eventually such pathological lesions are reversed. Still, the treated animals can develop chronic pathological lesions if they continue grazing in the high potential transmission sites as observed in endemic areas but without possibilities of developing symptoms of the acute disease. There is a need for carrying out studies in ruminants on the dynamics of schistosome egg granulomas and the subsequent development of severe liver fibrosis in order to find ways of minimising liver fibrosis that follows after anthelmintic treatment.

5.6 Distribution of *S. bovis* eggs in the small intestine

The results from the studies on the distribution of schistosome eggs in the small intestine of cattle compare well with those of Massoud (1971) who observed a

higher proportion of *S. bovis* eggs in the duodenum at seven weeks post infection than in the jejunum and *vice versa* at 18 weeks. Similarly, Saad *et al.* (1980) observed higher proportion of *S. bovis* eggs in the large intestine at six months than at six weeks post infection. Studies on *S. mattheei*, infections in cattle revealed similar results (Lawrence, 1977b). However, the migration of *S. mattheei* may involve urogenital organs (Condy, 1960; Van Wyk *et al.*, 1974, Obwolo and Rogers, 1988) that is very uncommon in *S. bovis* infections in cattle. So far no convincing explanations have been reached regarding the major factors responsible for the distribution of bovine schistosome eggs in different parts of the gastro-intestinal tract and other organs. Lawrence (1977b) has associated migration of *S. mattheei* during early infections from the anterior towards the posterior part of the intestine to the inflammatory reactions on the intestinal mucosa due to the immune response of the host. These inflammatory reactions such as haemorrhages and eosinophilic and mononuclear cell infiltrations are alleged to alter the composition of the blood in the veins draining the affected areas and thus creating an unfavourable habitat for the flukes.

The age of infection could also contribute to such distribution. For instance, although *S. mattheei* predominantly resides in the mesentery; few incidents that were observed in the urogenital organs were associated with the older cattle as compared to immature ones (Pitchford, 1963). Studies in *S. bovis* infection in cattle have shown that old chronically infected cattle had relatively much higher numbers of tissue egg counts, which were more widely distributed so as to involve rare organs such as gall bladder, compared to the animals exposed for short periods (6 to 9 months) (Makundi, *et al.*, 1998). Apart from mucosal inflammatory reactions, the blockage

and narrowing of the mesenteric veins due to inflammatory reactions against the schistosome eggs is not an uncommon feature in *S. bovis* infections in cattle (Semuguruka, 1992). Such blockage of the veins as seen in the present study would logically start appearing in the proximal part and it may be postulated that, the female worms would search for alternative patent vessels located further posteriorly. As this process continues, increasing number of blocked or narrowed mesenteric veins would appear as the infection progressed. This may explain the common feature observed in intestinal schistosomosis in domestic ruminants where the proportion of eggs in the liver increases as the infection progresses without actual shifting of the worms into this organ (Lawrence, 1977d).

Distribution of schistosome eggs has also been associated with the intensity of infection. It has been postulated that higher number of worms would lead to an overflow of worms into unsuitable sites such as fore stomachs and urinary bladder in *S. mattheei* infections (Lawrence, 1977d). Therefore, it seems quite likely that the blockage and narrowing of the veins as a result of inflammatory reactions are the major factor governing such movement rather than actual changes in blood composition. Examination of eggs across the intestinal wall showed that most of the eggs were found in the mucosa. The results are contrary to those of Saad *et al.* (1980) who showed that eggs were more in the mucosa than in the sub-mucosa during early stages of infection and *vice versa* in old infections. Such disagreement is possible with regard to the different techniques used. However, mucosal scraping technique employed in the present study in the quantification of eggs seems to be more efficient than the histopathological sections apparently due to the limited amount of tissue examined by the latter technique.

Regarding the small number of animals used in the study, the data obtained is rather insufficient to make any explicit conclusions at this stage. Nevertheless, it was demonstrated that most of the *S. bovis* eggs are deposited in the anterior part during early stage of infection and as the infection progresses there is a shift towards the distal part and that most of eggs are found in the mucosa.

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

The key observation from the snail studies is that *B. natalensis* is the only bulinid and most abundant freshwater snail present at the Kilima pond in Lulanzi village. The abundance of this snail species occurs mainly during the rainy season while high snail infection rates with *S. bovis* occur mostly towards the end of the rainy season. These findings have practical implications for high morbidity of infection in cattle during the dry season and explain why deaths of fluke-infected animals were seen at the farm during this period. In addition, the present study confirms and extends the available information that *B. natalensis* can transmit *S. bovis* under certain natural conditions such as interacting with other trematodes.

The results from the present field and experimental animal studies on the treatment of *S. bovis* with praziquantel support the available information that this drug is highly effective in reducing worm burden, tissue and faecal eggs but has little effect against immature flukes. Notwithstanding the high efficacy of this drug, the observations in the treated calves have provided further evidence that treatment transiently causes more severe liver pathology in comparison to no treatment. However, the observed significant reduction in schistosome tissue eggs within six weeks and the fast regression of the intestinal lesions suggest that anthelmintic treatment may prevent development of acute disease. Apart from preventing acute disease, the field animal studies have shown that anthelmintic treatment of natural

trematode infections prevents further deterioration of body weights and haematological parameters, which occur mainly during the dry season. Additionally, the observed reduction in faecal egg excretion for seven months in the treated calves has a significant epidemiological impact of reducing transmission intensity of the parasite in the environment. Regarding immunity of cattle against schistosome infections, evidence from the present studies suggest that effective anti-schistosomal treatment does not abolish or interfere with the development of immunity, which is mainly an anti-fecundity effect. As regards anthelmintic treatment of *Fasciola* species in cattle, the present has shown that triclabendazole is a highly effective drug against both mature and immature flukes.

So far *B. natalensis* has never been observed to serve as an intermediate host for schistosomes. Therefore, these findings raise the need for further studies about this snail species. Such future studies should include detailed taxonomy (based on molecular biology) and interaction of trematode larval stages within the snail. Since anthelmintic treatment of schistosomosis in cattle leads to more severe liver fibrosis in comparison to untreated cattle, future studies devoted to reducing the extent of liver fibrosis such as the use of anti-pathology or anti-fibrotic vaccines, drugs and adjuvants combined with drugs are recommended. As observed in the present study, paramphistomes are highly prevalent in the area and with no effect of praziquantel, triclabendazole and commonly used anthelmintics, this parasite clearly deserves further attention regarding its epidemiology, immunity, pathology and control studies.

In summary, the information available in the present study on transmission biology and impact of anthelmintic interruption of early bovine fasciolosis and schistosomosis provides a basis for the planning of an effective control strategy for these infections in endemic areas.

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