Co-joint trematode infections in cattle from Kafue and Zambezi river basins of Zambia

By

Yabe John

A dissertation submitted to the University of Zambia in partial fulfillment of the requirements of the degree of Master of Science in Veterinary Pathology

THE UNIVERSITY OF ZAMBIA

SCHOOL OF VETERINARY MEDICINE

PARACLINICAL STUDIES DEPARTMENT

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DECLARATION

I, **Yabe John**, do hereby declare that the contents of the dissertation being submitted herein are my original work and they have not been previously submitted to any university for the award of a degree or any other qualification.

Signature: ---------- Date: 16/10/07

CERTIFICATE OF APPROVAL

This dissertation submitted by Yabe John, is approved as fulfilling the requirements for the award of the degree of Masters of Science in Veterinary Pathology of the University of Zambia.

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Chairman (Board of Examiners)

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ABSTRACT

This study investigated the heterologous interaction among *Fasciola gigantica*, *Schistosoma* spp. and *Amphistomum* spp. co-joint infections in cattle based on egg and worm counts. A total of 315 cattle from trematode endemic areas along the Kafue and Zambezi flood plains were screened at Turn Pike abattoir for either *F. gigantica* in the liver, *Schistosoma* spp. in the mesenteric veins and/ or *Amphistomum* spp. in the rumen. One hundred and thirty three of the abattoir examined cattle harboured one, two or all three trematodes. Faecal samples, livers, mesenteries, lungs, kidney, spleen, abomasum and rumens from fifty randomly selected trematode infected cattle were collected for further laboratory examination. There were heavy *Amphistomum* (mean ± SEM = 622.08 ± 97.87), low *Schistosoma* (mean ± SEM = 33.68 ± 7.44) and low *Fasciola* (mean ± SEM = 19.46 ± 4.58) worm burdens. There was no significant difference (χ^2 =575.34, p =0.923) between *F. gigantica* and *Schistosoma* worm burdens. A significant difference (χ^2 =1210, p =0.038) was observed between *F. gigantica* and *Amphistomum* worm burdens. A total of 32% (n = 50) harboured all the three trematodes, 66% had *F. gigantica* and *Amphistomum* spp. infections, 52% had *Schistosoma* spp. and *Amphistomum* spp. infections while 32% had *F. gigantica* and *Schistosoma* infections. A positive correlation (p = 0.014) was obtained between *F. gigantica* and *Amphistomum* worm burdens. There was no correlation between *Amphistomum* and *Schistosoma* worm burdens (r = 0.15, p = 0.302) and between *F. gigantica* and *Schistosoma* worm burdens (r = -0.12, p = 0.390). *Schistosoma* faecal egg and tissue egg counts had positive correlations (p < 0.001) with worm burden. *Fasciola* eggs were also detected in the liver, lung, kidney, mesenterium and spleen. *Fasciola* tissue egg counts were

significantly higher than faecal egg counts ($p < 0.001$). Histopathological examination of the lungs did not confirm the presence of *Fasciola* eggs in the tissue.

Based on these findings, it may be concluded that there is no significant cross-protection among these trematodes in cattle in endemic areas. Therefore, existence of co-joint infections and lack of cross-protection among *F. gigantica*, *Schistosoma* and *Amphistomum* infections in endemic areas should be considered when formulating control measures of trematode infections.

DEDICATIONS

This dissertation is dedicated to my mother who did not give up but continued with the struggle to give me this opportunity to reach this far. I also dedicate it to my cousin Dr. D.C. Mimba who groomed me and inspired me to study veterinary medicine. A special dedication of this dissertation goes to my wife Susan, and to my son Malumbo Yabe who lifted my spirit and put a smile on my face whenever I felt discouraged and frustrated.

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Last but not the least, I would like to extend my profound gratitude to the meat inspector at Turn Pike abattoir, Mr. S Banda and the abattoir management for their co-operation and assistance during the survey.

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CHAPTER ONE

1.0 Introduction

Trematodes are some of the most economically important pathogens responsible for causing helminth diseases, which severely affect productivity in domesticated ruminants worldwide (Vercruysse and Claerebout, 2001). *Fasciola* (liver fluke), *Schistosoma* (blood fluke) and *Amphistomum* (rumen flukes) are the most important trematodes affecting cattle, sheep and goats (Soulsby, 1982).

Fasciolosis represents a recognized agricultural problem responsible for economic losses estimated at more than US $2 billion per year to rural agricultural active communities and commercial livestock producers worldwide (Dalton *et al.*, 1996). It has been observed that liver fluke infections can be highly pathogenic and can cause severe morbidity and even death of the host (Kofta *et al.*, 2000). Moreover, the diminished capacity of infected animals for draught power also impacts on crop production, particularly in regions where animal draught power is heavily depended upon for land preparation and transport (Spithill *et al.*, 1999).

Although some adverse effects on ruminant health have been attributed to infection by *Amphistomum* spp. (Singh and Parihar, 1988), amphistomosis is still underestimated and generally there is limited information about the infection (Diaz *et al.*, 2006). However, amphistomosis has been observed to cause severe disease and mortalities especially in young animals, due to the migratory habits and feeding behaviour of the immature parasites (Agosti *et al.*, 1980).

Schistosomosis is a common parasitic infection in cattle, mainly in Africa and Asia (Christensen *et al.*, 1980) where it is estimated that at least 165 million animals are infected (Lawrence, 1976; De Bont and Vercruysse, 1997). Out of the 10

Schistosoma species that have been reported to naturally infect cattle, only *Schistosoma mattheei*, *S. bovis*, *S. nasale* and *S. spindale* have received particular attention, mainly because of their recognized veterinary significance (Van der Werf *et al.*, 2003). *Schistosoma leiperi* and *S. margrebowiei*, which are primarily parasites of antelopes (*Kobus* spp.), have occasionally been isolated from cattle living in areas within the distribution of *Kobus* spp. (Pitchford, 1976). In areas where conditions favour the transmission of trematode infections, prevalence rates of *Schistosoma* infection in cattle may range from 40 to 70% (Christensen *et al.*, 1980).

Molluscun snails are the intermediate hosts for *Fasciola*, *Schistosoma* and *Paramphistomum* infections (Soulsby, 1982). Since these infections may share the same intermediate snail hosts, they may not occur in isolation (Chingwena *et al.*, 2002). Agosti *et al.* (1980) reported a mixed infection of *F. hepatica* and *Paramphistomum daubney* in France. Follow up studies by Szmidt-Adjide *et al.* (2000) recorded a positive association between these two trematodes. Recently in Zambia, Phiri *et al.* (2006a) observed that *Fasciola gigantica* and *Amphistomum* spp. mainly occur as mixed infections in cattle reared on the Kafue and Zambezi river basins.

On the contrary, heterologous resistance between *Fasciola* and *Schistosoma* infections in cattle has been reported (Sirag *et al.*, 1981). Moreover, *Schistosoma* antigens have been observed to cross-react with *Amphistomum* antigens in sheep (Singh *et al.*, 2004). Available literature on trematode cross-resistance in cattle is largely from experimental studies and as such the actual mechanisms of protection are not fully explained. To our knowledge, there has been no study on co-joint *Fasciola*, *Schistosoma* and *Amphistomum* infections in naturally infected cattle.

Therefore, the implication of cross-protection among *Fasciola*, *Schistosoma* and *Amphistomum* on the epidemiology of these parasites in endemic areas is not well elucidated. There was a need to study the dynamics of *Fasciola*, *Schistosoma* and *Amphistomum* infections and the effects of cross-protection on the epidemiology of co-joint trematode infections in naturally infected cattle living under conditions of continuous moderate challenge. The main objective of this study was therefore to investigate the effects of cross-protection in co-joint trematode infections in naturally infected cattle in endemic areas. The specific objectives of the study were (i) to determine the occurrence and extent of co-joint *Fasciola gigantica*, *Schistosoma* and *Amphistomum* infections in slaughtered cattle, (ii) to investigate the interaction of *Fasciola*, *Schistosoma* and *Amphistomum* in cattle and how this influences their relative infection intensities, and (iii) to study the histopathological effects of *Fasciola* and *Schistosoma* eggs in selected tissues.

CHAPTER TWO

2. Literature Review

2.1 The parasites

Most digenetic trematodes parasitizing livestock are inhabitants of the gastro-intestinal tract, or its associated organs, especially the liver, bile duct, gall bladder, lungs, pancreatic duct, ureter and urinary bladder; where they feed on blood, bile, mucus and intestinal debris (Smyth, 1996). Morphologically, most trematodes are flattened dorso-ventrally; some have fleshy bodies while others are round in section. The major hosts of these parasites are cattle, sheep, goats and buffalo (Soulsby, 1982).

2.1.1 *Fasciola* Species

The two known *Fasciola* spp. which cause fasciolosis in domestic animals, are *Fasciola hepatica* (30 x 12 mm) and the larger *Fasciola gigantica* (75 x 12 mm). *Fasciola hepatica* is common in the temperate regions and higher altitude areas in the tropics, especially Kenya, South Africa and Lesotho while *F. gigantica* occurs in the tropical regions and is the most common and widely distributed liver fluke in Africa (Soulsby, 1982; Urquhart *et al.*, 1989). *Fasciola* spp. have a predilection for the liver, inhabiting the bile ducts in their mature stages and are thus commonly called 'liver flukes'. They are dorso-ventrally flattened, unsegmented and leaf-shaped (Fig. 2.1). Liver flukes look grayish-brown and usually possess a spiny integument (Soulsby, 1982; Urquhart *et al.*, 1989; Hansen and Perry, 1994). The adult flukes have two suckers used for attachment: the oral sucker at the anterior end surrounding the mouth and the ventral sucker at the level of the 'shoulders' of the fluke (Urquhart

et al., 1989). Liver flukes have a blind alimentary tract with their branched internal organs lying in a parenchyma. They are hermaphrodites and both cross- and self-fertilization may occur.

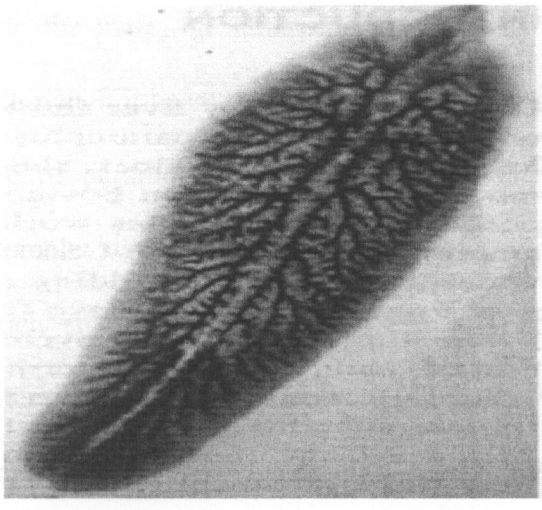


Figure 2.1: An adult *Fasciola gigantica* worm with flattened, unsegmented, leaf-like body.

2.1.2 *Schistosoma* Species.

In endemic areas, *Schistosoma mattheei*, *S. bovis*, *S. nasale*, *S. curassoni*, *S. indicum* and *S. spindale* have been reported to naturally infect cattle (Lawrence, 1976). Schistosomes show morphological and physiological peculiarities that distinguish them from other trematodes (Smyth, 1996). They have an elongate structure, but as for other trematodes, they possess oral and ventral suckers, the latter being larger and more muscular in the male than the female. The alimentally canal of schistosomes is unusual in that the paired intestinal caeca rejoin about the middle of the body and continue as a single winding tube ending blindly posteriorly (Smyth, 1996). The

other intriguing feature about schistosomes is that they are dioecious. The male and female worms have to come together in the definitive host to mate thus enabling the female worm to produce eggs (Webster *et al.*, 2005). The female worm lives in permanent copula with the male, the latter curving its body ventrally to form the *gynaecopholic canal* in which it bears the long narrow body of the female (Fig. 2.2). Pairing appears to be of paramount importance to the development and sexual maturation of the female worm. Female *Schistosoma* worms from unisexual infections remain much smaller and their vitellaria less developed than female worms from mixed infections (Taylor, 1971). Pairing between worms is not confined to male and female, multiple pairing between males/males and females/females can also occur. Heterospecific interactions sometimes occur between male and female schistosomes of different species if they infect the same definitive host. The outcome of such heterospecific interactions depends upon the phylogenetic distance of the two species involved, usually resulting in either parthenogenesis or hybridization (Southgate, 1997). A number of crosses between different species have been obtained in ruminants; *Schistosoma mattheei* and *S. mansoni* (Taylor, 1970), *S. mattheei* and *S. haematobium* (Wright and Ross, 1980), *S. guineensis* and *S. haematobium* (Webster *et al.*, 2005). In Zambia, *S. mattheei* and *S. haematobium* mixed infections have been reported to occur naturally (Hira and Patel, 1981).

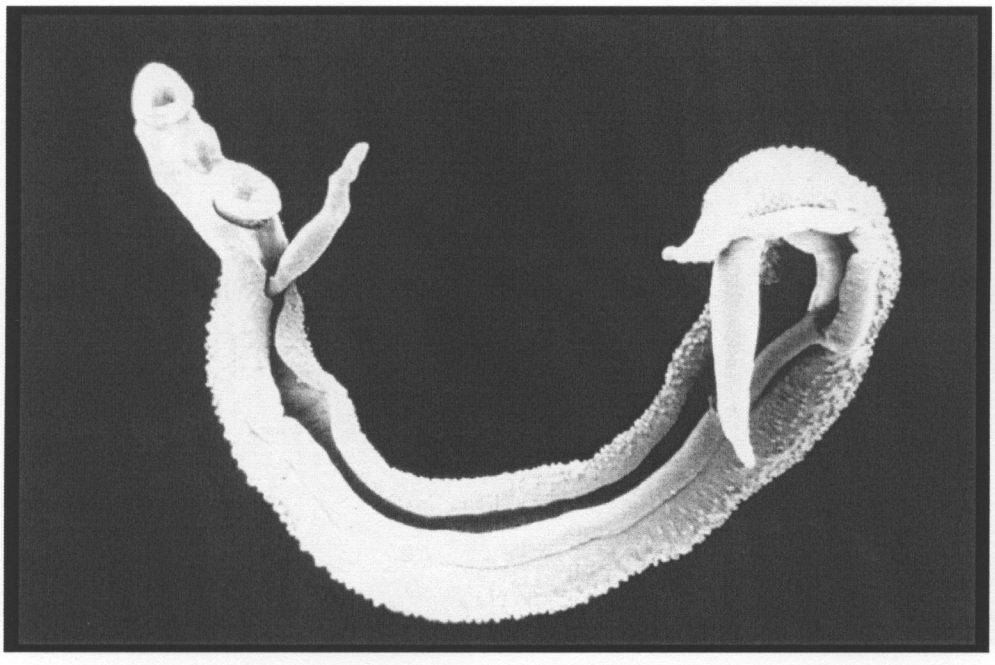


Figure 2.2: *Schistosoma* spp., showing the larger male worm carrying the female worm in its gynaecopholic canal.

2.1.3 *Paramphistomum* (*Amphistomum*) Species.

Paramphistomes have a reddish-pink, thick, short and fleshy maggot-like structure (Fig. 2.3). They possess both anterior and posterior suckers. The posterior (ventral) sucker, which is situated at or close to the posterior extremity, may be strongly developed. Paramphistomes have a spineless tegument and the worm measures about 5 - 13 by 2-5 mm (Urquhart *et al.*, 1989). They appear as clusters between the papillae of the rumen and the reticulum where they may survive for many years without significant effects to the host, as not all of their species are pathogenic (Hansen and Perry, 1994). However, *Paramphistomum cervi,* which parasitizes cattle with cosmopolitan distribution, is considered one of the most important species (Rangel-Ruiz *et al.*, 2003).

Figure 2.3: *Amphistomum* species appearing as reddish-pink, thick, short and fleshy maggot-like structures.

2.2 Trematode life cycles

Fasciola, *Schistosoma* and *Paramphistomum* spp. have a complex indirect life cycle that shows an obligatory dependence on an aquatic environment in the pre- and intra-molluscan stage (Urquhart *et al* 1989). In the egg, the embryo develops into a ciliated miracidium and light plays a major role in stimulating the hatching process (Smyth, 1996). The life cycle may give rise to five larval stages, miracidium, sporocyst, redia, cercaria and metacercariae (Smyth, 1996). After penetration of a suitable intermediate snail host, the miracidium sheds its ciliated plates and develops into a motile, vermiform sac-like larva, the sporocyst. Sporocysts may give rise to daughter sporocysts, the latter giving rise directly to cercariae and rediae are not formed. If sporocysts give rise to rediae, the rediae produce a second or even a third

generation of rediae before producing cercariae (Smyth, 1996). Sporocysts and rediae from a single egg may ultimately give rise to up to a million cercariae due to the phenomenon of paedogenesis, i.e. the production of new individuals by single larval forms (Urquhart *et al.*, 1989; Smyth, 1996). A single miracidium hatched from the *Fasciola* egg can produce up to 4 000 cercariae (FAO, 1994). Cercariae are essentially young flukes, which bear many of the features of the adult fluke. When fully developed, they emerge from the molluscan host. While the cercariae of *Schistosoma* spp. are infective and actively penetrate the skin of the definitive host, the tadpole-like motile cercariae of *Fasciola* and amphistomes swim until they make contact with herbage and encyst into infective metacercaria that are ingested together with herbage and water (Soulsby, 1982; Smyth, 1996; Urquhart *et al.*, 1989).

2.2.1 *Fasciola* life cycle.

The preferred snail intermediate host of *F. hepatica* is *Lymnaea truncatula*, the amphipod snail that is adapted to the cooler areas of the Africa's eastern highlands, whereas the aquatic snail, *Lymnaea natalensis* which occurs throughout tropical and subtropical Africa, is the snail intermediate host for *F. gigantica* (Smyth, 1996; Esteban *et al.*, 2003). *L. natalensis* is found in relatively large, deep, permanent, and moderately polluted water bodies with relatively dense surface vegetation cover or in irrigation channels and marshy swamps (Urquhart *et al.*, 1989; Esteban *et al.*, 2003). In the warm tropical conditions, it takes 75 days for the development of the *F. gigantica* larval forms in the snail (Soulsby, 1982). The miracidia undergo mitosis inside the snail and develop into a sporocyst, by repeated divisions. Each germinal cell within the sporocyst forms a redia, which also forms second-generation redia.

The redia gives rise to tailed cercaria, which swim out of the snail and encyst on surface of herbage to become infective metacercaria (Fig. 2.4).

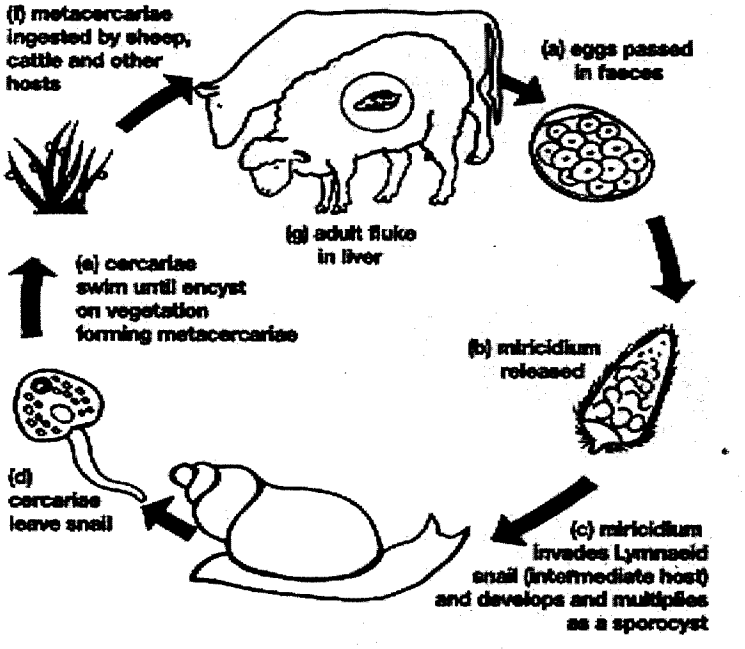


Figure 2.4: Genealised life cycle of *Fasciola* species in ruminants.

Metacercariae of *Fasciola* show little morphological differences from cercariae and may survive for up to four months on stored plants (Soulsby, 1982). When ingested, the metacercaria move to the duodenum where they excyst. The juvenile liver flukes penetrate the intestinal wall into the peritoneal cavity within 24-72 hours after excystment. During the next 48-90 hours the flukes move through the peritoneal cavity and penetrate the liver parenchyma (Kofta *et al.*, 2000). Then they migrate through the liver tissue causing extensive hemorrhages and fibrosis (Dalton *et al.*, 1996). Finally, 9 to 12 weeks after infection, they migrate to the bile ducts where

they mature into egg-laying adults (Soulsby, 1982). The produced eggs are expelled with the bile in the intestines and then with faeces to the outside environment. *Fasciola* eggs are oval and have a yellow shell with an indistinct operculum and embryonic cells. The eggs of *Fasciola gigantica* measure 190 by 100 μm while those of *F. hepatica* measure 150 by 90 μm (Urquhart *et al.*, 1989).

2.2.2 *Paramphistomum* life cycle.

A wide range of species of *Bulinus* and *Biomphalaria* have been reported to act as snail intermediate hosts for amphistomes in Africa (Wright *et al.*, 1979; Chingwena *et al.*, 2002). *Paramphistomum* eggs take 12 to 21 days to hatch (Soulsby, 1982). Development in the snail intermediate host is similar to that of *Fasciola* (Urquhart *et al.*, 1989) and can be completed in four weeks under favourable conditions (26-30°C). After ingestion of encysted metacercariae with herbage, development in the final host occurs entirely in the alimentary tract. Following excystment in the duodenum the young flukes attach to the duodenal mucosa and feed for about six weeks before migrating to the rumen. They mature in the rumen and produce eggs that are shed directly in the faeces (Urquhart *et al.*, 1989). Although *Paramphistomum* eggs have morphological similarities to *Fasciola* eggs, they are larger, measuring 114-176 by 73-100 μm. They also have transparent shells and more distinct opercula. Their embryonic cells are clearer and there is frequently a small knob at the posterior pole (Soulsby, 1982).

2.2.3 *Schistosoma* life cycle.

Mature schistosomes inhabit the mesenteric veins and during the period of egg laying, the female worm enters the small blood vessels of the gut wall where the eggs are deposited. The deposited eggs attach to the intima of the blood vessels using the spine to avoid being swept away by the blood current. When laden with eggs, the vessels eventually rupture so that the eggs penetrate the vessel wall and migrate to the intestinal lumen (Smyth, 1996). This migration takes several days to weeks, during which time non-embryonated eggs develop. On their way to the intestinal lumen, about half of the eggs are carried away with the blood stream and get trapped in the liver, spleen and other organs such as the lungs (Urquhart *et al.*, 1989). The eggs that reach the rumen are passed in faeces and hatch within minutes of coming in contact with water (Smyth, 1996). The released miracidia penetrates an appropriate snail intermediate host of which *Bulinus* spp. is particularly important in the transmission of bovine schistosomosis (Wright *et al.*, 1979; Urquhart *et al.*, 1989). Intramolluscan development takes about five weeks and it occurs without the redial forms. It involves asexual multiplication of daughter sporocysts resulting in the emergence of numerous infective cercariae from the snail (Fig. 2.5).

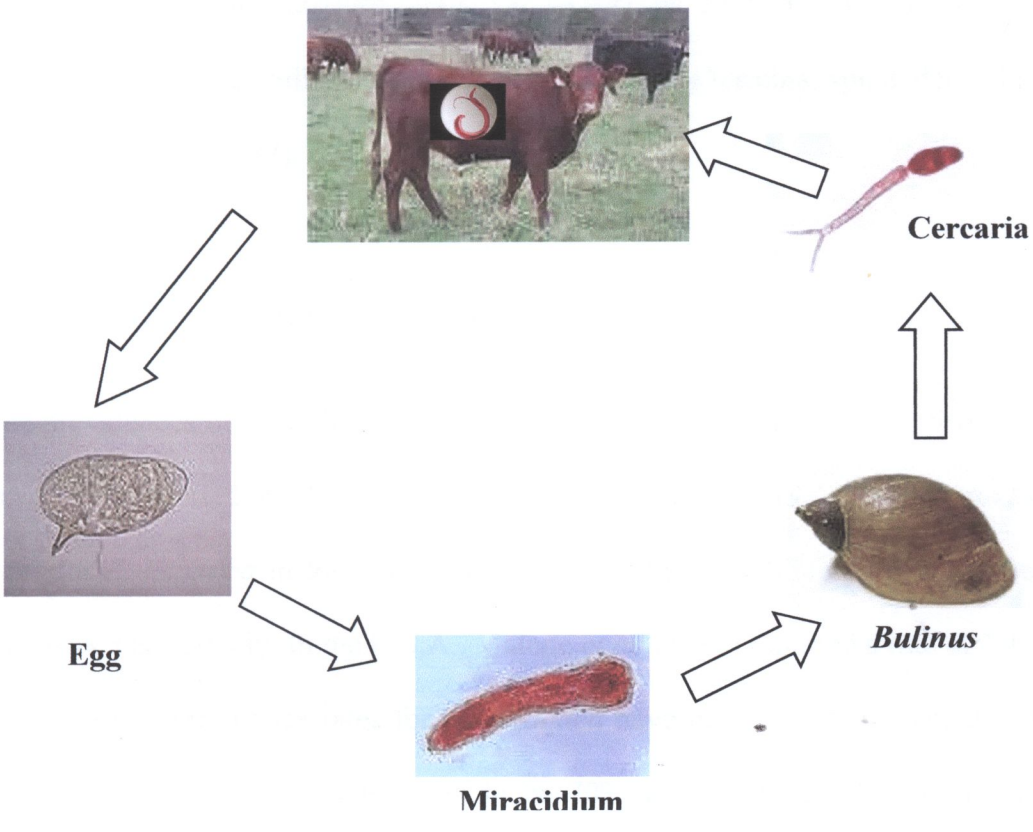
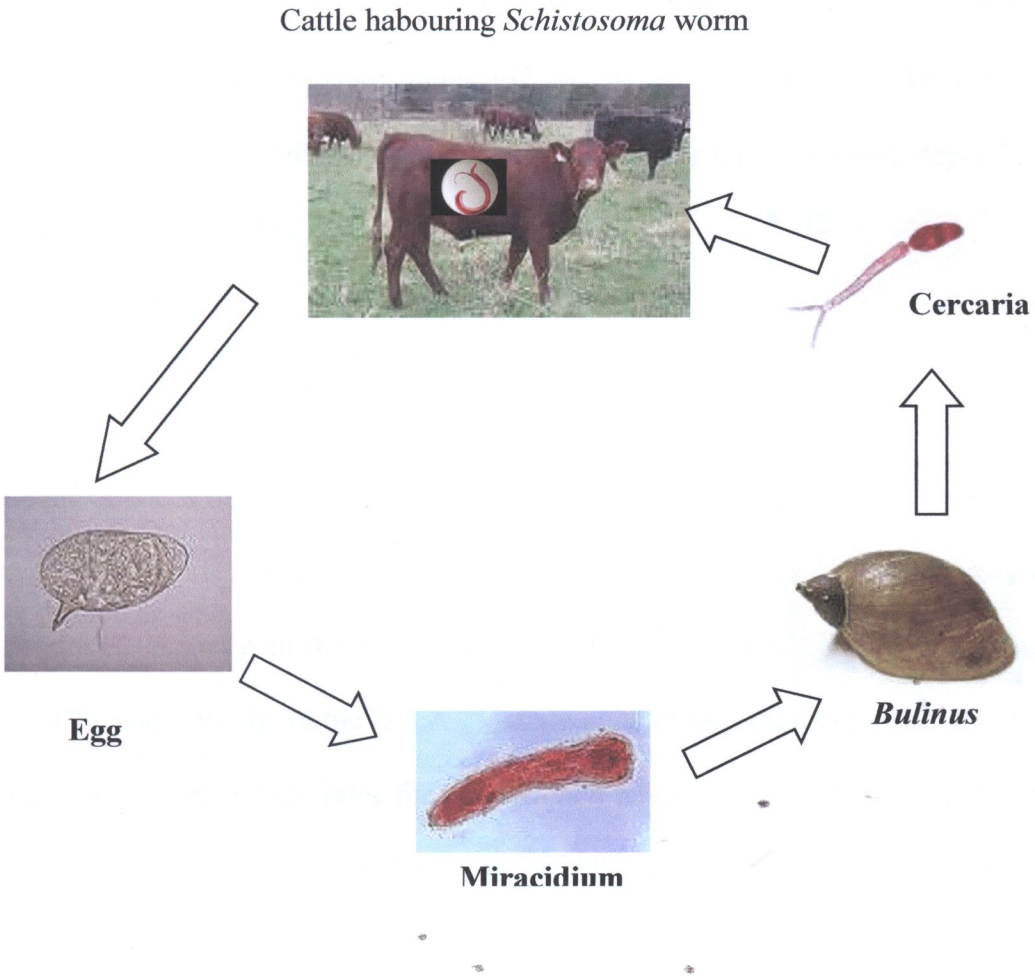


Figure 2.5: Generalized life cycle of *Schistosoma* species in cattle.

Infection of ruminants by cercariae is usually accomplished by active penetration of the skin, although it has been shown that per-oral infection while drinking cercariae contaminated water may also be of importance (Kassuku *et al.*, 1986). After penetrating the skin or mucosa the cercariae shed their tail and transform to schistosomula that migrate via the vascular system through the heart and lungs to the systemic circulation. Schistosomes mature in the hepatic portal veins, mate and migrate to the mesenteric veins where egg production starts, after 6 to 7 weeks of infection (Urquhart *et al.*, 1989). Female *Schistosoma* worms lay only one egg at a



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time and a pair of worms produces about 100 to 300 eggs daily (Smyth, 1996). The eggs of *Schistosoma* measure about 132 to 247 by 38 to 60 μm. They have no operculum but are spindle-shaped and have a lateral or terminal spine, depending on species (Soulsby, 1982).

2.3 Prevalence of trematode infections

The prevalences of fasciolosis caused by both *F. hepatica* and *F. gigantica* in cattle have been reported world wide (Soulsby, 1982). Dargie (1986) found prevalence rates for *F. hepatica* in the United Kingdom and the Republic of Ireland to be 6% and 38%, respectively. In the U.S.A., Malone *et al.* (1982) reported a rate of 4.4%. In Asia, various prevalence rates for *F. gigantica* have been reported; Kendall (1954) found a prevalence rate in Pakistan to be 70% while Mahato (1993) working in Nepal found a prevalence rate of 18.5%.

In Africa, Megard (1975) reported prevalence rates of 33% for Kenya, 37% for Sudan, 45% for Cameroon, 30 to 90% for Ethiopia, 16% for Uganda, 62% for Central African Republic, 50% for Rwanda and 4% for Tawanca. In Ghana, McCullough (1965) quoted authorities as estimating the annual incidence at 10%. In a field report study of cases at 12 major abattoirs in Nigeria between 1971 and 1976, in which 1.2 million cattle were slaughtered 30,000 cattle or 2.5% were found positive for fasciolosis (Ogunrinade *et al.*, 1981).

In Zambia, *Fasciola gigantica* is endemic in cattle, sheep and goats particularly in Western province where Silangwa (1973) and Pandey and Ahmadu (1998) recorded

Fasciola prevalence of 89% and 51%, respectively. Recently, Phiri *et al.*, (2005) recorded a *F. gigantica* prevalence of 53.9% in cattle slaughtered at selected abattoirs in Central, Southern and Western provinces of Zambia.

Information available on the geographical distribution of *Schistosoma* species has been reviewed by Rollinson and Southgate (1987). *S. spindale*, *S. nasale* and *S. incognitum* have been reported in Asia while *S. japonicum* is endemic in several countries of the Far East. *S. mattheei* has been reported in south-eastern Africa, from the cape province of South Africa northwards to Tanzania. *S. bovis*, which occurs in the Mediterranean region and Middle East, is also common in northern, western and eastern Africa extending southwards to central Angola. The occurrence of cattle schistosomosis within their range is known to be discontinuous and obviously depends on the presence of intermediate snail hosts, their level of infection and the frequency of water contacts (De Bont, *et al.*, 1994). Regional surveys based on postmortem or faecal examinations in areas where conditions are favourable show *Schistosoma* species prevalence rates ranging from 40-70% (De Bont, 1995).

Four species of schistosomes have been reported from cattle in Zambia, i.e. *Schistosoma mattheei*, *S. leiperi*, *S. margrebowiei* and *S. bovis* (Dinnik and Dinnik, 1965; De Bont *et al.*, 1994). Bovine schistosomosis, mainly caused by *Schistosoma mattheei*, is widely distributed in Zambia (De Bont *et al.*, 1994). The estimated prevalence of 51% is however characterized by low levels of infection; 93% of the infected animals having less than 100 worm pairs in the mesenteric veins (De Bont *et al.*, 1994).

Paramphistomosis in cattle has a wide geographic distribution (Smyth, 1986). *Paramphistomum daubneyi* has been reported as the most frequent paramphistome

species present in Europe, causing significant gastrointestinal disease, drop in production or even death (Rieu *et al.*, 2007). Recent epidemiological surveys in Central France (Mage *et al.*, 2002) demonstrated that the prevalence of natural infections with *Paramphistomum* in cattle significantly increased from 5.2% (in 1990) to 44.7% (in 1999).

In India, paramphistomiasis is a serious helminthic problem and fatal outbreaks of the disease in ruminants have been reported (Chhabra *et al.*, 1978). Although 44 species of amphistomes have been reported, *Paramphistomum cervi* is the species most common in ruminats in India (Dutt, 1980).

The most widely distributed *Paramphistomum* species in Africa is probably *P. microbothrium* (Brown, 1980). The presence of *Paramphistomum microbothrium* has been documented in Nigeria (Fagbeni, 1984) and in Zambia (Wright *et al.*, 1979).. Recently in Zambia, Phiri *et al.* (2006a) recorded a *Paramphistomum* species prevalence of 51.6% in cattle from communal grazing areas along the Zambezi and Kafue river basins in Zambia. The authors observed that the prevalence of paramphistomosis in Zambia ranks among the highest in Africa.

2.4 Pathology of trematode infections

2.4.1 Pathology of fasciolosis

2.4.1.1 Gross pathology

Lesions produced by *F. gigantica* and *F. hepatica* are most prominent in the liver (Phiri *et al.*, 2007). Occasionally, the parasites may reach the lungs and other tissues where they are found within the abscesses they cause (Jones and Hunt, 1983; Phiri *et al.*, 2006b). Pneumonia, fibrous pleuritis and pleural fluid are commonly reported in many animals where lung lesions are involved (Boray, 1969). The gross and microscopic changes associated with fasciolosis take two main forms: the parenchymal phase, which occurs during migration of the immature flukes through the liver parenchyma causing extensive perforation, and the biliary phase, which coincides with the presence of mature flukes in the bile ducts, causing thickening of the bile ducts (Phiri, 1997; Kofta *et al.*, 2000).

In calves and small ruminants, the acute form of the disease is associated with traumatic hepatitis caused by migration of immature flukes in the parenchyma leading to high mortality (Soulsby, 1982). Migration of immature flukes within the liver produces dark red hemorrhagic tracts of necrotic liver parenchyma (Carlton and McGavin, 1995). A variety of untoward sequelae can follow these migrations, including acute peritonitis, hepatic abscesses, the proliferation of spores of *Clostridium haemolyticum* or *C. novyi* in necrotic tissue and the subsequent development of bacillary hemoglobinuria or infectious necrotic hepatitis and death of the host (Carlton and McGavin, 1995). As the migrating fluke grows, the size of its track through the liver also increases, exacerbating the damage and the inflammatory response (Behm and Sangster, 1999). The hemorrhagic tracts become paler than the

surrounding parenchyma with time and the liver pathology is characterized by hepatic fibrosis and hyperplastic cholangitis, pallor and firmness, with the ventral lobe being most affected and reduced in size (Urquhart *et al.*, 1989).

Because flukes may pass through the same part of the liver twice or more during their migrations, fresh and resolving lesions caused by the sequential insults may be found in the same section of tissue and thus several flukes become trapped in the parenchyma. Even after a high infection, only about 5% of the inoculum reaches the bile ducts and thus, only few eggs are passed (Ross *et al.*, 1966). In the bile ducts, flukes feed on blood by penetrating the bile duct epithelium (Kofta *et al.*, 2000). The mature flukes then cause cholangitis or cholangiohepatitis to develop in the bile ducts, which appear dilated and thickened. Chronic cholangitis and bile duct obstruction lead to ectasia and stenosis of the ducts, and periductular fibrosis that thickens the walls so that the ducts become increasingly prominent as calcified white cords which when opened, display dirty dark brown or nearly black contents within which the flukes are easily seen (Phiri *et al.*, 2006b). Obstruction of the ducts leads to extrahepatic cholestasis (Carlton and McGavin, 1995). *Fasciola* eggs can also provoke a granuloma-like reaction that can result in obliteration of the affected bile ducts (Urquhart *et al.*, 1989). Calcium deposits start to form in the duct walls after 16 to 20 weeks of infection enlarging the ducts up to 3 cm in diameter and they become prominent on the surface of the liver. 'Stove pipe or clay pipe' livers are typical and apt descriptions. The lumina of the ducts are variously dilated and stenosed and the epithelium shows ulceration and hemorrhage (Behm and Sangster, 1999).

2.4.1.2 Histopathology

Microscopically, the lesions generated by liver flukes are visible on Haematoxylin and Eosin (H& E) stained sections of the liver (Cervi *et al.*, 2001). These lesions appear as migratory tracts filled with blood, fibrin and cellular debris surrounded by coagulative necrosis. In some of the tunnels, sections of flukes may be observed (Martinez-Moreno *et al.*, 1999; Phiri *et al.*, 2006b). The cellular inflammatory infiltrate surrounding these fresh migratory tunnels is composed of eosinophils, neutrophils and macrophages laden with hemosiderin pigment. In chronic infections, lesions include moderate to severe fibrous perihepatitis and moderate number of white migratory tracts in the hepatic surface and parenchyma. These tracts are composed of abundant infiltrate of eosinophils, numerous macrophages with hemosiderin pigment, lymphocytes as well as proliferation of fibrous connective tissue (Martinez-Moreno *et al.*, 1999). Chronic cholangiohepatitis and proliferative mesoendophlebitis are also observed (Ferreras *et al.*, 2000). Degenerating flukes and fluke eggs attracting foreign body giant cells surrounded by macrophages, lymphocytes and fibrous tissue forming nodules of various sizes can also be observed (Singh and Parihar, 1988; Phiri *et al.*, 2006b). Numerous micro-abscesses containing *Fasciola* larvae or eggs can also be found accompanied by cellular reactions and calcification (Uzoukwu and Ikeme, 1978). Granulomas composed of multinucleate giant cells, macrophages, eosinophils and lymphocytes are also features of chronic infections. Cirrhosis featuring loss of lobular architecture, bile duct proliferation with a marked epithelial hyperplasia, fibrosis and abundant lymphoplasmacytic infiltrate replacing hepatic parenchyma and granulation tissue are common features in healing lesions (Cervi *et al.*, 2001).

2.4.2 Pathology of schistosomosis

2.4.2.1 Gross Pathology

The intestinal and hepatic forms of schistosomosis are characterized by the presence of adult flukes in the portal, mesenteric, and intestinal submucosal and subserosa veins (Ferreras *et al.*, 2000). In the intestinal form, passage of eggs through the gut wall leaves numerous diffuse mucosal hemorrhages. In other cases the intestinal wall of infected animals appears apparently normal (Ferreras *et al.*, 2000). The liver pathology associated with *Schistosoma* infections is a direct sequela of inflammatory responses to the deposition of eggs in the intrahepatic portal circulation and subsequent encapsulation granulomas in the liver (Ferreras *et al.*, 2000). In infected animals, yellowish-white small foci (1 mm in diameter or larger) are evident at the liver surface and into the parenchyma followed by marked portal fibrosis in the host (Esteban *et al.*, 2003).

2.4.2.2 Histopathology

Schistosoma lesions are observed in both the intestinal and hepatic forms. *Schistosoma* egg-induced granulomas appear in the lamina propria of the small intestines and within the perilobular zones of the liver (Ferreras *et al.*, 2000). In that study, four types of granulomatous lesions were observed in the small intestine and liver of *Schistosoma* infected lambs, based on their histological appearance. Granuloma type 1 was mainly composed of a central schistosome egg surrounded by macrophages, occasionally foreign body giant cells, some eosinophils, and a peripheral infiltrate that comprised a low or moderate number of lymphocytes and

plasma cells. The schistosome eggs in this category showed no alterations or variable degree of alterations (Ferreras *et al.*, 2000). Granuloma type 2 consisted of a central necrotic schistosome egg (including empty shells and residues of eggs) surrounded by macrophages, some foreign body giant cells and eosinophils. The main difference between type 1 and 2 granulomas was the constant presence of a large number of lymphocytes surrounding the granuloma and infiltrating the associated intestinal lamina propria and liver perilobular zones in granuloma type 2 (Ferreras *et al.*, 2000). In many cases lymphoid aggregates were seen. Granuloma type 3 was small in size and was composed mainly of a necrotic and calcified center surrounded by fibrous tissue. Granuloma type 4 contained schistosome eggs in the lamina propria with a slight inflammatory response. This category also included the presence of free eggs without an appreciable cellular reaction, which was always seen together with fibrous granuloma type 3.

2.4.3 Pathology of paramphistomosis

2.4.3.1 Gross pathology

The size of the paramphistome burden is the most important factor determining the degree of small intestinal pathology (Hansen and Perry, 1984). The immature stages of paramphistomes can cause severe pathology and have been documented to cause death of the host in heavy infections (Silvestre *et al.*, 2000). They are attached to the wall of the small intestine causing marked inflammation. Digestion and absorption are affected, and appetite is also depressed, resulting in diarrhoea, anorexia, anaemia and weakness (Spence *et al.*, 1996). There is little evidence regarding the pathogenicity of the adult amphistomes in the rumen of their hosts (Hansen and

Perry, 1994), but severe damage to the mucosa of the rumen is provoked in heavy infections. Studies done by Singh *et al.* (1984) demonstrate that tissue changes in the rumen only appear after 80 days post infection. They observed amphistomes lying on the mucosal surface of the rumen, either in between, or attached to the villi, which showed desquamation.

2.4.3.2 Histopathology

A comprehensive study carried out by Singh *et al.* (1984) on *Paramphistomum cervi* in goats demonstrated the histopathological effects of the infection in ruminants. They observed that in the early stages of the infection, there were slight changes in the duodenum. The parasites were seen embedded in the Brunner's gland just beneath the muscularis mucosae. Between days 40 and 60 post infection, the authors observed intense tissue reaction in the duodenum accompanied by diffuse cellular infiltration of mononuclear cells (macrophages) and lymphocytes in the lamina propria and interstitial tissue of Brunner's gland. There was also desquamation of intestinal villi epithelium causing atrophy of the mucosa. This was attributed to the embedding-in and penetration of the immature flukes through the mucosa. In long standing infection, Singh and co-workers (1984) observed that the muscular and serosal layers of the duodenum appeared almost normal.

2.5 Socio-economic significance of trematode infections

2.5.1. Fasciolosis

The losses in livestock productivity due to fasciolosis can be very high, particularly in areas where the local environmental conditions are favourable to sustaining the mollusc snails, which exacerbates exposure of cattle to high rate of acute fasciolosis leading to high morbidity and mortalities (Sewell, 1966). The estimated economic losses due to *Fasciola* are mostly based on calculations obtained from prevalence reports derived from abattoir or faecal egg examination studies (Spithill *et al.*, 1999). With a few exceptions, estimates of economic losses have only been based on the value of infected livers condemned at slaughterhouses as unfit for human consumption, or on the value of meat lost through lower carcass weights of infected animals (Sewell, 1966; Phiri, 2006c). These figures bear little association to the actual number of animals infected as some livers with light infections are often not totally condemned, instead only the affected parts are removed (Froyd, 1959). Since the efficiency of detecting light infections also varies with the training and experience of the meat inspector, chances of missing light infections are very high (Phiri *et al.*, 2006b).

Chronic fasciolosis leads to loss in livestock productivity through direct and indirect losses, which include liver condemnation and reduced growth rates. In Zambia, tremendous losses from fasciolosis in cattle are attributed to condemnation of livers during meat inspection and indications are that the losses are continually on the increase (Pandey and Ahmadu, 1998; Phiri, 2006c). In western province alone, bovine liver condemnation rates of between 33% and 73% were recorded due to

fasciolosis between 1980 and 1994 (Pandey and Ahmadu, 1998). During the same period, out of 95,018 cattle slaughtered at Mongu abattoir, 48,692 livers (51%) were totally condemned because of fasciolosis resulting in an estimated loss of up to US$ 228, 244. Indirect losses due to diminished weight gain in relation to the level of infection, the age and sex of animals, the interaction between level of nutrition and pathogenic effect, and the differences between breeds and within breeds are well documented (Spithill *et al.*, 1999). Sewell (1966) reported a linear relationship in yearling zebu cattle between the burden of adult *F. gigantica* and weight gain. It was observed that each fluke reduces the potential annual weight gain by about 200g. It has also been estimated that anaemia caused by fasciolosis can reduce work output by 7 to 15% (Sewell, 1966). Reduced draught power performance due to fasciolosis significantly imparts negatively on production of crops in South East Asia and Africa where ruminants provide 80% of the draught power (Spithill *et al.*, 1999). Fasciolosis has also been reported to reduce fertility and lower feed conversion efficiency in infected animals (Hillyer, 2005).

2.5.1.1 Public Health significance of fasciolosis

Human fasciolosis, which was considered a secondary disease with only approximately 2,000 cases reported between 1970 and 1990 (Esteban *et al.*, 2003), has now been well recognized with a number of reported cases having increased worldwide. It is estimated that 2.4 to 17 million people are infected worldwide (Spithill and Dalton, 1998, Mas-Coma *et al.*, 1999). Outbreaks of human fasciolosis have been reported in developed countries including France, Portugal, Spain and Australia (Hillyer, 2005). Although both human and animal infections with the

tropical liver fluke (*F. gigantica*) have been reported in Africa and Asia (Hillyer, 2005), the high prevalence of human infections that have been observed recently do not seem to necessarily be associated with areas where fasciolosis poses a great veterinary problem (Mas-Coma, 2005). Chronicity and superimposed repetitive liver fluke infections in humans tend to pose additional pathologic complications, making fasciolosis to be no longer considered as mere secondary zoonotic disease, but as an important human parasitic disease.

2.5.2 Schistosomosis

Most of the research work done on some of the major bovine *Schistosoma* species, *S. bovis*, *S. Spindale*, *S. nasale* and *S. mattheei* indicate that schistosome infections represent a subclinical form characterised by high prevalence of moderate worm burdens in the host population (Lawrence, 1976; De Bont *et al.*, 1994). Although abattoir surveys done by Makundi (1993) in Tanzania also indicated lower infection levels in cattle, Kassuku *et al.* (1986) reported heavy infection levels of *S. bovis* on some Tanzanian farms. In earlier studies, McCauley *et al.* (1983) observed high levels of mortality in Sudanese cattle due to schistosomosis. Makundi (1993) concluded that abattoir surveys grossly provide an underestimation of the true morbidity and economic impact of bovine schistosomosis. McCauley *et al.* (1983) observed that the pathological significance of schistosome infections in cattle is not only restricted to isolated outbreaks of clinical symptoms and mortality in susceptible populations, following intense transmission. High prevalence rates of subclinical schistosome infections also cause significant losses due to long-term

effects on animal growth and productivity and increased susceptibility to other parasitic or bacterial diseases.

2.5.2.1 Public Health significance of schistosomosis

Human schistosomosis (Bilharzia) is one of the most prevalent parasitic diseases in the world and it is endemic in 76 countries of the tropics and sub-tropics (Southgate *et al.*, 2005). It is estimated that there are in excess of 200 million people infected with schistosomosis worldwide, with children being at particular risk (WHO, 2002). Human schistosomosis caused by adult *S. japonicum* depositing eggs in blood vessels surrounding the gut, is one of the most important parasitic diseases in china (Zhondao *et al.*, 2004). In sub-Sahara Africa, *Schistosoma haematobium* and *Schistosoma mansoni* are widespread human parasites (WHO, 2002). Along the shores of Lake Victoria and Lake Albert, *Schistosoma mansoni* can be considered to be hyper-endemic and the prevalence of infection within school-age children can be nearly universal (Kabatereine *et al.*, 2005). Estimates of mortality are difficult to calculate, owing to the limited data available, but may be as high as 150,000 per year as a result of non-functioning kidney (*Schistosoma haematobium* infection) and 130,000 from haematesis (*Schistosoma mansoni* infection) (Van der Werf et al., 2003). The global burden of the disease is 4.5 million disability-adjusted years, but recent studies suggest that this figure is severely underestimated (WHO, 2002).

2.5.3 Paramphistomosis

Although the infection by paramphistomes in cattle has not been thoroughly evaluated, it affects livestock productivity by provoking a lower nutritious conversion resulting in loss of weight and/or a decrease in milk production (Rangel-Ruiz *et al.*, 2003). Clinical outbreaks of paramphistomosis caused by *Paramphistomum microbothrium* have been recorded in Africa (Hansen & Perry, 1994) and the disease has been observed to cause mortality (Agosti *et al.*, 1980). The frequent outbreaks of clinical paramphistomosis are reported to be due to *Paramphistomum cervi* in ruminants and relate to the immature phase of intestinal development of the flukes (Dutt, 1980). Soulsby (1982) reported a mortality rate of 80 to 90% in cattle due to paramphistomosis in some areas of India, the Republic of South Africa and Australia.

2.6 Resistance to trematode infections

2.6.1 Resistance to *Fasciola* infection

Resistance to fasciolosis in cattle based on adult worm recoveries has been considered since the early sixties, where a number of high levels of acquired protection ranging from 60 to 84% were observed (Doyle, 1971). Based on the observation of occurrence of cattle livers with lesions of chronic fasciolosis, but which contained no or very few liver flukes, Doyle (1971) suggested that hepatic fibrosis resulting from the primary infection might be an important factor contributing to resistance to homologous challenge infections. Doyle (1971) also pointed out that the duration of the primary infection, and hence the extent of liver fibrosis, correlated with the level of resistance. Findings from an abattoir survey in

Kenya considered that age immunity, or more likely, an acquired resistance to re-infection with *F. gigantica* had occurred in many places (Bitakaramire, 1973). Using irradiated metacercaria as the immunizing antigen in cattle, Bitakaramire (1973) reported a 98% reduction in worm burdens following a challenge infection in vaccinated calves. Follow up studies provided strong evidence that cattle acquire resistance to both *Fasciola hepatica* and *F. gigantica* infection following a primary infection or vaccination using irradiated metacercaria or parasite extracts (Haroun & Hillyer, 1986; Spithill *et al.*, 1999; Knox *et al.*, 2001).

It is now established that after ingestion of metacercaria by the host, the animal is exposed to a variety of *Fasciola* antigens as the migrating fluke undergoes both morphological and physiological changes to its outer tegument (glycocalyx), thus a defined sequence in the development of these antigens (Sewell, 1966). During these stages, the parasite produces various excretory/secretory (E/S) products (Phiri *et al.*, 2006) that play an important role in the host-parasite relationship since they form the interface between the living parasite and the host's immune system (Smith *et al.*, 1993). These E/S products (e.g. cysteine proteinases) are very important enzymes in the host-parasite interactions as they play a role in parasite feeding, migration through the host's tissues as well as immune evasion such as antibody degradation (Smith *et al.*, 1993; Kofta *et al.*, 2000). However, these proteins are also immunogenic and thus are targets of the host's immune response warranting their experimental use as defined antigens (Spithill *et al.*, 1999; Mulcahy & Dalton, 2001; Hillyer, 2005).

About half a dozen purified, native and recombinant *Fasciola* antigens have been shown to have immunoprophylactic potential against fasciolosis (Hillyer, 2005).

These include fatty acid binding proteins (FABP), glutathione S-transferases (GST), cathepsin L proteases (catL), haemoglobin, leucine aminopeptidase (LAP) and a saposin-like protein denoted SAP-2 (Spithill and Dalton, 1998). Experimental works using FABP as the immunizing antigen was able to protect calves with up to 55% reduction in challenge infection with *Fasciola hepatica* (Hillyer *et al.*, 1988). Further studies concluded that a recombinant *F. hepatica* FABP induced protective (worm burden reductions), anti-fecundity (immature flukes) and anti-pathology (less liver lesions) effects in rabbits and may serve as a model for the immunoprophylaxis of fasciolosis (Hillyer, 2005). Similar studies using cysteine (cathepsin) proteases demonstrated that both cathepsin L1 and L2 antigen preparations elicited protection against challenge infection, with the highest being 72% fluke reduction when cathepsin L2 was used in combination with parasite haemoglobin. In a large series of vaccine trials on both sheep and cattle with purified native *F. hepatica* cathepsin L1 and L2, Dalton *et al.* (2003) observed that these enzymes could induce protection to challenge infection with *F. hepatica* metacercaria ranging from 33 to 79%, and produced a very potent anti-embryonation/hatch rate effect that would block parasite transmission. Anti-fecundity, anti-embryonation and lower faecal egg counts are the other observed features of cathepsin L vaccines in cattle and sheep (Mulcahy & Dalton, 2001). These findings strongly demonstrate the ability by cattle to acquire protective immunity against fasciolosis through cellular, humoral and inflammatory components (Dalton and Mulcahy, 2001). Protection in vaccinated cattle correlates with the induction of high titres of IgG1 and IgG2 antibody isotypes and production of gamma IFN, indicating a mixed Th1/2 response (Clery *et al.*, 1996; Mulcahy *et al.*, 1998; Mulcahy *et al.*, 1999; Espino *et al.*, 2005). These differences in antibody

isotype responses of cattle to fasciolosis may play an important role in the "self cure" phenomenon and resistance against challenge infection observed in *Fasciola* spp. (Phiri *et al.*, 2006).

2.6.2 Resistance to *Schistosoma* infection

There is overwhelming evidence that acquired immunity to *Schistosoma* infection in cattle exists and that it is of major importance in the regulation of infection intensity under natural conditions (De Bont & Vercruysse, 1998). With increasing duration of exposure to continuous challenge, cattle have been observed to become less susceptible to reinfection (De Bont *et al.*, 1995c). Gabriel and co-workers (2007) observed perinatal priming of calves born to *Schistosoma mattheei*-infected dams. This perinatal priming was thought to have important consequences for subsequent immunity and protection of the newborn in endemic areas. Earlier, Gabriel *et al.* (2004) observed that seropositive-born calves from infected dams, suspected of intra-uterine priming, had lower faecal egg and tissue egg counts and lower schistosome circulating antigen levels in their serum after natural *S. mattheei* challenge around the age of 5 months, indicating a protective memory response.

Cattle residing in endemic areas show a typical pattern in faecal egg counts where faecal egg excretion starts between 4 and 8 months of life and rapidly increase to reach a maximum around the age of 6–15 months and then decrease markedly by the age of 18 months (De Bont and Vercruysse, 1997). Lawrence's investigations (1973) suggested that development of an acquired immunity mainly acts through a reduction of the fecundity of the female worm, expressed as reduced faecal and tissue egg counts, with few effects on worm burden. While worm burden tends to increase with

the age of the host, faecal egg counts and tissue egg counts have been observed to remain low (Majid *et al.*, 1980). Immune responses against schistosomosis have a well-established anti-embryonation effect on the eggs (De Bont *et al.*, 2003). Earlier, Capron *et al.* (1995) observed that the capacity of the schistosomosis immune response in reducing the female worm fecundity and egg viability occurs through the production of neutralizing antibodies, IgA and IgG. Recent vaccination trials in cattle against schistosomosis using the recombinant *S. japonicum* GST antigen demonstrated a reduction in the fecundity of the female worm characterized by a significant decrease in tissue egg counts and a reduction in the number of miracidium hatched per 50 g faeces (Zhongdao *et al.*, 2004).

Soluble egg antigen triggers a transient increase in gamma IFN likely generated by Th1 and Natural Killer (NK) cells, which is followed by secretion of the Th2 products such as Interleukin (IL)-4, IL-5, IL-10 and IL-13 (Cervi *et al.*, 2001). Mice immunized with IL-2 and eggs of *S. mansoni* inhibit the formation of granuloma and strongly reduce the fibrosis caused by natural infection of the homologous species (Wynn *et al.*, 1995). Recent evidence indicates that the Th1 and then Th2 subsets of soluble eggs antigen-specific CD4 + lymphocytes and their products dominate the cascade of events leading to inflammation of granuloma (Cervi *et al.*, 2001). Granulomas form around the eggs, but as infection proceeds their size decrease with a concurrent abatement of the disease (Dalton *et al.*, 1996). The inverse relationship between level of infection and the granuloma size observed in mice (Hirata *et al.*, 1993) and the different morphological appearance of *Schistosoma bovis* egg granulomas in the small intestines and the liver, was observed to support a local immunological response (Ferreras *et al.*, 2000). Thus, schistosome egg-induced

granulomas represent a form of chronic delayed type-hypersensitivity (Abbas *et al.*, 1994). It has been established that IgG and IgE are directly involved in the killing of schistosome larvae in association with macrophages and platelets (Vilar *et al.*, 2003). IgG1 has been identified as the potential effector of immune protection in cattle (Gabriel, 2005).

2.6.3 Resistance to *Paramphistomum* infection

There are very little immunological studies that have been done in paramphistomes (Diaz *et al.*, 2006). It has been documented that strong immunity to paramphistome infections is developed in cattle (Urquhart *et al.*, 1996) and that repeated infections generally produce an almost complete immunity, resulting in a marked reduction in the fluke burdens from challenge infection. Recently, Diaz *et al.* (2006) demonstrated an IgG response in cattle naturally infected with *Calicophoron daubneyi*. It was observed that the IgG response in infected cattle did not increase after reinfection, as occurs in fasciolosis and schistosomosis (Castro *et al.*, 2000; Gabriel *et al.*, 2004). This observation was explained by a small trematode burden that developed after the challenge infection and migrated to the rumen, involving very little antigenic stimulus insufficient to increase the production of IgG (Diaz *et al.*, 2006). Mavenyengwa and co-workers (2003) demonstrated that cattle immunized by *Calicophoron microbothrium* metacercaria can mount resistance to re-infection. The study assessed development of immunity by comparing the severity of both gross and histopathological lesions, amphistome recovery rates and trends in the clinico-haematological findings between the immunized and challenged animals. It

was observed that differences in response to re-infection between the two groups were significant.

2.6.4 Cross-resistance in trematode infections

Studies that have been done on *Fasciola* and *Amphistomum* indicate that there is a close association between these two trematodes in cattle (Keyyu *et al.*, 2006). A positive correlation between *Fasciola* and *Amphistomum* has been demonstrated in co-joint infection of cattle by these two related trematodes (Szmidt-Adjide *et al.*, 2000; Phiri *et al.*, 2006a). The higher prevalence of amphistomes than of *Fasciola* observed by Keyyu *et al.* (2006) in naturally infected cattle in endemic areas in Tanzania concurred with other studies (Szmidt-Adjide *et al.*, 2000). The differences in the prevalence of *Fasciola* and *Paramphistomum* infections in cattle might be influenced by the density and uptake of their metacercariae by the host and/or the specific development of metacercariae in the definitive host (Szmidt-Adjide *et al.*, 2000). The higher prevalence of amphistomes than of *Fasciola* has been attributed to the high biological potential of the amphistome snail intermediate host (Dinnik and Dinnik, 1965), the direction of control measures mainly against *Fasciola* and the lack of effective drugs against amphistomes (Mage *et al.*, 2002).

Heterologous resistance between *Schistosoma* spp. and *Fasciola* spp. has been observed in several experimental and farm animals. Reciprocal cross-protection has been demonstrated between *F. hepatica* and *S. mansoni* using the criteria of worm burden and/or egg-producing capacity evaluation procedure in mice (Hillyer, 1984). Earlier studies by Hillyer (1981) and Christensen *et al.* (1980) observed a marked reciprocal resistance between *F. hepatica* and *S. bovis* in mice. It was observed that

single-sex or prepatent *Schistosoma* infections and immature *Fasciola* infections did not induce heterologous protection. Based on this finding, they suggested that in experimental mouse model, the resistance induction might be dependent upon pathological changes, such as liver fibrosis caused by primary infections.

In natural host-parasite interactions, Sirag *et al.* (1981) found that Jersey calves with a mature, primary infection with *S. bovis*, showed significant resistance to a challenge with *F. hepatica*, as judged from adult worm recovery and histopathology. Based on the low density of schistosome eggs in the liver and lack of microscopically obvious liver fibrosis, Sirag *et al.* (1981) disputed that, unlike the mice model, heterologous resistance between *Fasciola* and *Schistosoma* may not be dependent upon the pathological consequences of the primary infection in the normal definitive hosts. Using the criteria of adult worm recoveries, Monrad and colleagues (1981) described heterologous resistance between *S. bovis* and *F. hepatica* in sheep where it was reported that significant resistance to *Fasciola* challenge was demonstrated in a *S. bovis* infected European breed of sheep. Both the non-patent (two to three-week-old) and newly patent (seven to eight-week-old) primary schistosome infections were protective, whereas the more chronic (16 to 17-week-old) schistosome infections were not protective. Monrad and colleagues (1981) observed lack of resistance when the infection challenge with *F. hepatica* was carried out during the schistosome patent period in sheep. They speculated that a protective mechanism triggered by *F. hepatica* may be acting on the primary stages of *S. bovis* in their schistosomula form and not by an established adult worm presence in the host. Ferreras *et al.* (2000) observed that sheep harboring primary patent *F. hepatica* infection acquired resistance to heterologous challenge with *S.*

bovis. They suggested that this protection could be due to the killing of pre-adult migrating schistosome stages.

Other investigations on heterologous resistance between the tropical *Fasciola* species *F. gigantica* and *S. bovis* in Sudanese zebu cattle also recorded significant results (Yagi *et al.*, 1986). The authors observed a marked level of reciprocal resistance between *F. gigantica* and un-irradiated *S. bovis*. Their earlier findings demonstrated a 94.2% *S. bovis* worm count reduction after a challenge infection in calves harboring a *F. gigantica* infection for eight weeks. A worm count reduction of 84.3% was also observed in heterologous challenge with *F. gigantica* in calves infected with *S. bovis* for eight weeks. The level of heterologous resistance stimulated by primary *S. bovis* infection was markedly higher than that of 30% recorded by Sirag *et al.* (1981). Similarly, Rodrigues-Osorio *et al.* (1993) showed that sheep infected with *F. hepatica* acquired resistance to challenge with *S. bovis* with a significant reduction in the *S. bovis* burden. This primary infection of sheep with *F. hepatica* was not able to induce protective immunity to homologous challenge. Rodrigues-Osorio *et al.* (1993) suggested that *F. hepatica* might evade the host's immune system by eliciting humoral and cellular responses to nonessential epitopes that might prove vital for *S. bovis*.

The purified, native and recombinant *Fasciola* antigens (FABP, GST and SAP-2), which have been shown to have immunoprophylactic potential against fasciolosis have also been shown to cross-protect against schistosomes (Hillyer, 2005). Several reports have demonstrated efficacy of *Fasciola* fatty acid binding protein (FABP) antigen against *S. mansoni* and *F. hepatica* (Hillyer *et al.*, 1988), and *F. gigantica* (Estuningsih *et al.*, 1997). The same *Fasciola* FABP antigen that was able to induce

protection in mice with up to 78% reduction and in calves with up to 55% reduction, against challenge infection with *F. hepatica*, also protected vaccinated mice against *S. mansoni* with up to 81% reductions (Hillyer, 1987). Studies carried out by Lopez-Aban *et al.* (2000) have shown that the protection obtained in experimental murine schistosomosis of *S. bovis* with the FABPs of *F. hepatica* was superior to that obtained with other candidate molecules for anti-*Schistosoma* vaccines. Both the native Fh12 molecule and the recombinant Fh15 FABP molecule, gave percentages of protection of greater than 70% in terms of reduction of worm burden. A close correlation between the intensity of the hepatic lesions and the reduction in the number of worms was observed. In further studies, vaccination with the recombinant *S. bovis* 28-kDa glutathione S-transferase (rSb28GST) also appeared to protect calves against natural *F. gigantica* infection (De Bont and Vercruysse, 1997). An FABP homologue from *S. mansoni* was also found to be able to protect experimental animals against *F. hepatica* (Smooker *et al.*, 2001).

Rodrigues-Osorio and co-workers (1993) considered the mechanism of cross-protection between *Fasciola* and *Schistosoma* to the existence of common antigens. This fact was earlier established by Hillyer (1979) who discovered that cross-reactive antigens isolated from *F. hepatica* protect against *S. mansoni* and that some of these antigens share common epitopes with *S. bovis*. Antibodies against *S. bovis* are reported to recognize a polypeptide antigen of 12 kDa on antigenic extracts from both *S. bovis* and *F. hepatica*. This molecule was observed to present, equally, cross reactivity between *F. hepatica* and *S. mansoni* (Lopez-Abane *et al.*, 2000). The recombinant FhSAP-2 is also an 11.5kD *Fasciola/Schistosoma mansoni* cross-reactive antigen that contains three-intra-chain disulfide bonds and is expressed by

the fluke at an early stage of infection of its mammalian host (Espino and Hillyer, 2003). More recent studies (Espino *et al.*, 2005) have demonstrated that since the anti-parasite IgE antibody confers protective immunity to both *Fasciola* and *Schistosoma*, the ability by a primary infection with one of these related trematodes to induce an IgE response, should be expected to induce a protective immunity to a challenge infection by the other parasite. These studies confirm the reported conservation of immunoprotective epitopes between *Fasciola* and *Schistosoma* antigens (Hillyer, 1979).

2.7 Diagnosis of trematode infections

2.7.1 Fasciolosis diagnosis

Diagnosis of fasciolosis is based primarily on clinical signs, a previous history of infection on the farm or the identification of snail habitats (Urquhart *et al.*, 1989). In acute cases, especially in calves and small ruminants, the animal dies suddenly; blood stained froth appears at the nostrils and blood is discharged from the anus (Soulsby, 1982). In chronic cases, especially in cattle, constipation is marked and the faeces are passed with difficulty, being hard and brittle. Diarrhoea is seen only in extreme stages. Emaciation increases rapidly, while dullness and weakness soon lead to prostration (Soulsby, 1982). Definitive diagnosis of infection with *Fasciola* is usually achieved parasitologically by detecting the fluke eggs in the faeces (Raina *et al.*, 2006). The differential flotation technique described by Hammond and Sewell (1990) and the sieving and sedimentation technique with a glass beads layer described by Taira *et al.* (1983) are commonly used in field routine work. Although the incubation period and presentation of clinical signs can take a few days, *Fasciola*

spp. require a period of at least 3 to 4 months to attain sexual maturity and release eggs in the faeces (Hillyer, 1988). Parasitological diagnosis can only detect patent infections and yet most damage to the animal is done during the parenchymal phase when migration of the immature flukes through the liver parenchyma causes traumatic hepatitis, before patency (Velusamy *et al.*, 2004). Parasitological methods have other limitations due to lack of uniformity and consistency in low, medium and high worm burdens after faecal egg counts (Boray, 1969). Fluctuations in faecal egg count per gramm (EPG) have been attributed to the numerous pathophysiological changes including peribiliary fibrosis, granulation or calcification of the liver that could easily lead to obstruction of the egg passage (Ross *et al.*, 1966; Boray, 1969). The egg counts rise rapidly by 7-9 weeks post infection, but become variable thereafter and may even be very low for a heavily infected animal (Sewell, 1966).

Sero-diagnosis of fasciolosis can detect early prepatent infections thus enabling timely chemotherapeutic intervention and also preventing the contamination of water bodies with the parasite eggs (Raina *et al.*, 2006). Field diagnosis of fasciolosis based on the antibody detection has some inherent limitations, as it does not differentiate between the current and the previous infection due to the persistence of *Fasciola* antibodies for a prolonged period of time after treatment of the infected animal (Fagbemi *et al.*, 1995). Antigen detection assays are now known to avoid giving false positive results due to previous exposure (Goubadia and Fagbemi, 1997). They also give a more accurate indication of current infection rather than past infection as they appear prior to antibody in host body fluids (Velusamy *et al.*, 2004). The main sources of potential immunodiagnostic antigens in fasciolosis are the metabolic antigens released in the excretory-secretory (E/S) products of adult

parasites e.g. cathepsin L cysteine proteinases (Fagbemi and Guobadia, 1995; Fagbemi *et al.*, 1995; Rodriguez-Perez and Hillyer, 1995). Several cathepsin L genes constituting a cysteine proteinase gene family have been identified in both *Fasciola hepatica* and *F. gigantica*, and regulate the development of the parasite in its host environment (Sriveny *et al.*, 2006). Using the antigen capture sandwich ELISA as a diagnostic tool, Velusamy *et al.* (2004) demonstrated that the 54 kDa *F. gigantica* antigen has a great potential for early diagnosis of bovine fasciolosis as it could be detected as early as 2 weeks post-infection in calves, irrespective of dose of infection. The *Fasciola hepatica* and *F. gigantica* cysteine proteases have been assayed in the immuno-diagnosis of fasciolosis in ruminants and have given satisfactory levels of sensitivity and specificity (Ruiz *et al.*, 2003). Recently, Sriveny *et al.* (2006) demonstrated the immuno-diagnostic potential of cathepsin L cysteine proteinase as a suitable antigen in the detection of *F. gigantica* infection in cattle without showing cross-reactivity with antibodies to *Paramphistomum epiclitum*, *Gigantocotyle explanatum* and hydatid cyst antigens in buffaloes infected with these mono-infections.

2.7.2 Schistosomosis diagnosis

Symptoms and history alone are insufficient to distinguish schistosomosis from other debilitating diseases (De Bont, 1995). Acute heavy infections are manifested by profuse diarrhoea, dehydration and anorexia. Anaemia and hypoabuminaemia may be followed by oedema (Soulsby, 1982). Chronically infected animals are emaciated, with marked decrease in production (Smyth, 1996). Diagnosis is confirmed by the presence and identification of *Schistosoma* eggs in the faeces of the infected animal (Smyth, 1996). At post-mortem, macroscopic examination of the mesenteric veins for the presence of adult worms or microscopic examination of scrapings of the intestinal mucosa or crushed and digested liver tissue for the presence of schistosome eggs may be useful (De Bont, 1995). In epidemiological studies, different quantitative parasitological techniques for faecal egg counting are still commonly used (Lawrence, 1970; De Bont, 1995). Low faecal egg excretion is commonly observed in chronic *Schistosoma* infections resulting in a reduction in the sensitivity of detecting eggs in faeces in aging animals (De Bont and Vercruysse, 1997). This was attributed to acquired resistance, which causes decline in female worm fecundity (Lawrence, 1976). The existence of acquired reduction in female worm fecundity and other factors such as crowding effects or reduction of egg output from each worm in heavy infections (Lawrence, 1976), implies that faecal egg counts cannot be used as a good indicator of the worm burden (De Bont, 1995).

Quantitative miracidial hatching techniques have been preferred to faecal egg counts for the diagnosis of schistosome infections in cattle (Kassuku *et al.*, 1986). Large faecal samples or pooled samples are used for miracidia detection, offering a better sensitivity for the detection of light or old infections, or infection at the herd level

(De Bont, 1995). The technique also provides information on the viability of the eggs excreted in the faeces (Kassuku *et al.*, 1986).

Serological tests for the detection of antibodies and circulating schistosome antigens have been developed for the diagnosis of cattle schistosomosis (Barsoum *et al.*, 1992). Detection of circulating antigens, which are produced in the gut of schistosomes, by ELISA techniques, is now widely applied for immunodiagnostic and seroepidemiological studies (Barsoum *et al.*, 1992). De Bont *et al.* (1995b) concluded that circulating antigen determination is an excellent diagnostic tool for *S. mattheei* infections in cattle, especially in aging infections when demonstration of eggs in the faeces may be difficult. It has also been demonstrated that there is a good correlation between antigen levels, egg excretion and worm burden (Qian and Deelder, 1983).

2.7.3 Paramphistomosis diagnosis

Diagnosis of paramphistomosis in cattle is based on the history of grazing around snail habitats and clinical signs. The clinical signs consist of profuse foetid diarrhoea, marked weakness and frequently death (Soulsby, 1982). Diagnosis may be based on the presence of immature paramphistomes in the fluid faeces, usually involving young animals in the herd (Urquhart *et al.*, 1989). Although the pathogenic effects are caused by the immature flukes, presence of paramphistome eggs in the faeces may be indicative of the disease as a large number of adult worms may accompany the immature burden (Soulsby, 1982). Parasitological diagnosis of amphistomosis is of little value since the disease occurs during the prepatent period (Urquhart *et al.*, 1989). Confirmation can be obtained by a post mortem examination

and recovery of immature flukes from the duodenum and/or adult flukes from the rumen (Urquhart *et al.*, 1989). Recently, Diaz *et al.* (2006) demonstrated that serological diagnosis of amphistomosis in cattle can be done as *Calicophoron daubneyi* induces a notable IgG response assessable by an ELISA technique.

2.8 Control of trematode infections

Control methods such as strategic antihelmintic treatment, grazing management, application of molluscicides, and fencing off or draining of swampy areas have been used against trematodes for many years (Phiri, 1997; Spithill *et al.*, 1999). Basically, control of trematodes can either be therapeutic management of infected hosts and/or controlling of the snail intermediate host (Simpson *et al.*, 1985).

2.8.1 Therapeutic management of trematode infections

It has been established that triclabendazole (TCB) is the most effective fasciolicide on the market (Hammond and Sewell, 1990). Although prohibitively expensive for the small-scale livestock producers, triclabendazole has a high efficacy against both mature and immature *F. gigantica* (Estuningsih *et al.*, 1990). It is advised that all animals at risk be treated at the beginning of the dry season and, all the exposed animals at the beginning of the wet season (Schillhorn van Veen, 1980). The latter treatment is of strategic importance because it reduces pasture contamination at this critical time when the snails are more likely to be infected with miracidium

(Schillhorn van Veen, 1980). Although chemotherapy against fasciolosis has been available for decades and has helped to reduce morbidity rates, it has only achieved a palliative measure. The exorbitant cost of TCB is hampering its wide use, and thus its failure to effectively control the morbidity of fasciolosis in livestock (Hillyer, 2005). The actual transmission rates have remained unaltered due to continuous re-infections in endemic areas (Vilar *et al.*, 2003). Increasingly, drug resistance in areas where TCB has been in regular use is being reported in sheep infected with *F. hepatica* (Espino *et al.*, 2005). It is feared that such resistance may induce cross-resistance to other antihelmintics such as benzimidazole carbamates, which are used in the control of trichostrongylidae and other nematodes (Fernandez *et al.*, 2004).

Niclosamide, Oxyclozanide plus levamosole have been successfully used in the treatment of cattle against paramphistomosis (Rolfe and Boray, 1987). The authors reported a significant reduction in IgG and EPG values, with a significant increase in the haematic parameters in cattle naturally infected with Paramphistomes.

Praziquantel is the current drug of choice for the control of human schistosomosis (Ndyomugyenyi and Minjas, 2001; Southgate *et al.*, 2005). In cattle, the drug has been successfully used against *Schistosoma bovis* (Bushara *et al.*, 1982). Treatment trials of nasal schistosomosis using a single oral administration of praziquantel at a dose rate of 20 mg/kg body weights gave unsatisfactory results (De Bont *et al.*, 1989). Chemotherapy alone cannot prevent re-infection and therefore its application is often restricted to scarce outbreaks of clinical schistosomosis for economic reasons (Zhongdao *et al.*, 2004).

2.8.2 Control of snail intermediate host

Snail control is one of the widely used control strategies in the control of trematode infections (WHO, 2002). However, moderate reduction of snail numbers may result in an increased proportion of the remaining snails becoming infected (Brown, 1994).

2.8.2.1 Chemical control of snails

Molluscicides are mainly used to control the snail intermediate hosts in dams. The more extensive habitats such as rivers and irrigated rice fields make certain molluscicide application cost prohibitive and poses toxic threat to non-targeted animals and plants in the vast habitat (Spithill *et al.*, 1999). Nevertheless, molluscicides are effective in temporarily reducing snail populations in schistosomosis control interventions (McCullough, 1992). The efficacy of four molluscicides: Sodium Pentachlorophenate (NaPCP), N-tritymorpholine (Frescon), Copper sulphate, Niclosamide (Bayluscide®) and Yurimin on *Lymnea ollula* were evaluated and Bayluscide® was the most effective (Harada, 1974). However, the indiscriminate application of molluscicides to water bodies without much consideration for other aquatic fauna may cause adverse exposure of non-targeted organisms to the harmful effects of the molluscicides, thereby upsetting the balance of the ecosystem. Copper sulphate is known to be toxic to both livestock and fish when applied at a molluscicide rate of 0.1 to 0.2 ppm (Soulsby, 1982).

2.8.2.2 Environmental control

Considerable success has been achieved in reducing habitats for *Oncomelania*, the amphibious intermediate host for *Schistosoma japonicum*, through environmental control. Such control methods have not been widely applied in Africa where the aquatic snail hosts are abundant (Brown, 1994). Removal of favourable vegetation from water and stream banks, drainage and canalization of the source areas of streams are some of the methods used to eliminate persistent snail habitats. Efficient management of irrigation schemes has also been reported to achieve snail control, as removal of aquatic vegetation promotes free flow of water and also removes shelter and food sources for snails (Brown, 1994).

2.8.2.3 Integrated Management control

Integrated farm management and environmental intervention of the snail habitats is the most economically feasible method of preventing fluke infection in areas where the eco-system permits (Phiri, 1997). The number of suitable final and snail intermediate hosts in an area, except where these are kept entirely apart, largely control the incidence of fasciolosis (Hammond, 1970). This means that the more animals and the snails are found together at one site, the more likely the disease will be propagated. Separating these hosts offers a major control aspect of the disease. In areas where the main source of infection is irrigated rice fields, after harvest feeding management, which denies livestock access viable metacercaria and cercaria in paddy fields is a particularly relevant control strategy (Spithill *et al.*, 1999). This can

be achieved by providing good drainage to the low-lying areas, fencing off water swamps or by constructing water troughs for animals (Simpson *et al*, 1985).

2.8.3 Immunological control of trematode infections

Although control of parasite infections by parasiticides is efficient, parasites develop resistance to antihelmintics and concerns about residues of such parasiticides in food of animal origin are growing (Waldvogel *et al.*, 2004). Immune prophylaxis for the control of parasites has been proposed.

Vaccines and good serodiagnostics have not been well developed resulting in the continuous increase of incidence and prevalence of bovine schistosomosis (Chitsulo *et al.*, 2000). McManus (2000) observed that control of bovine schistosomosis by vaccination in most endemic areas is an attractive goal and would reduce transmission of this parasite to humans. Studies conducted in experimental animals and humans from endemic populations show that immunity-based intervention of schistosomosis may be feasible and that humoral immunity plays a major role in resistance to infections (Nyame *et al.*, 2003). Several researchers have explored the utilization of defined schistosome-derived protective antigens as they are easy to standardize and to deliver (De Bont, 1995). The promising capacities of the schistosome-derived glutathione S-transferases (GST) as protective antigens against experimental schistosomosis have been underlined (Capron *et al.*, 1995). A recent report by Zhongdao *et al.* (2004) suggest that it may be feasible to annually immunize domestic animals against schistosomosis using recombinant schistosome GST antigens during the non-flooded season when chemotherapy is also administered. This is expected to lower the re-infective density of domestic animals

to marshlands in the endemic areas, which in turn reduce the transmission of schistosomosis in animals and humans.

In cattle, immunity against fasciolosis can be acquired after exposure to attenuated/abbreviated metacercaria infections or by immunization with crude adult parasite extracts (Spithill and Dalton, 1998). Defined *Fasciola* antigens like glutathione-S-transferase (GST), fatty acid binding protein (FABP), parasite hemoglobin, cathepsin L, paramyosin and others have also been exploited for immunoprophylaxis (Nyame *et al.*, 2003, Hillyer, 2005). The fatty acid binding protein that has shown some promise in inducing protection against trematode infections in various vaccine trials has been recognized as a prominent vaccine candidate (Estuningsih *et al.*, 1997).

CHAPTER THREE

3. Materials and Methods

3.1 Study area

Zambia is situated in Southern Africa and lies between the latitudes 8°S and 18°S and longitudes 22°E and 34°E. The country is landlocked and has borders with Angola, Botswana, Democratic Republic of the Congo, Malawi, Mozambique, Namibia, Tanzania, and Zimbabwe. It occupies an area of 75 million hectares or about 752 610 km^2. The land constitutes a series of plateaux that undulate between 900 and 1600 metres above sea level.

Zambia has four seasons; rainy (December to February), post rainy (March to May) cold and dry (June to August) and hot and dry (September to November). The average temperature ranges between 10°C to 25°C during the cold dry season and 18°C to 33°C during the hot dry and the rainy seasons. October is the hottest month and June is the coldest.

The study was done at Turn Pike abattoir, 60 km south of Lusaka. The abattoir slaughters animals from Gwembe and Siavonga districts along the Zambezi river basin, and Kafue, Mazabuka, Monze and Namwala districts along the Kafue river basin. The animals reared in these areas are predominantly traditional cattle, the local Tonga breed and some mixed breeds. Unlike commercial farmers, traditional farmers in these areas rarely practice any form of worm control in their livestock.

3.2 Study design

The study involved weekly visits to Turn Pike abattoir from June 2005 to July 2006. On each visit more than ten cattle were slaughtered and the first ten animals presented for slaughter were numbered for identification and examination. Their origin and sex were recorded. Faecal samples were collected from the rectum of each of the first ten animals. After slaughter, thorough meat inspection was conducted on each carcass with much emphasis on the liver and the mesenterium. The carcasses were examined for fasciolosis (presence of lesions and/or liver flukes in the bile ducts) or schistosomosis (presence of worms in the mesenteric veins). Cattle that were positive for either fasciolosis or schistosomosis were sampled. If more than three animals were positive per visit, random selection of three cattle from the positive animals was conducted for total worm counts and faecal egg counts. The visceral organs (i.e. from esophagus/trachea to the rectum) from the three selected animals were purchased. The visceral organs and faecal samples from these selected animals were clearly labelled, recorded and transported to the University of Zambia School of Veterinary Medicine parasitology laboratory for further examination and analysis.

3.3 Sample collection

3.3.1 Faecal samples

Faecal samples were collected from the rectums of the first ten animals presented for slaughter. Appropriately labelled pregnancy diagnosis gloves were used for faecal sample collection. The samples were transported to the laboratory in a cooler box with ice packs to arrest hatching before coproscopic examination. Consideration was

made for the faecal samples to be analyzed not more than three hours after collection.

3.3.2 Abattoir inspection and worm counts

The intestines and fore-stomach from the selected animals were removed from the abdominal cavity as one structure. The small and large intestines were then separated and placed on the table for *Schistosoma* worm examination and worm counts in the abattoir. The rumens were opened and washed lightly to expose the amphistomes attached to the mucosa of the rumen. The rumen samples were then packed in appropriately labelled refuse bags for *Amphistomum* recovery and counting. The trachea, lungs, diaphragm, kidneys, spleen and liver were removed from the thoracic and abdominal cavities as the other structure and placed on a table in the abattoir. Then the liver was separated and the gall bladder was removed from the liver. Visual examination and palpation of the liver was preceded with a sharp incision on its surface and through the major bile ducts into the parenchyma. The exposed bile ducts were squeezed and examined for the presence of liver flukes. The selected *Fasciola* infected livers were placed in appropriately labelled refuse plastic bags and taken to the laboratory for dissection and *Fasciola* worm counts. The lungs, kidneys and spleen were also separated and transported to the laboratory for thorough examination and sampling in the School of Veterinary Medicine parasitology laboratory.

3.3.3 Sample preparation for digestion

The intestines were washed with tap water and the intestinal contents were flushed out. The entire length of the intestines was straightened to facilitate separation of the mesentery, which was later weighed. A representative sample of 5% of the mesenterium was collected from various portions along its entire length. The liver, lungs, spleen and kidneys were weighed and 5% of tissue was sampled from various portions of each organ according to the technique of De Bont *et al.* (2002). The abomasum was spread and the mucosa from half of the abomasum was scrapped off using a spatula and collected. The samples were individually collected in 1L digestion pots for subsequent digestion in potassium hydroxide (KOH).

3.3.4 Histopathology samples

The liver and lung sections showing pathological lesions like nodules/masses, abscesses, calcification and hardening were sampled. These portions were fixed in 10% formalin for at least one week in preparation for histopathological examination. The formalized samples were trimmed to less than 3 mm thickness suitable for the size of the glass slide as well as the cover glass.

3.4 Coproscopic examination

3.4.1 *Fasciola* and *Amphistomum* eggs per gram of faeces

A sieving and sedimentation technique for the detection and quantification of *Fasciola* and *Amphistomum* eggs in faeces as described by Taira *et al* (1983) was

used, with minor modifications. Number of eggs was recorded as eggs per gram of feces (EPG). Briefly, this technique, which compromises three steps (pre-treatment, selective sieving and sedimentation), includes the use of beads 590-710 μm in diameter and 2.5 specific gravity. Centrifuge tubes with rounded bottoms of 60 mℓ capacity, 30 mm inside diameter, and 100 mm height were used. The pretreatment stage involved placing 1g of faeces into centrifuge tube A before mixing it with tap water. The suspension was transferred to tube B containing a layer of glass beads (about 1/5 of the tube volume), through a size 60 μm mesh. Tube B was filled with the water that had been used to rinse tube A. The suspension in tube B was allowed to stand for 5 minutes to allow for sedimentation. The selective sieving stage involved placing tube B into rack of an electric rotator and rotating it five times at a velocity of about 10 seconds per rotation. All the supernatant containing debris on the beads layer was siphoned off using a pipette and discarded. The beads were then agitated with a 'jet' of tap water. As the beads settled, the suspension containing the eggs was transferred to tube A and allowed to sediment for 5 minutes. After sedimentation was complete, the resultant supernatant was siphoned off leaving 2 mℓ of the sediment. The sediment was then stained with 0.2% aqueous methylene blue on an EPG trough and examined under a compound microscope using 100 times magnification. The *Fasciola* and *Amphistomum* eggs were then counted and recorded. Although both eggs of *Fasciola* and *Amphistomum* are oval and operculated, *Fasciola* eggs are golden brown with fine granules while amphistome eggs are large, grayish and contain large granules (Soulsby, 1982).

3.4.2 *Schistosoma* eggs per gram of faeces

The *Schistosoma* faecal egg counts were determined using a modification (De Bont *et al.*, 1995b) of the concentration technique of Lawrence (1970). Two grams of the faecal sample were thoroughly mixed in a beaker with 50 mℓ of 10% formalin and left to stand for 30 minutes at room temperature. This mixture was then washed through Endicott sieves of 212 and 38 μm apertures. The material on the 38 μm sieve was collected in a graduated test tube and centrifuged at 1300 rpm for five minutes. The supernatant was discarded and the residue was topped up with 2% methylcellulose added to the 4 mℓ mark. On two separate occasions, schistosome eggs were counted in 0.1 mℓ of the thoroughly mixed suspension on glass sides under the microscope using 100 times magnification. Each egg counted represented 10 eggs per gram (De Bont *et al.*, 1995b).

3.4.3 *Schistosoma* faecal miracidia counts

Quantitative miracidial counts were done according to De Bont *et al.* (1991), on faecal samples from all the animals in the sample group. The technique involved dissolving 20 g of faecal sample from each animal in 100 mℓ of 0.85% normal saline to prevent hatching of the trematode eggs. This mixture was passed through a tea strainer to remove course sediments. The supernatant was then passed through two laboratory test sieves of 212 and 38 μm respectively.

The sediment from the 212 μm sieve was discarded while that from the 38 μm sieve was further examined. The sediments containing trapped schistosome eggs were placed in a 500 mℓ sedimentation beaker to which 500 mℓ normal saline was added

and then allowed to stand for 30 minutes. The supernatant was decanted and the beaker was again filled with normal saline and allowed to stand for another 30 minutes, this process was repeated for a third time. After decanting, the beaker was then refilled with tap water and the contents were transferred to Erlenmeyer flasks, which were then insulated, from light by covering them with aluminium foil (Fig. 3.1). The miracidia collecting side tubes (pipes) of the flasks were filled with tap water to allow miracidia to collect at the tip of the tubes. The flasks were placed directly under a light source (bulb) with the un-insulated tip of the side tube facing the light source.

This technique allowed the schistosome eggs to hatch in the dark in water and since miracidia got attracted towards the light, they congregated at the tip of the tube where there was light. The apparatus was left to stand for 2 hours before 1 mℓ of the water was drawn with a pastuer pipette from the tip of the side tube. The collected water was then placed on miracidial counting pots and examined for the presence of miracidia using a stereomicroscope. This procedure was repeated every 30 minutes until no more miracidia were detected. The total number of miracidia was counted.

Figure 3.1 Erlenmeyer flask apparatus covered in foil so that *Schistosoma* eggs hatch into miracidia in the dark.

3.5 Trematode worm counts

3.5.1 *Schistosoma* worm counts

The mesenterium was spread out for the examination of the mesenteric veins. A small portion of the intestines was held with both hands and spread to enable thorough examination of the veins for schistosomes (Fig. 3.2). The intestines were held against the source of light for a clear view especially in fat animals where the veins were covered with fat. The visible *Schistosoma* worms were counted in-situ in the abattoir and the total worm count recorded.

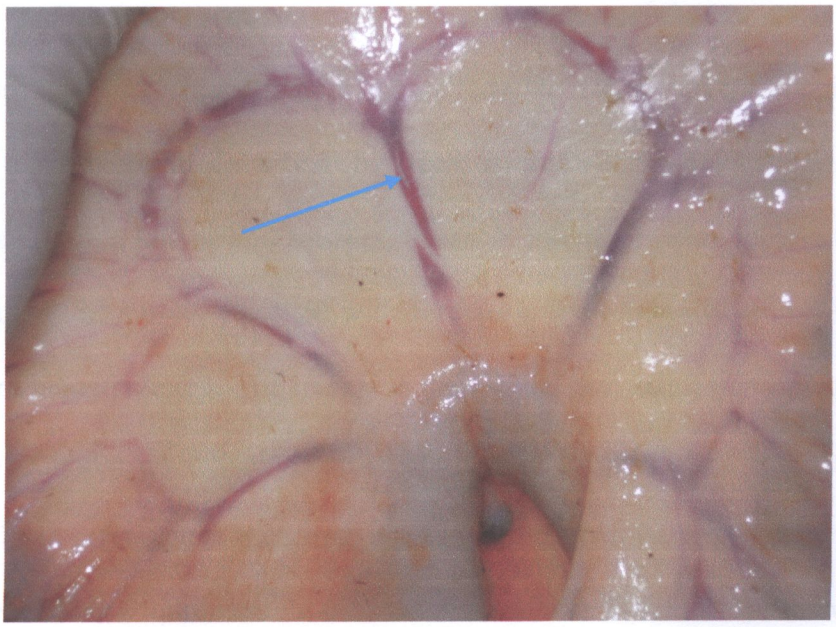

Figure 3.2 Examination of mesenterium in the abattoir. The mesenterium is spread out for thorough examination for the presence of *Schistosoma* worms (arrow heads) in the veins.

3.5.2 *Amphistomum* worm counts

The amphistomes were picked from the rumen using tissue forceps. In heavy *Amphistomum* burden, the rumens were divided into two equal parts. The flukes were picked and counted from one half, and the total count was doubled. However, where the amphistomes were not evenly distributed or in light infections, the flukes were counted without dividing the rumen. After counting, the flukes were stored in 70% ethanol.

3.5.3 *Fasciola* worm counts

To determine the fluke burden, liver dissections were done on a chopping board according to a method described by Hansen and Perry (1994), with minor modifications. Using knives and scissors, the main bile ducts were incised to expose the liver flukes, which were picked with forceps and temporarily stored in normal saline before being counted. The liver was then sliced into small portions of about 3 by 2 cm to examine the hepatic parenchyma and to locate flukes in the small bile ducts. The liver pieces were then immersed in a bucket filled with warm water and allowed to stand for 30 minutes. In order to extract liver flukes, the slices were then individually removed from the water and simultaneously squeezed over the bucket so as to collect any small flukes or broken fluke pieces back in the water. The water containing the flukes that were extracted from these tissues was passed through a sieve (tea strainer) to collect the trapped flukes. Only the anterior ends of flukes whose bodies were not intact after processing the livers were counted. After counting, all the flukes were preserved in 70% ethanol.

3.6 Tissue digestions for *Schistosoma* and *Fasciola* egg counts

The liver, lung, abomasum, mesenterium, kidney and spleen were separately digested in appropriately labelled 1L digestion pots and examined under the microscope to detect and count *Schistosoma* and *Fasciola* eggs trapped in the tissues using the digestion technique by De Bont *et al.* (2002), with minor modifications. The organ samples were individually chopped into small pieces with a pair of scissors and then homogenized using a tissue grinder (Comfort, Philips). The tissue grinder was thoroughly washed with running tap water after each sample to avoid

contaminations. The homogenates from each organ were separately digested in 800 mℓ of 5 % Potassium Hydroxide (KOH) for 12 hrs at 37 °C. Each digestion fluid was then thoroughly mixed with 50 mls of 40 % formalin to terminate the digestion process. The digested and homogenized samples were washed with tap water through laboratory test sieves of 212 and 38 μm apertures to collect the *Fasciola* and *Schistosoma* eggs. As a significant number of tissue eggs were found sticking to undigested fat particles remaining in the sieves, the fat particles were therefore dissolved after a first passage in a concentrated soap solution heated at 60 °C before passing them again through the same set of sieves. The solution was collected in well-labelled beakers that were filled with tap water and allowed to stand for 30 minutes. After decanting the supernatant, the sediment was transferred to graduated test tubes, which were filled with tap water and allowed to stand for another 30 minutes before centrifuging at 1300 rpm for five minutes. The supernatant was discarded and the residue topped up with a 2% methylcellulose solution added to the 4 mℓ mark. The viscosity of the latter solution allowed the formation of a uniform suspension of the fluke eggs. Four drops of 0.1 mℓ of the thoroughly mixed suspension were separately placed on a glass slide and coved with a cover slip. *Schistosoma* and *Fasciola* eggs were then counted under a microscope (100 times magnification). Each *Schistosoma* egg counted in the digestion fluids of the mesenterium, abomasum and liver represented 200 tissue eggs for the said organ (De Bont *et al.*, 2002).

3.7 Histopathological examination

The formalin fixed liver and lung sections were processed and stained with Haematoxylin & Eosin (H & E) and Periodic Acid Schiff (PAS) stains for histopathological examination as described by Bancroft and Stevens (1982) with minor modifications. Tissues were first washed with running water to remove the formalin. Dehydration was accomplished by treating tissues in ascending grades of alcohol in an automatic tissue processor. Clearing of liver tissues was done after treatment with xylene. In order to make tissues firm enough for cutting and embedding, they were passed through three changes of molten wax and attached to the embedding mould. The moulds were then put in the refrigerator at 4°C for thirty minutes. Embedded tissues were blocked out after cutting around each tissue. Tissues were cut into sections using a microtome set at 3 microns thickness dial gauge. Each section was transferred by floating it in two beakers of warm and cool distilled water before mounting it on to a slide. H & E and PAS stains were used for staining the prepared sections. The stained sections were then mounted with Eukitt mountant and examined under a microscope for lesions produced by eggs in tissues.

3.8 Statistical analysis

Recovered *Fasciola*, *Schistosoma* and *Amphistomum* flukes were counted and their frequencies calculated using the Statistical Package for Social Scientists (SPSS® Version 11.0; Chicago, Illionois, USA). Faecal egg counts and tissue egg counts from the sampled animals were recorded. Data were transformed to logarithms (count +1) to stabilize variances and analysed with Microsoft Excel and SPSS. Pearson's correlation coefficient and linear regression were used to determine and

describe the associations among *Fasciola*, *Schistosoma* and *Amphistomum* worm counts. Pearson's correlation coefficient and linear regression were performed to determine and describe the relationships between EPG and fluke burden as well as the relationships of tissue egg counts with fluke burden and EPG. A significant relationship was denoted by a P value of 0.05.

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4. Results

4.1 Coproscopic studies

A total of 315 cattle were examined from June 2005 to July 2006 at Turn Pike abattoir. Out of the number examined, 133 cattle were positive for trematode infections. From the 133 infected cattle, 50 were randomly selected for faecal egg counts. On coproscopic diagnosis, 58% of the animals were positive for fasciolosis, 22% were positive for schistosomosis while 76% were positive for amphistome eggs. Eggs for *Fasciola*, *Schistosoma* and *Amphistomum* were recorded as eggs per gram of faeces (EPG). The intensity of infections using faecal egg count varied among *Fasciola*, *Amphistomum* and *Schistosoma* infections (Table 4.1). Faecal egg counts also varied in individual trematode infections.

Table 4.1: Summary of *Fasciola*, *Schistosoma* and *Amphistomum* faecal egg counts (n = 50).

	Fasciola	***Schistosoma***	***Amphistomum***
EPG range	1-95	10-90	1-183
Mean	8.02	7.0	17.7
S.D	15.8	17.17	31.72

4.1.2 Correlation between worm burden and EPG

There was evidence of an association between *Fasciola* worm and *Fasciola* EPG counts (Fig. 4.1). The positive slope of the regression line in the scatter graph

indicates that an increase in the *Fasciola* worm count was associated with an increase in the *Fasciola* faecal egg count.

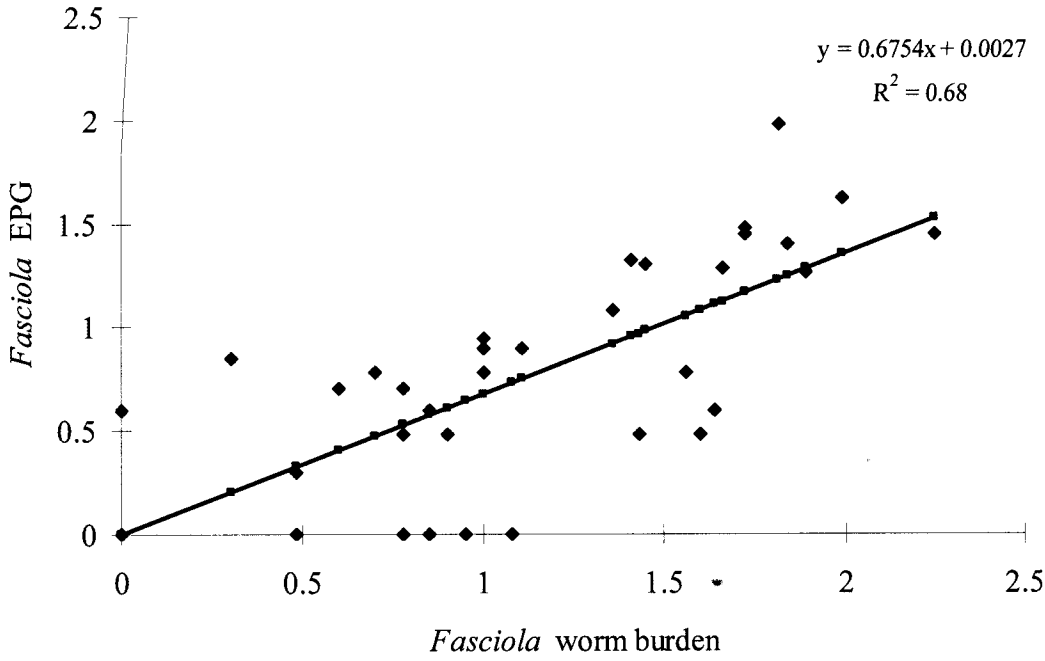

Figure 4.1: Log transformed *Fasciola* worm burden and EPG counts, showing the fitted linear regression line, linear regression equation and R^2 value.

Table 4.2 shows that a significant positive relationship ($r = 0.83$, $p < 0.001$) between *Fasciola* worm count and EPG was found. The high correlation coefficient ($r = 0.83$) and measure of goodness of fit of linear regression ($R^2 = 0.68$) suggests that the tightness of the linear relationship between *Fasciola* worm burden and EPG was strong.

Table 4.2: Summary of Correlation (r) and Regression (R^2) analysis for log transformed *Fasciola*, *Schistosoma* and *Amphistomum* worm burdens and EPG values.

	r	R^2	p value
Fasciola: EPG	0.83	0.68	<0.001
Schistosoma: EPG	0.64	0.41	<0.001
Amphistomum: EPG	0.60	0.36	<0.001

Similarly, there was a close association between *Amphistomum* worm count and *Amphistomum* EPG. The scatter graph indicates that an increase in the *Amphistomum* worm burden was associated with an increase in the *Amphistomum* EPG counts (Fig. 4.2).

A similar significant positive relationship ($r = 0.60$, $p < 0.001$) was obtained between *Amphistomum* worm burden and EPG (Table 4.2). The measure of goodness of fit of linear regression ($R^2 = 0.36$) was however, much lower.

Similarly, there was a significant positive relationship ($r = 0.64$, $p < 0.001$) between *Schistosoma* worm burden and EPG although the linear regression ($R^2 = 0.41$) value was lower.

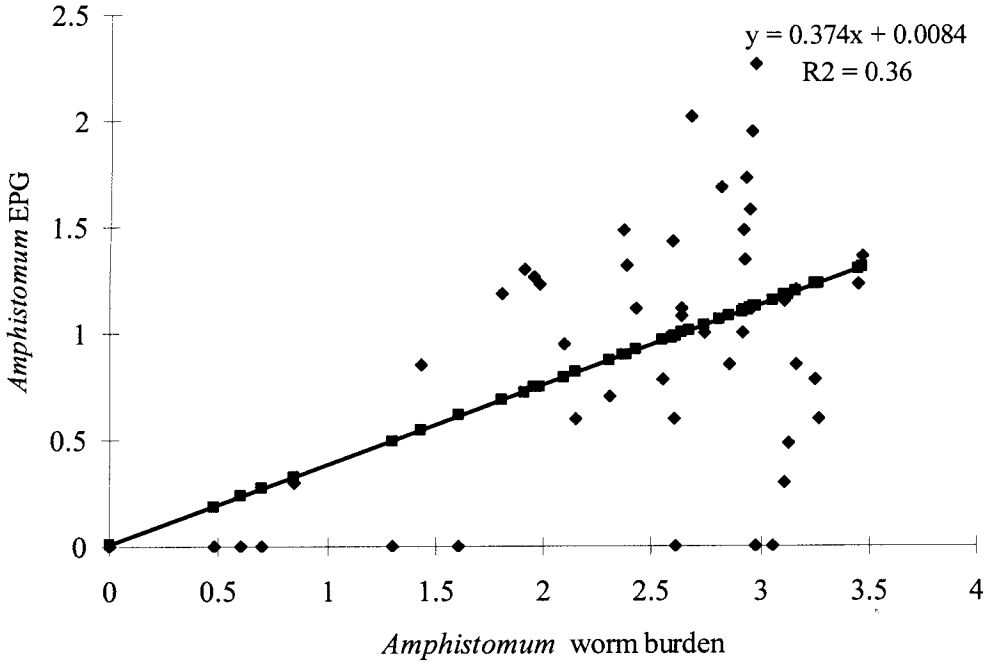

Figure 4.2: Scatter graph for log transformed *Amphistomum* worm burden and EPG indicating the fitted regression line, regression equation and R^2 value.

4.1.2 *Schistosoma* faecal miracidial counts

The *Schistosoma* faecal miracidial count was significantly lower than the *Schistosoma* worm burden ($\chi^2 = 97.97$, $p < 0.001$). As seen in Table 4.3, *Schistosoma* miracidial count had significant positive relationships with *Schistosoma* worm counts and EPG.

Table 4:3: Summary of Correlation (r) and Regression (R^2) analysis for log transformed *Schistosoma* miracidial count with *Schistosoma* worm and EPG counts.

	r	R^2	p value
Miracidia: worm count	0.549	0.301	< 0.001
Miracidia: EPG	0.505	0.255	< 0.001

4.2 Worm burdens of co-joint *Fasciola*, *Schistosoma* and *Amphistomum* infections

Out of the 50 cattle sampled, 68% were positive for *Fasciola* while 56% for schistosomosis and 92% were positive for paramphistomes (Table 4.4).

Table 4.4: Summary of *Fasciola*, *Schistosoma* and *Amphistomum* worm burdens in the 50 cattle selected for sampling at Turn Pike abattoir.

	Fasciola	***Schistosoma***	***Paramphistomum***
Range	1-176	1-168	2-2974
Mean	19.46	33.68	622.08
S.D	32.36	52.58	692.03

Heavy worm burdens characterized *Paramphistomum* infections with 48% of the animals harbouring more than 500 paramphistomes in the rumen. Figure 4.3 shows

the extent of *Paramphistomum* worm burden observed in the rumen of one of the sampled animals during the survey.

Figure 4.3: Rumen of one of the sampled animals showing the extent of *Paramphistomum* infection in cattle. Paramphistomes (arrow heads) were found attached to the mucosa of the rumen and were not evenly distributed.

In contrast, low worm burdens characterized *Schistosoma* infections with 68% of the *Schistosoma* infected animals (n = 28) harbouring less than 100 worms in the mesenteric blood vessels. Despite the higher prevalence of fasciolosis as compared to schistosomosis, there was no significant difference (χ^2 =575.34, p =0.923) between the *Fasciola* and *Schistosoma* worm burdens in the sampled cattle.

The occurrence of mixed *Fasciola*, *Schistosoma* and *Paramphistomum* infections was a common finding in the sampled animals. Out of the total number of sampled animals (n = 50), 32% harboured all the three worms. Dual trematode infections were also common. *Fasciola* and *Amphistomum* co-joint infections were the highest (66%) while *Fasciola* and *Schistosoma* co-joint infections were the lowest (32%). *Schistosoma* and *Amphistomum* co-joint infections were represented by 52% (n = 50). Only 6% (n = 50) of the sampled animals had *Paramphistomum* infections, 36% (n = 50) had *Fasciola* infections and 24% (n = 50) had *Schistosoma* infections only. Two animals presented with typical *Fasciola* lesions but had no live flukes in the liver.

The negative slope of the regression line in the scatter graph of log transformed *Fasciola* and *Schistosoma* worm burdens shows that an increase in the *Fasciola* worm burden in cattle was associated with a decrease in the *Schistosoma* worm burden (Fig. 4.4).

Correlation analysis was done using worm burdens of *Fasciola* and *Schistosoma* to determine the relationship between the two trematode infections in cattle. Although the correlation coefficient (r = -0.12) obtained indicated a negative association between *Fasciola* and *Schistosoma* worm burdens, the relationship between the two trematodes was not significant (p = 0.390).

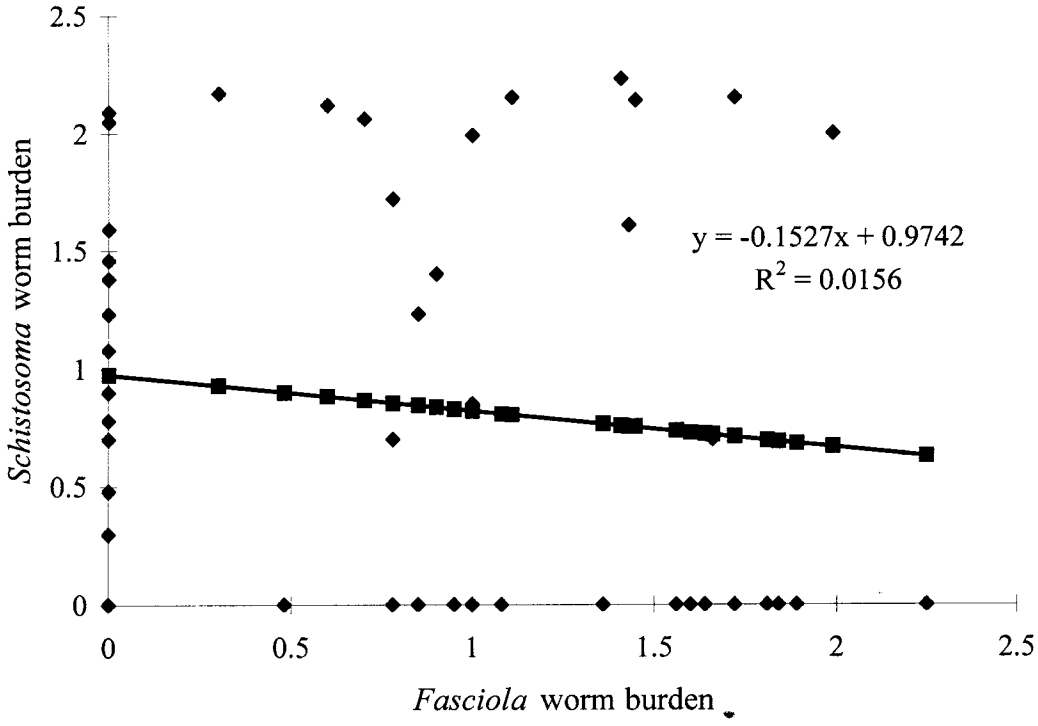

Figure 4.4: Scatter graph of log transformed *Fasciola* and *Schistosoma* worm burdens in cattle showing a negative best-fit line of linear regression.

The scatter graph illustrating the *Fasciola* and *Amphistomum* co-joint infection shows a positive association between the two trematodes (Fig. 4.5).

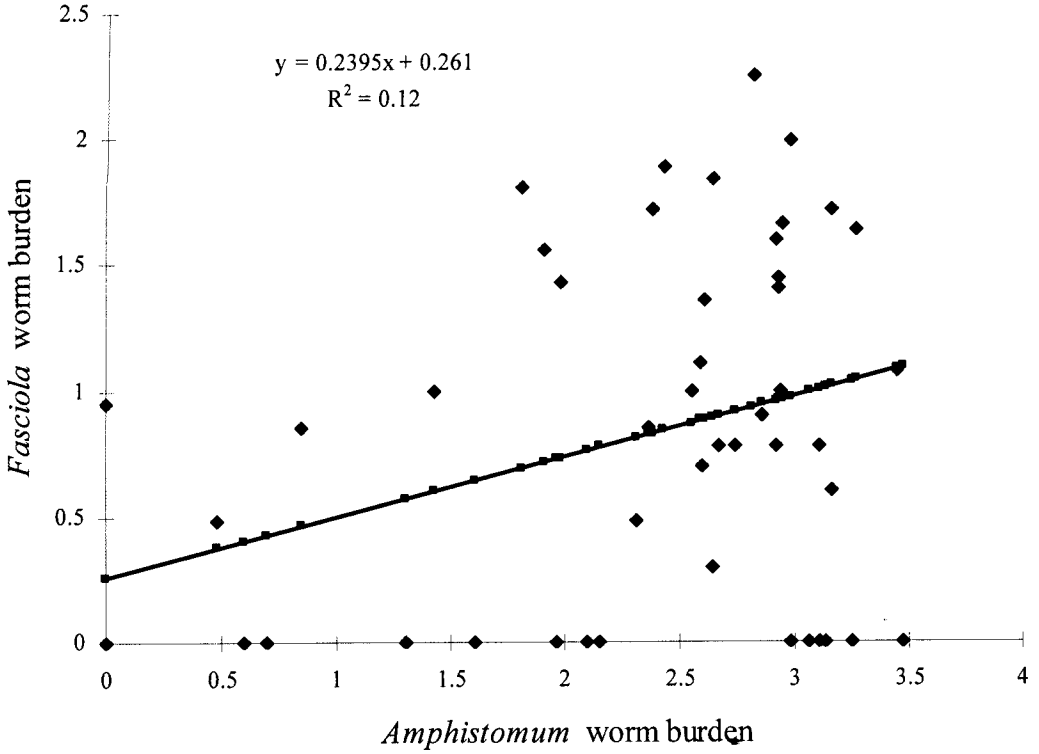

Figure 4.5: Scatter graph of log transformed *Fasciola* and *Amphistomum* worm burdens, showing the best-fit line, regression equation and R^2.

On correlation analysis, a positive relationship ($r = 0.35$, $p = 0.014$) between *Fasciola* and *Amphistomum* worm counts was found. A positive association ($r = 0.560$, $p < 0.001$) was also obtained between *Fasciola* and *Amphistomum* faecal egg counts. An increase in *Fasciola* faecal egg counts was observed to be associated with an increase in *Amphistomum* faecal egg count. Additionally, a positive association ($r = 0.593$, $p < 0.001$) was obtained between *Fasciola* worm burden and *Amphistomum* faecal egg counts.

Although *Schistosoma* and *Amphistomum* co-joint infections were common, there was no significant relationship between *Amphistomum* and *Schistosoma* worm burdens. As shown in Fig. 4.6, the points in the graph were irregularly scattered and the best-fit line did not show a relationship between the two trematodes.

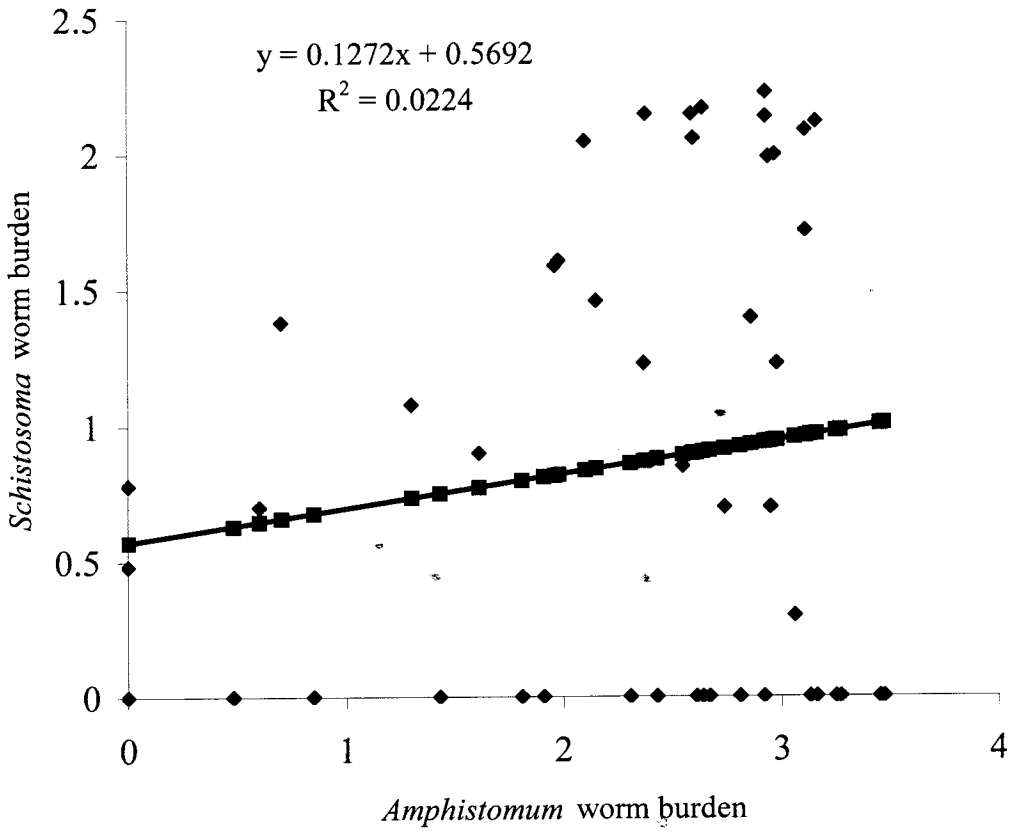

Figure 4.6: Scatter graph of log transformed *Amphistomum* and *Schistosoma* worm burdens in cattle, showing the best-fit line, regression equation and the R^2 value.

Correlation analysis revealed that there was no significant relationship ($r = 0.15$, $p = 0.302$) between *Schistosoma* and *Amphistomum* worm burdens.

4.3 *Schistosoma* and *Fasciola* tissue egg counts

4.3.1 *Schistosoma* tissue egg counts (TEC)

Schistosoma eggs were detected in the liver, abomasum and mesenterium after digestion of representative samples of these tissues. *Schistosoma* eggs in tissues were spindle-shaped and had terminal spines (Fig. 4.7).

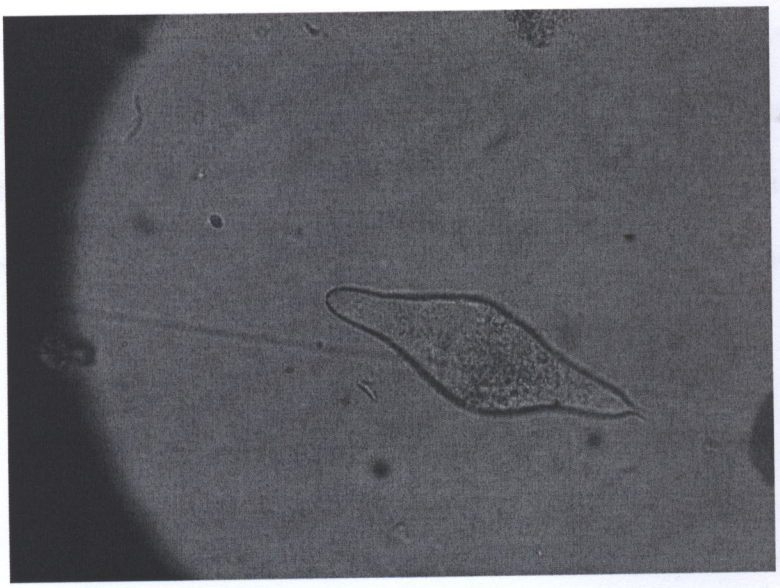

Figure 4.7: Spindle-shaped *Schistosoma* egg in the liver tissue with a terminal spine after digestion of the tissue in KOH.

The liver had a significant ($\chi^2 = 186.94$, $p < 0.001$) higher egg count than the other organs. For example one animal had 513,800 eggs deposited in the liver (Table 4.5).

Table 4.5: Summary of *Schistosoma* Tissue Egg Counts (TEC) in the 50 sampled animals

	Liver	Abomasum	Mesenterium
Range	800 - 513800	200 - 8600	800 - 5000
Mean	16900.00	272.00	136.00
S.E.M	10339.1	188.1	102.5

4.3.1.1 Association between *Schistosoma* TEC and worm burden

A significant relationship ($r = 0.83$, $p < 0.001$) between *Schistosoma* worm burden and liver egg count was obtained. The measure of goodness of fit of linear regression was also high ($R^2 = 0.69$). There was also a significant positive association ($r = 0.40$, $p = 0.004$) between log transformed *Schistosoma* worm burden and the abomasum egg counts. However, there was no correlation between worm burden and *Schistosoma* mesenteric egg counts.

4.3.1.2 Associations among *Schistosoma* TEC, EPG and miracidia counts

Schistosoma tissue egg counts in the liver and abomasum had significant relationships with EPG and miracidial counts (Table 4.6). However, there was no correlation ($r = -0.12$, $p = 0.420$) between EPG and mesenterium egg counts.

Table 4.6: Summary of correlation (r) and regression (R^2) analysis for log transformed *Schistosoma* EPG and Tissue Egg Counts.

	r	R^2	p value
Liver eggs: EPG	0.68	0.46	< 0.001
Abomasum eggs: EPG	0.48	0.23	< 0.001
Liver eggs: Miracidia	0.42	0.17	0.003
Abomasum: Miracidia	0.38	0.14	0.007

4.3.2 *Fasciola* tissue egg counts

Fasciola tissue eggs were detected in the liver, lungs, mesenterium, kidney and spleen after digestion of the tissues in KOH. *Fasciola* eggs in tissues appeared oval, golden-yellow, operculated and measured 193 by 104 μm (Fig. 4.8).

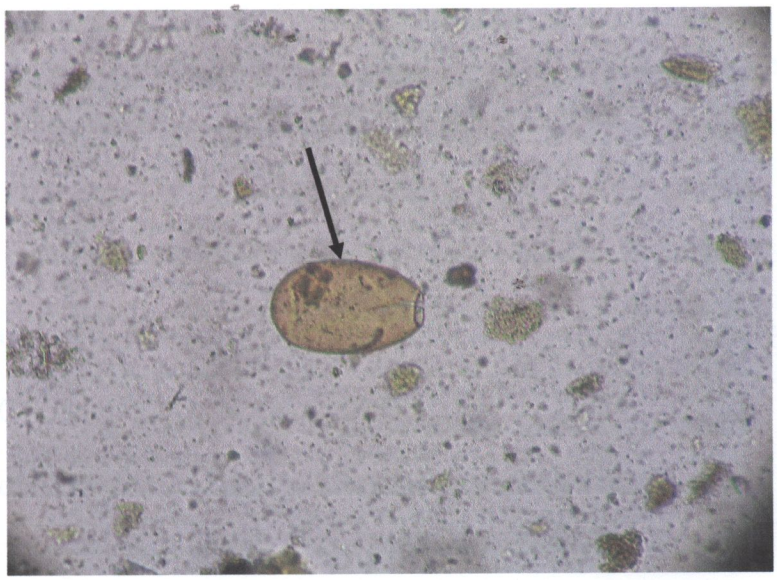

Figure 4.8: Golden-yellow, oval and operculated *Fasciola gigantica* egg (arrow head) in the lung tissue.

The distribution of *Fasciola* eggs in tissues was uneven. *Fasciola* egg counts among the tissues ranged from 23 to 1390, with the liver egg count being the highest (Fig. 4.9).

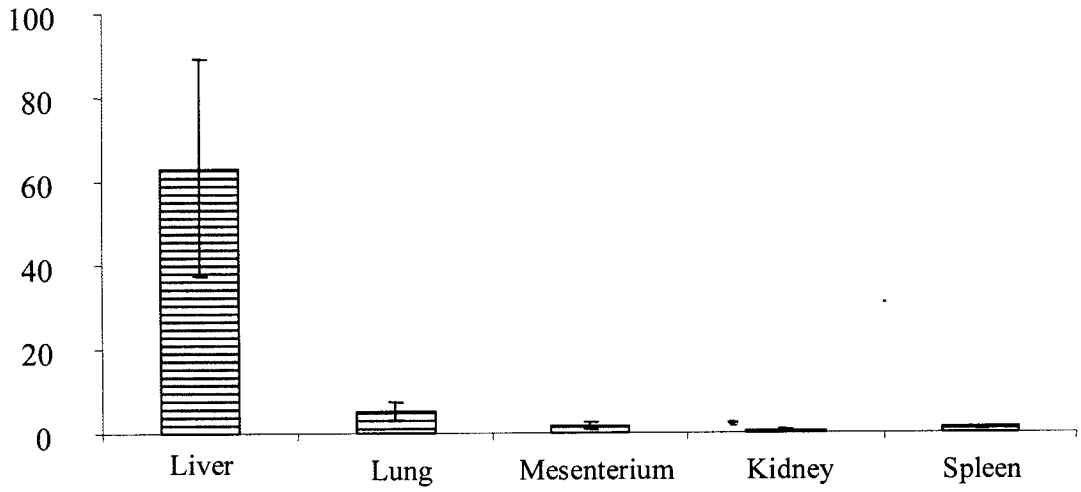

Figure 4.9: Mean tissue egg counts (TEC) (± SEM) values of *Fasciola* in the liver, lung, mesenterium, kidney and spleen.

The liver egg count was significantly higher ($\chi^2 = 136.43$, $p < 0.001$) than the lung egg count. The *Fasciola* tissue egg count in the liver was also significantly ($\chi^2 = 146.54$, $p = 0.001$) higher than *Fasciola* EPG.

The spleen and the kidney had the lowest mean tissue egg counts.

4.3.2.1 Association of *Fasciola* TEC and worm burden

Significant positive relationships were obtained between log transformed *Fasciola* tissue egg counts and log transformed *Fasciola* worm burden (Table 4.7). There were variations in the tightness of the relationships between *Fasciola* worm count and the various tissues with the R^2 value ranging from 0.39 to 0.74.

Table 4:7: Summary of Correlation and Regression analysis for log transformed *Fasciola* worm counts and log transformed *Fasciola* Tissue Egg Counts (TEC).

	r	R^2	p value
Worm counts: Liver eggs	0.86	0.74	< 0.001
Worm counts: Lung eggs	0.77	0.59	< 0.001
Worm counts: Mesenterium eggs	0.63	0.39	< 0.001
Worm counts: Kidney eggs	0.65	0.43	< 0.001
Worm counts: Spleen eggs	0.64	0.41	< 0.001

Fasciola liver egg count and worm burden had a strong positive relationship as shown in Fig. 4.10. The positive slope of the regression line indicates that *Fasciola* worm burden was associated with an increase in liver egg count.

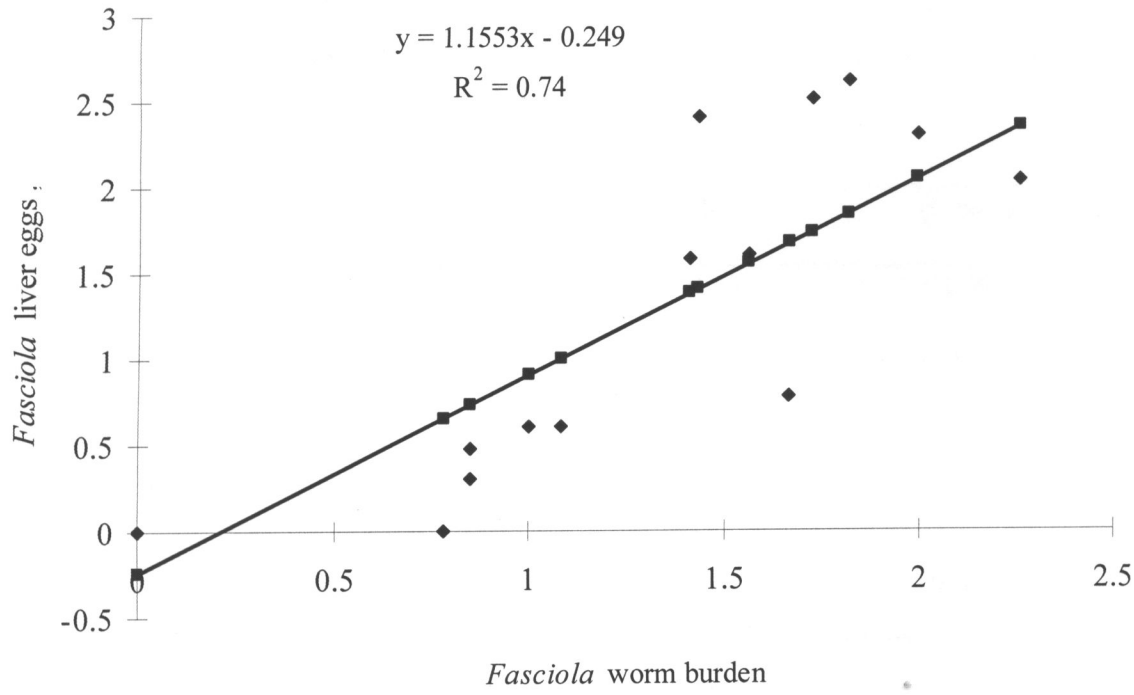

Figure 4.10: Scatter graph of log transformed *Fasciola* liver egg count and worm burden showing best-fit line, regression equation and R^2 value.

The association between log transformed *Fasciola* worm burden and lung tissue egg counts had a similar pattern as that of liver egg count and worm burden.

4.3.2.2 Association between *Fasciola* TEC and EPG

Tissue egg counts in the liver, lung, mesenterium, kidney and spleen had positive relationships with *Fasciola* EPG (Table 4.8). The relationships had correlation coefficients (r) ranging from 0.52 to 0.79.

Table 4.8: Summary of Correlation and Regression analysis for log-transformed log transformed *Fasciola* Tissue Egg Counts (TEC) and *Fasciola* EPG.

	r	R^2	p value
Liver eggs: EPG	0.79	0.63	< 0.001
Lung eggs: EPG	0.68	0.46	< 0.001
Mesenterium eggs: EPG	0.66	0.44	0.001
Kidney eggs: EPG	0.55	0.31	0.008
Spleen eggs: EPG	0.63	0.40	0.002

4.3 Histopathology

On PAS stain of the lung tissue, there was no evidence of trematodes or their eggs in the tissue. The main lesions observed on H & E stain were an infiltration of moderately degenerated neutrophils and fibrin in alveoli and bronchioles. In addition to focal alveolar haemorrhage, there was also congestion and focal necrosis of the interalveolar septa. Multifocal thickening of interalveolar septa with oedema and moderate infiltration of lymphocytes, monocytes and neutrophils were observed. Focally, there were several microcolonies of rod-shaped bacteria (1μm). Focal necrosis of bronchiolar epithelium as well as oedema of interlobular septa was also observed.

In the *F. gigantica* affected livers, the main lesions observed were chronic cholangiohepatitis and thickening of the walls of bile ducts that were infiltrated with lymphocytes and plasma cells with diffuse fibrosis around them. Haemorrhagic tracts

filled with blood, fibrin and cellular debris surrounded by coagulative necrosis were observed (Figure 4.11). In other liver sections, white migratory tracts composed of abundant infiltrate of eosinophils, numerous macrophages with hemosiderin pigment, lymphocytes as well as proliferation of fibrous connective tissue were also observed. The liver parenchyma was surrounded by neutrophilic reaction with adjacent hepatocytes showing degenerative changes. In some places, hepatocytes were completely replaced by fibroblasts and connective tissue, while in other places; they revealed various stages of cellular degeneration.

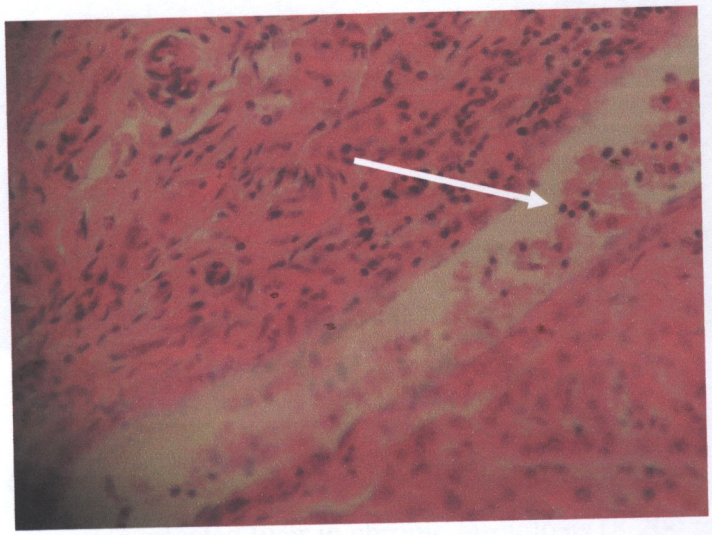

Figure 4.11: Picture showing haemorrhagic tracts filled with blood and cells (arrow) in the liver parenchyma due to acute fasciolosis (× 100 magnification).

Degenerating flukes and fluke eggs attracted foreign body giant cells, macrophages, lymphocytes and fibrous tissue thereby forming various sized nodules in the hepatic

parenchyma (Figures 4.12). Calcium deposits were also seen as brownish-black patches in some liver sections.

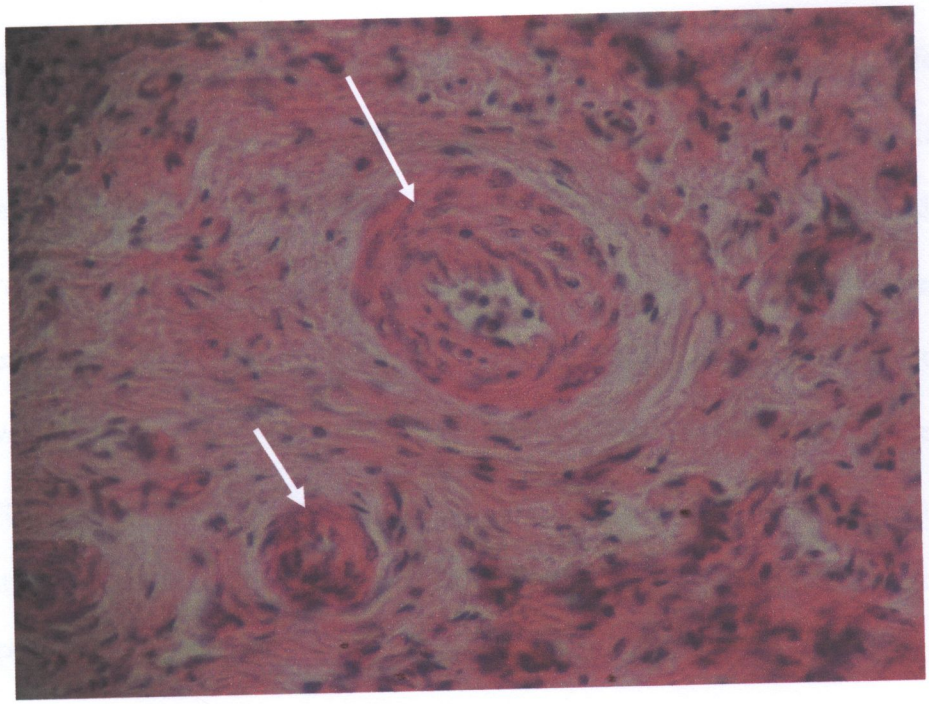

Figure 4.12: Granuloma formation in the hepatic parenchyma of a *Fasciola gigantica* infected liver in chronic fasciolosis (× 200 magnification).

CHAPETR FIVE

5. Discussion and Conclusions

5.1 Co-joint trematode infections

The results obtained in this study reveal the presence of mixed *Fasciola*, *Schistosoma* and *Amphistomum* infections in cattle. The presence of these infections at meat inspection and the detection of their eggs after coproscopic examination indicate that the three trematodes are endemic in areas where the examined cattle came from. It was found that 32% of these cattle (n = 50) harboured mixed *Fasciola*, *Schistosoma* and *Amphistomum* infections. These findings confirm earlier reports by Chingwena *et al.* (2002) who attributed their findings to the fact that the snail intermediate hosts for the three trematodes tend to occur in the same environment and that trematode infections do not often occur in isolation. Recent studies carried out by Phiri (2005) in Zambia recorded the presence of *Lymnaea natalensis* (the snail intermediate host for *Fasciola gigantica*) and *Bulinus* spp. (the snail intermediate host for *Schistosoma* and *Amphistomum*) in the same habitat.

In this study we found that 66% of the sampled animals (n =50) had both *Fasciola* and *Amphistomum* infections. This is in agreement with previous observations where concomitant *Fasciola gigantica* and *Amphistomum* infections were reported in cattle in Tanzania (Keyyu *et al.*, 2006) and in Zambia (Phiri *et al.*, 2006a), demonstrating that the interactive infection between the two trematodes was mutually inclusive ($p = 0.014$). In France, Szmidt-Adjide *et al.* (2000) also found a positive association between *F. hepatica* and *P. daubney* infections in cattle. These observations suggest that *Fasciola* and *Amphistomum* worm burdens do not seem to have a negative

influence on each other as they infect and develop in the same host. Although an increase in *Fasciola* worm burden was associated with an increase in *Amphistomum* worm burden, the differences in the worm burdens of the two trematodes in the host could be due to the density and uptake of their metacercariae by the host and/or the specific development of metacercariae in the definitive host (Szmidt-Adjide *et al.*, 2000). The higher worm burden of *Amphistomum* than of *Fasciola* could also be attributed to the high biological potential of the amphistome snail intermediate host (Dinnik and Dinnik, 1965), the direction of control measures mainly against *Fasciola* and the lack of effective drugs against amphistomes (Mage *et al.*, 2002).

This study demonstrated positive relationships between *Fasciola* EPG and *Amphistomum* worm burden ($p = 0.005$), and also between *Fasciola* worm burden and *Amphistomum* EPG ($p < 0.001$). The tightness of relationship between the two parasites using actual worm burdens was stronger ($R^2 = 0.120$) than the relationship reported by Phiri *et al.* (2006) using EPG values of the two parasites ($R^2 = 0.043$). This demonstrates that actual worm count is a better indicator for determining the relationship between trematodes than using faecal egg counts. It was also observed that the egg production of *Fasciola* and *Amphistomum* in co-joint infections was not significantly affected. This was demonstrated by the higher percentage of animals that were positive for fasciolosis (94.3%) and amphistomosis (82.6%) at both meat inspection and coproscopic examination coupled with the positive relationships *Fasciola* and *Amphistomum* worm burdens had with their respective egg counts ($p < 0.001$).

Inspite of the positive relationships ($p < 0.001$) between worm burdens and EPGs observed in our study, there was generally low *Fasciola* and *Amphistomum* egg

outputs. Several factors could be attributed to the low mean faecal egg output in our study. Firstly, light infections could not be easily detected by examination of only one faecal sample from each animal. Secondly, as observed by Dumenigo *et al.* (2000), intermittent excretion of *Fasciola* and *Amphistomum* eggs coupled with relatively large amounts of faeces produced per day and different sampling periods could have contributed to the difficulties in faecal egg detections. Thirdly, some infections could have been prepatent, especially that low *Amphistomum* faecal egg counts were recorded despite heavy worm burdens.

Although the economic effects of amphistomosis in cattle have not been well studied, the heterologous interaction of *Amphistomum* and *Fasciola* observed in this study and past studies in Zambia (Phiri *et al.*, 2006a) as well as elsewhere (Szmidt-Adjide *et al.*, 2000; Keyyu *et al.*, 2006) may compound the economical effects of the liver fluke to the livestock industry. This may be caused by the multiple effects induced by the pathology caused by these individual trematode populations in cattle.

In this study we found that 52% of the examined animals (n = 50) had co-joint *Schistosoma* and *Amphistomum* infections. While low levels of infection, with a mean worm count of 33.8 characterized *Schistosoma* infections, heavy *Amphistomum* infections were observed, with 48% of the infected cattle harbouring more than 500 amphistomes in the rumen. Since there was no significant relationship between the two worm burdens, it means that the concurrent infection and establishment of *Amphistomum* and *Schistosoma* infections in the host may not cause significant cross-protection between the two trematodes in cattle. This is despite reports in experimental studies where *Schistosoma indicum/spindale* and *Paramphistomum* spp. antigens have been observed to cross-react in sheep (Singh *et al.*, 2004). Earlier,

Fagbemi and Goubadia (1995) reported cross-reactivity between *Schistosoma bovis* and *Paramphistomum microbothrium* antigens in cattle. Results from the present study seem to suggest that the cross-reactivity observed between *Schistosoma* and *Amphistomum* antigens in experimental studies may not have any significant effect on the transmission and establishment of the two trematodes in co-joint infections in endemic areas. This can be attributed to the sharing of the same snail intermediate host between the two trematodes, as it is well known that *Bulinus* spp. are the intermediate hosts for both *Schistosoma* and *Amphistomum* (Chingwena *et al.*, 2002) and that concurrent *Calicophoron microbothrium* and *Schistosoma bovis* infection in *B. tropicus* can occur (Knowles and Jones, 1989). Since animals in endemic areas are potentially exposed to both parasites simultaneously, development and establishment of both *Schistosoma* and *Amphistomum* infections could proceed independently in the host animal without significant effects on each other. Consequently, cross-protection between the two trematodes resulting in an apparent effect on their epidemiology is not likely to occur in endemic areas due to the simultaneous exposure of cattle to both trematodes.

This study has revealed that only 32% of the examined animals (n = 50) animals harboured co-joint *F. gigantica* and *Schistosoma* spp. This was less than half the number of animals infected with both *Fasciola* and *Amphistomum*. There was a negative relationship observed between *Fasciola* and *Schistosoma* worm burdens in this study as indicated by the negative correlation observed between the two trematodes. Although this relationship gave an indication that *Fasciola* worm burden in cattle co-jointly infected with *Schistosoma* was compromised, or vice versa, this association was not significant ($p = 0.390$). Thus, the effect of *Schistosoma* worm

burden on the course of *Fasciola gigantica* was not apparent from the worm burdens. However, experimental studies in ruminants have indicated that primary infection with *Schistosoma* or *Fasciola* resulted in enhanced resistance to heterologous challenge. In earlier studies, Bushara *et al.* (1978) observed that a primary *Schistosoma bovis* infection induced resistance to heterologous challenge with *F. gigantica* in cattle. Similarly, Sirag *et al.* (1981) found that Jersey calves harbouring a mature primary infection of *S. bovis* showed significant resistance to challenge with *F. hepatica* on the basis of adult worm recovery and histopathology. Additionally, Monrad (1981), Rodrigues-Osorio *et al.* (1993) and Ferreras *et al.* (2000) observed that sheep harbouring a primary *F. hepatica* infection acquired resistance to heterologous challenge with *S. bovis* on the basis of adult worm recoveries. These authors thought that the protective mechanism triggered by *F. hepatica* was acting on the primary stages of *S. bovis*. Other investigations on heterologous resistance between *F. gigantica* and *S. bovis* in Sudanese Zebu cattle also recorded significant results, where a marked level of reciprocal resistance between *F. gigantica* and *S. bovis* was observed (Yagi *et al.*, 1986).

The heterologous resistance between these two trematodes has been attributed to the existence of cross-reactive antigens that results in cross-protection between *Schistosoma* and *Fasciola* infections (Hillyer, 2005). This fact was earlier established by Hillyer (1979) who discovered that cross-reactive antigens isolated from *F. hepatica* protected animals against *S. mansoni* and that some of these antigens share common epitopes with *S. bovis*. Follow up studies established that the purified, native and recombinant *F. hepatica* fatty acid binding proteins (FABP) and glutathione S-transferases (GST) antigens which have immunoprophylactic potential

against fasciolosis, also cross-protect against schistosomes (Hillyer, 2005). During vaccine trials in experimental animals, Hillyer *et al.* (1988) demonstrated the efficacy of *Fasciola* FABP antigen against *S. mansoni*. Further vaccination studies found that recombinant *S. bovis* 28-kDa glutathione S-transferase (rSb28GST) protected calves against natural *F. gigantica* infection (De Bont *et al.*, 1997) and that a FABP homologue from *S. mansoni* was also protective against *F. hepatica* (Smooker *et al.*, 2001) on the basis of adult worm recoveries.

Failure to establish a significant relationship between *Fasciola* and *Schistosoma* in this study could be attributed to duration of infection and simultaneous exposure of cattle to both trematodes in endemic areas. It has been reported that effective immunity against *Schistosoma* infection occurs at least 18 weeks post-infection (De Bont *et al.*, 2005c). A long duration primary *Schistosoma* infection is therefore required to induce cross-protection against the more pathogenic *Fasciola* infection. Our study however, was done in naturally infected cattle of various age groups in endemic areas with no known duration of infections and it was also not possible for us to determine which trematode first infected the host. It is therefore, possible that some of our study animals had established immunity against challenge infections whilst others had not. Although it is generally assumed that cattle are exposed to the *Schistosoma* cercaria first before acquiring the *Fasciola* metacercariae, which has to first encyst on herbage before becoming infective, animals in endemic areas may continuously and simultaneously be exposed to both *Fasciola* metacercaria and *Schistosoma* cercaria. If simultaneous ingestion occurs, the two trematodes may get established in the host animal simultaneously with no apparent effect on the development of the other parasite. This means that observations of heterologous

resistance in experimental animals harbouring a primary *Schistosoma* infection of known duration and later challenged with *Fasciola* may have little bearing on the epidemiology of fasciolosis and schistosomosis in endemic areas.

5.2 *Schistosoma* and *Fasciola* tissue egg counts

Our findings revealed that both *Fasciola* and *Amphistomum* had little effect on the egg production of *Schistosoma* in conjoint infections. Numerous *Schistosoma* eggs were detected in the liver, abomasum and mesenterium and it was observed that the intensity of *Schistosoma* infection was positively associated ($p < 0.001$) with an increase in tissue egg counts. Since little is known about the different factors affecting the passage of eggs through the intestinal wall, and the proportion of eggs that are destroyed during their visceral migration, deposition of *Schistosoma* eggs in the tissues could have accounted for the low faecal egg and miracidial counts observed in our study. Tissue egg counts were significantly ($p < 0.001$) higher than faecal egg counts. Failure to account for *Schistosoma* eggs deposited in tissues when determining the effect of cross-protection in co-joint trematode infections may underestimate egg production. This means that the use of *Schistosoma* EPG without considering tissue egg counts has limitations in assessing cross-protection between *Schistosoma* and *Fasciola* worm burdens as the use of EPG alone underestimates the fecundity of the *Schistosoma* female worm.

Our study detected *Fasciola* eggs in the liver, lungs, mesenterium, kidney and spleen, with as many as 414 eggs deposited in the liver in one case. Immature liver flukes may occasionally migrate to the lungs and other tissues causing abscesses (Jones and Hunt, 1983) but no eggs have been found other than in the liver

parenchyma (Phiri, 1997). Erratic parasitism has been reported in the lungs of sheep at autopsy, 14 weeks after inoculation with *F. gigantica* (Yoshihara *et al.*, 1998). The high number of *Fasciola* eggs detected in the liver and other tissues in our study could be attributed to the pathology of *F. gigantica* in endemic areas. Cattle in endemic areas remain exposed to repeated infections and as such acute and chronic lesions of fasciolosis overlap (Kumar, 1999). It is therefore, possible that a mixture of adult flukes laying eggs in the bile ducts and immature flukes creating fresh perforations in the liver parenchyma and bile ducts may result in 'leakage' of *Fasciola* eggs in the liver parenchyma. Further deposition of *Fasciola* eggs in other tissues could also be due to the feeding habits of flukes in the bile ducts where it has been observed that mature liver flukes feed on blood by penetrating the bile duct epithelium causing extensive haemorrhages in the process (Kofta *et al.*, 2000). Moreover, tegumental spines and suckers of the fluke in heavy worm burden mechanically lacerate bile duct mucosal surface thereby causing constant leakage of whole blood in the gut (Jennings, 1976) and other organs. These extensive intrabilliary and intrahepatic haemorrhages, therefore, may lead to some *Fasciola* eggs getting trapped in the ruptured blood vessels and transported via the haematogenous route to other tissues such as the lungs, spleen and kidneys.

However, despite significant numbers of *Fasciola* eggs detected in the lung after digestion, our histopathological examination of the lung did not show any evidence of *Fasciola* egg deposits. Several reasons could be attributed to our failure to demonstrate *Fasciola* eggs on histopathological examination of the lung. Firstly, it was possible to miss the eggs deposited in the tissue because other disease conditions could have produced the lesions (nodules/masses, abscesses, and calcification and

hardening nodules) which were used to select lung samples for histopathological examination. Secondly, the density of *Fasciola* egg deposits in the lung was not very high and as such it was possible to miss the eggs on histopathological examination. Thirdly, cutting of tissue sections for microscopic examination was not done serially. This could have resulted in missing the deposited eggs.

In conclusion, the occurrence of co-joint *Fasciola*, *Schistosoma* and *Amphistomum* infections in cattle in Zambia has been confirmed in this study and that this may be common. *Amphistomum* infections are characterized by heavy worm burdens while *Schistosoma* infections represent a subclinical form characterized by low to moderate worm burdens. In natural infections of cattle, *Fasciola* and *Amphistomum* seem to be mutually inclusive in Zambia, thereby resulting in increased cases of dual *Fasciola* and *Amphistomum* infections.

The extent of co-joint *Schistosoma* and *Amphistomum* infections revealed in this study renders the impression that these trematodes are of little veterinary significance in cattle debatable.

In endemic areas, there is insignificant cross-protection between *Fasciola* and *Schistosoma* based on worm burdens and faecal egg counts. This means that the cross-protection observed in several experimental studies between *Schistosoma* and *Fasciola* may have little effect on the epidemiology of co-joint trematode infections in endemic areas.

Therefore, in order to come up with effective control programmes of fasciolosis, schistosomosis and amphistomosis, their design must consider the existence of co-joint infections and the lack of effective cross-protection among these trematodes in endemic areas.

In future studies, we recommend that large sample sizes be used in further studies on the heterologous interactions among *Fasciola*, *Schistosoma* and *Amphistomum* in endemic areas. Moreover, since *Fasciola* eggs were not detected in the lungs on histopathological examination, further studies on animals heavily infected with fasciolosis are required.

REFERENCES

Abbas, A.K., Lichtman, A.H. and Pober, J.S. (1994). Cellular and Molecular Immunology. W.B. Saunders Co., Philadelphia.

Agosti, M., Cavaletti, E. and Pozza, O. (1980). Clinica e epizootologia della paramphistomiasis bovina nella Provincia di Milano. *Clinical Veterinarian* **103**, 284-296.

Bancroft, J.D. and Stevens, A. (1982). Theories and practice of histological techniques. Churchill Livingstone, London, pp 42-53.

Barsoum, I.S., Bogitsh, B.J. and Colley, D.G. (1992). Detection of *Schistosoma mansoni* circulating cathodic antigen for evaluation of resistance induced by irradiated metacercaria. *Journal of Parasitology* **78**, 681-686.

Behm, C.A. and Sangster, N.C. (1999). Pathology, Pathophysiology and Clinical aspects. In: Dalton, J.P. (Ed.), Fasciolosis. CABI Publishing, Wallingford, UK, pp 185-224.

Bitakaramire P.K. (1973). Preliminary studies on the immunization of cattle against fasciolosis using gamma-irradiated metacercariae of *Fasciola hepatica*. In: Dalton, J.P. (Ed.), Fasciolosis. CABI Publishing, Wallingford, UK, 465-525

Boray J.C. (1969). Experimental fasciolosis in Australia. *Advances in Parasitology* **7**, 95-209.

Brown, D. (1994). Freshwater snails of Africa and their medical importance. 2nd Edition. Taylor and Francis, London. U.K. pp 340–343, 390-406, 437.

Bushara H.O., Hussein M.F., Saad A.M., Taylor M.G., Dargie J.D., Marshall T.F., de C. and Nelson G.S. (1978). Immunization of calves against *Schistosoma bovis* using irradiated cercaria or schistosomula of *S. bovis*. *Parasitology* **77**, 303-311.

Bushara H.O., Hussein M.F., Majid, M.A. and Taylor M.G. (1982). Effects of praziquantel and metrofinate on *Schistosoma bovis* infectiosn in Sudanese cattle. *Research in Veterinary Science* **33**, 125-126.

Capron A., Riveau G., Grzych J.M., Boulanger D., Capron M. and Pierce R. (1995). Development of a vaccine strategy against human and bovine schistosomiasis. Background and update. In: Zhongdao Wu., Shuxian Liu., Shaoji Zhang., Huanzhang Tong., Zulu Gao., Yiaoming Liu., Dandan Lin., Zide Liu., Guochang Wu., Hua Yi., Guangcehen Song., Yuxian Xu. 2004. Persistence of the protective immunity to *Schistosoma japanicum* in Chinese yellow cattle induced by the recombinant 26 kDa glutathione-S-transferase (reSjc26GST). *Veterinary Parasitology* **123**, 167-177.

Carlton, W.W. and McGavin, M.D. (1995). Thomson's Special Veterinary Pathology, Second Edition. Molsby, USA, 99-100.

Castro, E., Freyre, A. and Hernandez, Z. (2000). Serological responses of cattle after treatment and during natural re-infection with *Fasciola hepatica*, as measured by a dot-ELISA system. *Veterinary Parasitology* **90**, 201-208.

Cervi, L., Cejas, H. and Diana, T.M. (2001). Cytokines involved in the immunosuppressor period in experimental fasciolosis in rats. *International Journal of Parasitology* **31**, 1467-1473.

Chhabra, R.C., Gill, B.S. and Dutt, S.C. (1978). Paramphistomiasis of sheep and goats in the Punjab State and its treatment. *Indian Journal of Parasitology* **2**, 43-45.

Chingwena, G., Mukaratirwa, S., Kristensen, T.K. and Chambari, M. (2002). Larval trematode infections in freshwater snails from the highveld and lowveld areas of Zimbabwe. *Journal of Helminthology* **76**, 283-293.

Chitsulo L., Engels D., Montresor A. and Savioli L. (2000). The global status of schistosomiasis and its control. *Acta Tropica* **77,** 41-51.

Christensen, N.O., Monrad, J., Nansen, P. and Frandsen, F. (1980). *Schistosoma mansoni* and *Fasciola hepatica*: Cross resistance in mice. *Experimental Parasitology* **46**, 113-120.

Clery, D., Torgerson, P.R. and Mulcahy, G. (1996). Immune responses of chronically infected adult cattle to *Fasciola hepatica*. In: Dalton, J.P. (Ed.), Fasciolosis. CABI Publishing, Wallingford, UK, 341-375.

Dalton, P.J., McGonigle, S., Rolph, T.P. and Andrews, S.J. (1996). Induction of Protective Immunity in Cattle against Infection with *Fasciola hepatica* by Vaccination with Cathepsin L Proteinases and Haemoglobin. *Infection and Immunity*, 5066-5074.

Dalton, P.J and Mulcahy, G. (2001). Parasite vaccines: a reality. *Veterinary Parasitology* **98**, 149-167.

Dalton, P.J., Neill, S.O., Stack C., Collins, P., Walshe, A., Sekiya ,M., Doyle, S., Mulcahy, G., Hoyle, D., Khaznadji, E., Moire N., Brennan, G., Mousley, A., Kreshenko, N., Maules, A.G. and Donnely S.M. (2003). *Fasciola hepatica* cathepsin L-like proteinases: biology, function, and potential in the development of first generation liver fluke vaccines. *International Journal for Parasitology* **33**, 1173-1181.

Dargie, J.D. (1986). The impact on production and mechanisms of pathogenesis of Trematodes infection in cattle and sheep. *International Journal of Parasitology* **17**: 453-463.

De Bont, J., Van Aken, D., Vercruysse, J., Fransen, J., Southgate, V.R. and Rollinson D. (1989). The effects of praziquantel on *Schistosoma nasale* infections in cattle. *Journal of Veterinary Pharmacology and Therapeutics* **12**, 455-458.

De Bont, J., Vercruysse, J., Van Aken, D., Southgate, V.R., Rollinson, D. and Moncreff, C. (1991). The epidemiology of *Schistosoma spindale* Montgomery, 1906 in cattle in Sri Lanka. *Parasitology* **102**, 237-241.

De Bont, J., Vercruysse, J., Southgate, V.R., Rollinson, D. and Kaukas, A. (1994). Cattle schistosomiasis in Zambia. *Journal of Helminthology* **68**, 295-299.

De Bont. (1995). Cattle schistosomosis: Host-Parasite Interactions. A thesis for the degree of Doctor of Philosophy. Gent University.

De Bont, J., Vercruysse, J., Sabbe, F., Southgate, V.R. and Rollinson, D. (1995a). *Schistosoma mattheei* infections in cattle: changes associated with season and age. *Veterinary Parasitology* **57**, 299–307.

De Bont, J., Vercruysse, J., Sabbe, F. and Ysebaert M.T. (1995b). Observations on worm population dynamics in calves naturally infected with *Schistosoma mattheei*. *Parasitology* **111**, 485-491.

De Bont, J. and Vercruysse J. (1997). The epidemiology and control of cattle Schistosomiasis. *Parasitology Today* **13**, 255–262.

De Bont, J, and Vercruysse J. (1998). Schistosomiasis in cattle. *Advanced Parasitology* **41**, 286-364.

De Bont, J., Shaw, D.J. and Vercruysse, J. (2002). The relationship between faecal egg counts, worm burden and tissue egg counts in early *Schistosoma mattheei* infections in cattle. *Acta Tropica* **81**, 63-76.

De Bont, J., Claerebout, E., Riveau, G., Schacht, A.M., Smets ,K., Conder, G., Brake, D.A., Capron, A. and Vercruysse, J. (2003). Failure of a recombinant *Schistosoma bovis*-derived glutathione S-transferase to protect cattle against experimental *Fasciola hepatica* infection. *Veterinary Journal* **113**, 135-144. 63-76.

Diaz, P., Lomba, J., Pedreira, M., Arias, R., Sanchez-Andrade, J.L., Suarez, P., Diez-Bienos,. Morrondo, P. and Paz-Silva, A. (2006). Analysis of the IgG antibody response against Paramphistomatidae trematoda in naturally infected cattle: Application to serological surveys. *Veterinary Parasitology* **140**, 281-288.

Dinnik J.A. and Dinnik N.N. (1965). The schistosomes of domestic ruminants in Eastern Africa. *Bulletin of Epizootic Diseases of Africa* **13**, 341-359.

Doyle, J.J. (1971). Acquired immunity to experimental infection with *Fasciola hepatica* in cattle. *Research in Veterinary Science* **12**, 527-534.

Dumenigo, B.E., Espino, A.M., Finlay, C.M. and Mezo, M. (2000). Kinetics of antibody-based antigen detection in serum and faeces of sheep experimentally infected with *Fasciola hepatica. Veterinary Parasitology* **89** (1-2): 158.

Dutt, S.C. (1980). Paramphistome and Paramphistomiasis of Domestic Animals in India. In: Singh, R.P., Sahai, B.N. and Jha, G.J. (1984). Histopathology of the duodenum and rumen of goats during experimental infections with *Paramphistomum cervi. Veterinary Parasitology* **15**, 39-46.

Rieu, E., Recca, A., Be´net, J.J., Saana, M., Dorchies, P. and Guillot, J. 2007. Reliability of coprological diagnosis of *Paramphistomum* species infection in cows. *Veterinary Parasitology* **146**, 249–253.

Espino, A.M. and Hillyer, G.V. (2003). Molecular cloning of a member of the *Fasciola hepatica* saposin-like protein family. *Journal of Parasitology* **85**, 545-552.

Espino, A.M., Osuna, A., Gil, R. and Hillyer, G.V. (2005). *Fasciola hepatica*: Humoral and cytokine responses to a member of the saposin-like protein family following delivery as a DNA vaccine in mice. *Experimental Parasitology* **110**, 374-383.

Esteban, J.G., Gonzalez., C. Curtale, F., Munoz-Antoli, C., Valero, M.A., Bargues, M.D., El Sayed, M., El Wakeel, A., Abdel-Wahab, Y., Montresor, A., Engels, D., Savoili, L. and Mas-Coma, S. (2003). Hyperendemic fasciolosis associated with schistosomosis in villages in the Nile Delta of Egypt. *American journal of tropical medicine and hygiene* **69**, 429-437.

Estuningsih, S.E., Stevenson, P., Beriajaya, C. and Knox M.R. (1990). Triclabendazole in the treatment of *Fasciola gigantica* infection in swamp buffalo (*Bubalus bubalus*). *Australian Veterinary Journal* **67**, 234-235.

Estuningsih, S.E., Smooker, P.M., Wiedosari, E., Widjajanti, S., Vailano, S., Parrtoutomo, S. and Spithill, T.W. (1997). Evaluations of antigens of *Fasciola gigantica* as vaccines against tropical fasciolosis in cattle. *International Journal for Parasitology* **27**, 1419-1428.

Fagbemi, B.O. and Goubadia, E.E. (1995). Immunodiagnosis of fasciolosis in ruminants using 28 kDa cysteine proteinase of *Fasciola gigantica* adult worms. *Veterinary Parasitology* **57**, 309–318.

Fagbemi, B.O., Obarisiagbon, I.O. and Mbuh, J.V. (1995). Detection of circulating antigen in sera of *Fasciola gigantica* infected cattle with antibodies reactive with a *Fasciola* specific 88 kDa antigen. *Veterinary Parasitology* **58**, 235-246.

Fernandez, C.A., Fraga, J., Pointer, J.P., Yong, M., Sanchez, J., Gutierrez, C.A. and Theron A. (2004). Detection and genetic distance of resistance of populations of *Pseudosuccinea columella* (Mollusca: *Lymnaeidae*) to *Fasciola hepatica* (Trematoda: Digenea) using RAPD markers. *Acta Tropica* **92** 83-87.

Ferreras, M.C., Garcia-Iglesias, M.J., Manga-Gonzalez, M.Y., Perez-Martinez, C., Mizinska, Y., Romajo, V., Gonzalez-Lanza, M.C., Escudero, A. and Garcia-Marin, J.F. (2000). Histopathological and Immunohistochemical Study of Lambs Experimentally Infected with *Fasciola hepatica* and *Schistosoma bovis*. *Journal of Veterinary Medicine*. B **47**, 763-773.

Food and Agricultural Organization (FAO) (1994). Diseases of domestic animals caused by flukes. Rome, pp 2-6, 12, 13, 31, 32.

Froyd, G. (1959). The incidence of liver fluke and gastrointestinal parasites of cattle in Kenya. *Bulletin of Epizootic Diseases in Africa* **7,** 179–182.

Gabriel, S., Phiri, I.K., Van Dam, G.J., Deelder, A.M., Duchateau, L. and Vercruysse, J. (2004). Variations in the immune response to natural *Schistosoma mattheei* infections in calves born to infected mothers. *Veterinary Parasitology* **119**, 177-185.

Gabriel, S. (2005). Influence of *Schistosoma mattheei* infections in cows on offspring infection and immunity. PhD Thesis. University of Gent.

Gabriel, S., Ververken, C., Vercruysse, J., Duchateau, L., Phiri, I.K. and Goddeeris, B.M. (2007). Perinatal priming of calves born to *Schistosoma mattheei* infected dams. *Veterinary Parasitology* **144**, 61-67.

Goubadia, E.E. and Fagbemi, B.O. (1997). The isolation of *Fasciola gigantica* specific antigens and their use in the serodiagnosis of fasciolosis in sheep by the detection of circulating antigens. *Veterinary Parasitology* **68**, 269-282.

Hammond, J.A. (1970). Studies on fascioliasis with special reference to *F. gigantica* in East Africa. PhD Thesis, University of Edinburgh, UK.

Hammond, J.A. and Sewell, M.M.H. (1990). Diseases caused by helminths. In: Handbook on Animal Diseases in the Tropics, 4th edition (Eds. M.M.H. Sewell and D.W. Brocklesby). Bailliere Tindall. pp. 104-159.

Hansen, J. and Perry, B. (1994). The Epidemiology, Diagnosis and Control of Helminth Parasites of Ruminants (2nd Ed). ILRAD, Addis Ababa, Ethiopia. Pp 31-33, 117,118.

Harada, Y. (1974). Studies on the effects of various molluscicides against *Lymnaea ollula*, intermediate host of *Fasciola* spp. in Japan. II. Estimation of the efficiencies of five molluscicides against adult and juvenile snails and eggs of *Lymnaea ollula*. *Japanese Journal of Parasitology* **23**, 293.

Haroun, E.M. and Hillyer, G.V. (1986). Resistance to fasciolosis - a review. *Veterinary Parasitology* **20**, 63-93.

Hillyer, G.V. (1979). *Schistosoma mansoni*: Reduced worm burdens in mice immunized with isolated Fasciola hepatica antigen. *Experimental Parasitology* **48**, 287-295.

Hillyer, G.V. (1981). Effect of *Schistosoma mansoni* infections on challenge infections of *Fasciola hepatica* in mice. *Journal of Parasitology* **67**, 731-733.

Hillyer, G.V. (1984). Immunity to schistosomes using heterologous trematode antigens. *Veterinary Parasitology* **14**, 263-283.

Hillyer, G.V. (1987). Heterologous resistance in schistosomiasis. *Memorias do Instituto Oswardo Cruz* **82** (Supplement IV), 171-174.

Hillyer, G.V., De Galanes, M., Rosa, M.I. and Montealegre, F. (1988). Acquired immunity in schistosomosis with purified *Fasciola hepatica* cross-reactive antigens. *Veterinary Parasitology* **29**, 265-280.

Hillyer, G.V. (2005). *Fasciola* antigens as vaccines against fasciolosis and schistosomiasis. *Journal of Helminthology* **79**, 241-247.

Hira, P.R. and Patel, B.G. (1981). Transmission of Schistosomiasis in a rural area in Zambia. *Central African Journal of Medicine* **27**, 244-249.

Hirata, M., Kage, M., Takushima, M. and Fukuma, T. (1993). Different courses of granulomatous reactions around *Schistosoma japonicum* eggs in three stages of mice. *Journal of Parasitology* **79**, 266-273.

Jennings, F.W. (1976). The anaemias of parasitic infections. In: Soulsby, E.J.L. (ED.) *Pathophysiology of parasitic infection*. Academic press, New York, pp. 41-67.

Jones, T.C. and Hunt, R.D. (1983). Distomiasis (fascioliasis, liver fluke disease, liver rot fascioloidiasis dicrocoelisiasis). In: Veterinary Pathology. 5th ed., pp. 251–256. Lea and Febiger, Philadelphia.

Kabatereine, N.B., Tukahebwa, E., Kazibwe, F., Namwangye, H., Zaramba, S., Brooker, S., Stothard, J.R., Kamenka, C., Whanwell, J., Webster, J. and Fenwick, A. (2005). Fresh from the field; country wide control of schistosomiasis and soil transmitted helminthiasis (STH) in Uganda. In: **Southgate, V.R., Rollinson, D., Tchuem Tchuente, L.A. and Hagan P.** (2005). Towards control of schistosomiasis in sub-Saharan Africa. *Journal of Helminthology* **79**, 181-185.

Kassuku A.A., Christensen N.O., Monrad J., Nansen P. and Knudsen J. (1986). Epidemiological studies on *Schistosoma bovis* in Iringa Region, Tanzania. *Acta Tropica* **43**, 153-163.

Kendall, S.B. (1954). Fascioliasis in Pakistan. *Annuals of Tropical Medicine and Parasitology* **48**: 307-313.

Keyyu J.D., Kassuku, A.A., Msaliwa, L.P., Monrad J. and Kyvsgaard, N.C. (2006). Cross-sectional prevalence of helminth infections in cattle of tradional, small scale and large scale dairy farms in Iringa district, Tanzania. *Veterinary Research Communications* **30**, 45-55.

Knowles, R.J. and Jones, A. (1989). The influence of *Calicophoron microbothrium* on the susceptibility of *B. tropicus* to *Schistosoma bovis*. *Parasitological research* **75**, 381-391.

Knox, P.D., Redmond, D.L., Skuce, P.J. and Newlands, G.F.J. (2001). The contribution of molecular biology to the development of vaccines against nematode and trematode parasites of domestic ruminants. *Veterinary Parasitology* **101**, 311-335.

Kofta, W., Mieszczanek, J., Pluciennicczak, G. and Wedrychowicz, H. (2000). Successful DNA immunisation of rats against fasciolosis. *Vaccine* **18**, 2985-2990.

Kumar, V. (1999). Trematode infections and diseases of man and animals. Kluwer Academic publishers, London. UK.

Lawrence, J.A. (1970). Examination of ruminal faeces for schistosome eggs. *Rhodesia Veterinary Journal* **1**, 49-52.

Lawrence, J.A. (1973). *Schistosoma mattheei* in cattle: variations in parasite egg production. *Research in Veterinary Science* **14**, 402-404.

Lawrence, J.A. (1976). *Schistosoma mattheei* in the ox. Ph.D. Thesis, University of Rhodesia, Zimbabwe, pp 308.

Lopez-Aban, J., Oleaga, A., Romajo, V., Casanueva, J.L., Perez A., Hillyer G.V. and Muro, A. (2000). Vaccination of mice against *Schistosoma bovis* with a recombinant fatty acid binding protein from *Fasciola hepatica*. *Veterinary Parasitology* **91**, 33-42.

Mage, C., Bourgne, H., Toullien, J.M., Rondelaud, D. and Dreyfuss, G. (2002). *Fasciola hepatica* and *Paramphistomum daubneyi*: changes in prevalences of natural infections in cattle and in *Lymnaea truncatula* from central France over 12 years. *Veterinary Research* **33**, 439-447.

Mahato, S.N. (1993). Epidemiology and pathogenesis of Fasciolosis in eastern Nepal. PhD Thesis. University of Edindurgh. Scotland, U.K.

Majid, A.A., Marchall, T.F de C. and Hussein, M.F. (1980). Observations on cattle schistosomiasis in the Sudan, a study on comparative medicine. I. Epizootical observations on *Schistosoma bovis* in the White Nile Province. *American Journal of Tropical Medicine and Hygiene* **29**, 435– 441.

Makundi, M. (1993). Epidemiological studies on *Schistosoma bovis* infection in cattle in Iringa District, Tanzania. MSc. Thesis, Royal Veterinary and Agricultural University, Copenhagen, Denmark.

Malone, J.B., Loyacano, A.F., Armstrong, D.A. and Archibald, L.F. (1982). Bovine Fasciolosis: Economic and control in the Gulf Coast based on seasonal transmission. *Bovine Practitioner* **17**: 126-133.

Martinez-Moreno, A., Jimenez-Luque, V., Moreno, T., Redondo, E.S.H., Martin de las Mulas, J. and Perez, J. (1999). Liver pathology and immune response in experimental *Fasciola hepatica* infections in goats. *Veterinary Parasitology* **82**, 19-33.

Mas-Coma, S., Bargues, M.D. and Esteban, J.G. (1999). Human fasciolosis. In: Dalton, J.P. (Ed.), Fasciolosis. CABI Publishing, Wallingford, UK, pp. 411-434.

Mas-Coma, S. (2005). The Epidemiology of fasciolosis in human endemic areas. *Journal of Helminthology* **79**, 207-216.

Mavenyengwa, M., Mukaratirwa, S., Obwolo, M. and Monrda, M. (2003). The bovine gut cellular responses following primary and challenge infection with *Calicophoron microbothrium* metacercaria. p. 35. in *Proceedings of the 12th annual meeting of the ENRECA Livestock Helminths Research Project in Eastern and Southern Africa*, 21-24 June, Sokoine University of Agriculture, Morogoro, Tanzania.

McCauley, E.H., Tayed, A. and Majid, A.A. (1983). Owner survey of schistsomoiasis mortality in Sudanese cattle. *Tropical Animal Health and Production* **15**, 227-233.

McCullough, F.S. (1965). Annual Tropical Medicine. *Parasitology Journal* **59**: 320-336.

McCullough, F.S. (1992). The Role of Molluscicides in Schistosomiasis Control. Unpublished Document. WHO/SCHISTO/92, 107.

McManus, D.P. (2000). A vaccine against Asian schistosomiasis: the story unfolds. *International Journal for Parasitology* **30** (3), 265-271.

Megard, J.P. (1975). Fascioliasis in Black Africa- Merck, Shap Dohme Research Department, Paris.

Monrad, J., Christensen, N.O., Nansen, P. and Frandsen, F. (1981). Resistance to *Fasciola hepatica* in sheep harbouring primary *Schistosoma bovis* infections. *Journal of Helminthology* **55**, 261-271.

Mulcahy, G., O'Connor, F., McGonigle, S., Clery, D.G., Andrews, S.J. and Dalton, P.J. (1998). Correlation of specific antibody titre and avidity with protection in cattle immunized against *Fasciola hepatica*. *Vaccine* **16**, 932-939.

Mulcahy, G., Joyce, P. and Dalton, P.J. (1999). Immunology of *Fasciola hepatica* infection. In: Dalton, J.P. (Ed.), Fasciolosis. CABI Publishing, Wallingford, UK, 341-375.

Mulcahy, G. and Dalton, J.P. (2001). Cathepsin L-proteinases as vaccines against infection with *Fasciola hepatica* (liver fluke) in ruminants. *Research in Veterinary Science* **70**, 83-86.

Ndyomugyenyi, R. and Minjas, J.N. (2001). Urinary schistosomiasis in school children in Dar-es-Salaam, Tanzania, and the factors influencing its transmission. *Journal of Tropical Medicine and Parasitology* **7**, 697-706.

Nyame, K.A., Lewis, A.F., Doughty, L.B., Correa-Oliveira, R. and Cummings D.R. (2003). Immunity to schistosomiasis: glycans are potential antigenic targets for the immune intervention. *Experimental Parasitology* **104**, 1-13.

Ogunrinade, A.F., Okon, E.D. and Fasanmi, E.F. 1981. The prevalence of bovine fascioliasis in Nigeria. A 5- year analysis of abattoir records. *Bulletin of Animal Health and Production in Africa* 29: 381- 387.

Pandey, G.S. and Ahmadu, B. (1998). Prevalence, seasonal variation and economic importance of bovine fascioliasis in western province of Zambia. *Zimbabwe Veterinary Journal* **29**, 63-69.

Phiri, A.M. (2005). Factors influencing the prevalence of fasciolosis in the Kafue and Zambezi river basins. Mac Thesis, University of Zambia.

Phiri, A.M., Phiri, I.K., Sikasunge, C.S. and Monrad J. (2005). Prevalence of fasciolosis in Zambian cattle observed at selected abattoirs with emphasis on age, sex and origin. *Journal of Veterinary Medicine* B **52**, 414-416.

Phiri, A.M., Phiri, I.K. and Monrad, J. (2006a). Prevalence of amphistomiasis and its association with *Fasciola gigantica* infections in Zambian cattle from communal grazing areas. *Journal of Helminthology* **80**, 65-68.

Phiri, A.M., Phiri, I.K., Sikasunge, C.S., Chembensofu, M. and Monrad J. (2006b). Comparative fluke burden and pathology in condemned and non-condemned cattle livers from selected abattoirs in Zambia. *Onderstepoort Journal of Veterinary Research* **73**, 275-281.

Phiri, A.M. (2006c). Common conditions leading to carcass and offal condemnations at 3 abattoirs in the Western Province of Zambia and their

zoonotic implications to consumers. *Journal of the South African Veterinary Association* **77**, 28-32.

Phiri, I.K. (1997). Immunoglobulin isotype response of *Fasciola* spp. infected sheep and cattle to defined *Fasciola* spp. antigens. A thesis for the degree of Doctor of Philosophy. The University of Edinburgh.

Phiri, I.K., Phiri, A.M. and Harrison, L.J.S. (2006). Serum antibody isotype responses of *Fasciola*-infected sheep and cattle to the excretory and secretory products of *Fasciola* species. *Veterinary Parasitology* **141**, 234-242.

Phiri, I.K., Phiri, A.M. and Harrison, L.J.S. (2007). The serum glucose and β-hydroxylbutyrate levels in sheep with experimental *Fasciola hepatica* and *Fasciola gigantica* infection. *Veterinary Parasitology* **143**, 287-293.

Pitchford, R.J. (1976). Preliminary observations on the distribution, definitive hosts and possible relation with other schistosomes, of *Schistosoma margrebowiei*, Le Roux, 1933 and *Schistosoma leiperi*, Le Roux, 1955. *Journal of Helminthology* **50**, 111-123.

Qian, Z.L. and Deelder, A.M. (1983). *Schistosoma japonicum*: immunological characterization and detection of circulating polysaccharide antigens form adult worms. *Experimental Parasitology* **55**, 168-178.

Raina, O.K., Yadav, S.C., Sriveny, D. and Gupta, S.C. (2006). Immuno-diagnosis of bubaline fasciolosis with *Fasciola gigantica* cathepsin-L and recombinant cathepsin L 1-D proteases. *Acta Tropica* **98**, 145-151.

Rangel-Ruiz, L.J., Albores-Brahmas, S.T. and Gamboa, A. (2003). Seasonal trends of *Paramphistomum cervi* in Tabasco, Mexico. *Veterinary Parasitology* **116**, 217-222.

Rodriguez-Onsorio, M., Gomez-Garcia, Vi., Rojas-Gonzalez, J., Romajo-Martin, V., Manga-Gonzalez, Y. and Gonzalez-Lanza, C. (1993). Resistance to *Schistosoma bovis* in sheep induced by an experimental *Fasciola hepatica* infection. *Journal of Parasitology* **72**(2), 223-225.

Rodriguez-Perez, J. and Hillyer, G.V. (1995). Detection of excretory–secretory circulating antigens in sheep infected with *Fasciola hepatica* and with *Schistosoma mansoni*. *Veterinary Parasitology* **56**, 57-66.

Rolfe, P.F. and Boray, J.C. (1987). Chemotherapy of paramphistomosis in cattle. *Australian Veterinary Journal* **64**, 328-332.

Rollinson, D. and Southgate, V.R. (1987). The genus *Schistosoma*: A taxonomic appraisal. In: **De Bont.** (1995). Cattle schistosomosis: Host-Parasite Interactions. A thesis for the degree of Doctor of Philosophy. Gent University.

Ross, J.G., Todd, J.R. and Dow, C. (1966). Single experimental infections of calves with the liver fluke *Fasciola hepatica* (Linnaeus 1958). *Journal of Comparative Pathology* **76**, 67-81.

Ruiz, A., Molina, J.M., Gonzalez, J., Martinez-Moreno, F.J., Gutierrez, P.N. and Martinez-Moreno, A. (2003). Humoral response (IgG) of goats experimentally infected with *Fasciola hepatica* against cysteine proteinases of adult fluke. *Veterinary Research* **34**, 435–443.

Schillhorn van Veen, T.W. (1980). Fascioliasis (*Fasciola gigantica*) in West Africa: a review. *Veterinary Bulletin* **50**, 529-533.

Sewell, M.M.H. (1966). The pathogenesis of fasciolosis. *Veterinary Record* **78**, 98-105.

Silangwa, S.M. (1973). Incidence of liver fluke (*F. gigantica*) in Barotse cattle, Zambia. *Bulletin of Epizootic Diseases in Africa* **21**, 11-17.

Silvestre, A., Sauve, C. and Cabaret, J. (2000). Caprine *Paramphistomum daubney* (Trematoda) infection in Europe. *Veterinary Records* **146**, 674-675.

Simpson, J.R., Kunkle, W., Courtney, C.H. and Shearer, J.K. (1985). Economic analysis of controlling liver flukes. *Agriculture Practice-Parasitology* **6** (2), 20–24.

Singh, R.P., Sahai, B.N. and Jha, G.J. (1984). Histopathology of the duodenum and rumen of goats during experimental infections with *Paramphistomum cervi*. *Veterinary Parasitology* **15**, 39-46 39.

Singh, K.P. and Parihar, N.S. (1988). Pathology of fluke infestation in livers of sheep and goats. *Indian Journal of Animal Sciences* **58** (8), 890–894.

Singh, A., Singh A. and Chaudhri S. S. (2004). Visceral schistosomiasis of domestic animals in India: humoral immune status of infected cattle, sheep and goats against major polypeptide antigens of *Schistosoma indicum* and *S. spindale*. *Parasite Immunology* **26**, 167–175.

Sirag, S. B., Christensen, N.O., Nansen, P., Monrad, J. and Frandsen, F. (1981). Resistance of *Fasciola hepatica* in calves harbouring primary patent *Schistosoma bovis* infection. *Journal of Helminthology* **55**, 63-70.

Smith, A.M., Dowd, A.J., Heffernan, M., Robertson, C.D. and Dalton, P.J. (1993). *Fasciola hepatica*: a secreted cathepsin L-like proteinase cleaves host immunoglobulin. *International Journal of Parasitology* **23**, 977-983.

Smooker, P.M., Kennedy, N.J., Steeper, K.R. and Christopoulos, H. (2001). *Fasciola*: Kinetics and Quality of Humoral Responses to Fatty Acid Binding Protein and cathepsin L Following Delivery as DNA Vaccine in Mice. *Experimental Parasitology* **97**, 154-160.

Smyth, J.D. (1996). Animal parasitology. Third Edition, Cambridge University Press, 178-198.

Soulsby, E.J.L. (1982). Helminths, Arthropods and Protozoa of Domesticated Animals. 8th Edition. Bailliere and Tindall, Philadelphia, U.S.A. Pp 40-51.

Southgate, V.R. (1997) Schistosomiasis in the Senegal River Basin: before and after the construction of the dams at Diama, Senegal and Manantali, Mali and future prospects. *Journal of Helminthology* **71**, 125–132.

Southgate, V.R., Rollinson, D., Tchuem Tchuente, L.A. and Hagan P. (2005). Towards control of schistosomiasis in sub-Saharan Africa. *Journal of Helminthology* **79**, 181-185.

Spence, S.A., Fraser, G.C. and Chang, S. (1996). Response in milk production to the control of gastro-intestinal nematode and paramphistome parasites in dairy cattle. *Australian Veterinary Journal* **74**, 456-459.

Spithill, T.W. and Dalton, J.P. (1998). Progress in development of liver fluke vaccines. *Parasitology Today* **14**, 224-228.

Spithill, T.W., Smooker, P.M. and Bruce, C.D. (1999). *Fasciola gigantica*: Epidemiology, control, immunology and molecular biology. In: Dalton, J.P. (Ed.), Fasciolosis. CABI Publishing, Wallingford, UK. Pp. 465-525.

Sriveny, D.R., Yadav, O.K., Chandra, S.C., Jayraw, D., Singh, A.K., Velusamy, M. and Singh, R.B.P. (2006) Cathepsin L cysteine proteinase in the diagnosis of bovine *Fasciola gigantica* infection. *Veterinary Parasitology* **135**, 25-31.

Szmidt-Adjide, V., Abrous, M., Adjide, C.C. and Dreyfuss, G. (2000). Prevalence of *Paramphistomum daubney* in central France. *Veterinary Parasitology* **87**, 133-138.

Taira, R., Suzuki, K. and Boray, J.C. (1983). Detection and quantification of *Fasciola* eggs in cattle using the Beads technique. *Japanese Journal of Veterinary Parasitology* **32**, 279-286.

Taylor, M.G. (1970). Hybridization experiments on five species of African schistosomes. *Journal of Helminthology* **44**, 253-314.

Taylor, M.G. (1971). Further observations on the sexual maturation of female schistosomes in single-sex infections. *Journal of Helminthology* **45**, 89-92.

Urquhart, G. M., Armour, J., Duncan, J. L., Dunn, A.M. and Jennings, F. W. (1989). The laboratory diagnosis of parasitism. In: *Veterinary Parasitology* (Ed. Urquhart, G.M.), pp. 269-271. Longman Group, U.K. Ltd.

Urquhart, G.M., Armour, J., Duncan, J.L., Jennings, F.W. and Dunn, A.M. (1996). *Veterinary Parasitology*. Blackwell Science Inc., Oxford, U.K.

Uzoukwu, M. and Ikeme, M.M. (1978). Hepatic changes in naturally *Fasciola gigantica* infected Fulani zebu. *Bulletin of Animal Health and Production in Africa* **25**, 162-167.

Van der Werf, M.J., De Vlass, S.J., Brooker, S., Looman, C.W.N., Nagelkerke, N.J.D., Habbema, J.D.F. and Engels, D. (2003). Quantification of clinical morbidity associated with schistosome infection in sub-Saharan Africa. *Acta Tropica* **86**, 125-139.

Velusamy, R., Singh, B.P., Sharma, R.L. and Chandra, D. (2004). Detection of circulating 54 kDa antigen in sera of bovine calves experimentally infected with *Fasciola gigantica*. *Veterinary Parasitology* **119**, 187-195.

Vercruysse, J. and Claerebout, E. (2001). Treatment vs. non-treatment of helminth infections in cattle: defining the thresholds. *Veterinary Parasitology* **98**, 195-214.

Vilar, M.M., Barrientos, F., Almeida M., Thaumaturgo, N., Simpson, A., Garratt R. and Tendler M. (2003). An experimental bivalent peptide vaccine against schistosomiasis and fasciolosis. *Vaccine* **22**, 137-144.

Waldvogel, A.S., Lepage, M.F., Zakher, A., Reichel, M.P., Eicher, R. and Heussler, V.T. (2004) Expression of interleukin 4, interleukin 4 splice variants and interferon gamma mRNA in calves experimentally infected with *Fasciola hepatica*. *Veterinary Immunology and Immunopathology* **97**, 53–63.

Webster B.L., Tchuem Tchuente L.A., Jourdane J. and Southgate V.R. (2005). The interaction of *Schistosoma haematobium* and *S. guineensis* in Cameroon. *Journal of Helminthology* **79**, 193–197.

World Health Organization (WHO). 2002. Prevention and Control of schistosomiasis and soil-transmitted helminthiasis; a report of WHO Expert Committee. *WHO Technical Report Series* No. 912. Geneva, World Health Organization.

Wright, C.A., Southgate, V.R. and Howard, G.W. (1979). A note on the life cycle of some amphistome flukes in Zambia. *Journal of Helminthology* **53**, 251-252.

Wright C.A. and Ross G.C. (1980). Hybrids between *Schistosoma haematobium* and *S. mattheei* and their identification by isoelectric focusing of enzymes. *Transactions of the royal Society of Tropical Medicine and Hygiene* **74**, 326-332.

Wynn, T.A., Cheever, A.W., Jankovic, D., Poindexter, R.W., Caspar, P., Lewis, F.A. and Sher, A. (1995). An IL-12 based vaccination method for preventing fibrosis induced by schistosome infection. *Nature* **376**, 594-596.

Yagi, A.I., Younis, S.A., Haroun, E.M., Gameel, A.A., Bushara, H.O. and Taylor M.G. (1986). Studies on heterologous resistance between *Schistosoma bovis* and *Fasciola gigantica* in Sudanese cattle. *Journal of Helminthology* **60**, 55-59.

Yoshihara, S., Hung, N.H., Hung, N.P. and Goto, N. (1998). An erratic parasitism found in the lungs of sheep during experimental infection with *Fasciola gigantica* (Japanese strain). *Journal of Heminthology* **72** (3), 227-230.

Zhongdao, W., Shuxian, L., Shaoji, Z., Huanzhang, T., Zulu, G., Yiaoming, L., Dandan, L., Zide, L., Guochang, W., Hua, Y., Guangcehen, S. and Yuxian, X. (2004). Persistence of the protective immunity to *Schistosoma japanicum* in Chinese yellow cattle induced by the recombinant 26 kDa glutathione-S-transferase (reSjc26GST). *Veterinary Parasitology* **123**, 167-177.

APPENDICES

Appendix 1. Table of animals harbouring all the three trematode infections at meat inspection. The number of flukes harboured by each animal is indicated.

Animal #	*Fasciola*	*Schistosoma*	*Amphistomum*
1	3	132	1460
2	5	51	1300
3	7	24	726
4	4	115	396
5	9	6	353
6	9	96	872
7	27	136	843
8	12	139	387
9	1	147	432
10	5	4	554
11	51	140	238
12	96	100	926
13	45	4	898
14	26	40	94
15	25	168	849
16	6	16	232

Appendix 2 Dual *Fasciola* and *Amphistomum* infections in cattle at meat inspection.

Animal #	*Fasciola*	*Amphistomum*
1	43	1880
2	68	440
3	39	840
4	3	1460
5	5	1300
6	7	726
7	51	1430
8	4	396
9	22	402
10	2	2
11	76	266
12	2	204
13	9	353
14	20	412
15	9	872
16	27	843
17	12	387
18	1	432

Appendix 2 continued

Animal #	*Fasciola*	*Amphistomum*
19	5	554
20	51	238
21	5	837
22	11	2793
23	96	926
24	45	898
25	26	94
26	64	63
27	35	81
28	9	26
29	176	642
30	25	849
31	6	232
32	5	464
33	6	6

Appendix 3 Dual *Schistosoma* and *Amphistomum* infections in cattle at meat inspection.

Animal #	*Schistosoma*	*Amphistomum*
1	16	780
2	1	1160
3	132	1460
4	51	1300
5	24	726
6	115	396
7	121	1282
8	11	19
9	6	353
10	23	4
11	7	40
12	96	872
13	136	843
14	139	387
15	147	432
16	4	554
17	140	238
18	110	124

Appendix 3 continued

Animal #	*Schistosoma*	*Amphistomum*
19	100	926
20	4	898
21	40	94
22	168	849
23	16	232
24	28	140
25	4	3
26	38	91

Appendix 4 Dual *Fasciola* and *Schistosoma* infections in cattle at meat inspection.

Animal #	*Fasciola*	*Schistosoma*
1	3	132
2	5	51
3	7	24
4	4	115
5	9	6
6	9	96
7	27	136
8	12	139
9	1	147
10	5	4
11	51	140
12	96	100
13	45	4
14	26	40
15	25	168
16	6	16

Appendix 5: *Schistosoma* tissue egg counts in relation to worm burden, EPG and miracidial counts.

Animal #	*Schistosoma* worm count	EPG count	Miracidia count	Tissue egg counts		
				Liver	Mesenterium	Abomasum
1	16	0	0	6	5	1
2	0	0	0	0	3	0
3	0	0	0	0	0	0
4	1	0	0	4	0	0
5	0	0	0	0	0	0
6	0	0	0	0	0	0
7	132	10	10	27	0	43
8	51	50	7	79	0	0
9	0	0	0	0	0	0
10	24	0	0	70	0	0
11	0	0	0	0	0	0
12	115	30	42	26	0	1
13	0	0	0	0	0	0
14	0	0	1	0	0	0
15	0	0	0	0	0	0
16	121	10	7	1	0	0
17	2	0	0	5	0	0

Appendix 5 continued

Animal #	*Schistosoma* worm count	EPG count	Miracidia count	Tissue egg counts		
				Liver	Mesenterium	Abomasum
18	5	0	0	2	0	0
19	11	30	0	31	0	0
20	0	0	0	0	0	0
21	0	0	0	0	0	0
22	6	0	0	5	0	0
23	23	10	7	138	0	0
24	0	0	0	12	0	0
25	7	0	0	32	0	0
26	96	0	4	102	2	0
27	136	40	14	271	0	3
28	139	10	0	50	0	0
29	147	30	0	62	0	0
30	4	0	0	1	0	0
31	140	40	1	351	0	0
32	110	0	4	215	25	0
33	0	0	0	4	0	0
34	0	0	0	0	0	0
35	0	0	0	0	0	0

Appendix 5 continued

Animal #	*Schistosoma* worm count	EPG count	Miracidia count	Tissue egg counts		
				Liver	Mesenterium	Abomasum
36	100	0	0	102	0	0
37	4	0	0	20	0	0
38	40	0	0	13	0	0
39	0	0	0	0	0	0
40	0	0	0	0	0	0
41	0	0	0	0	0	0
42	0	0	0	0	0	0
43	168	90	0	2569	0	20
44	16	0	0	6	0	0
45	0	0	0	0	0	0
46	0	0	0	0	0	0
47	28	0	0	0	0	0
48	0	0	0	0	0	0
49	4	0	0	0	0	0
50	38	0	0	11	0	0

Appendix 6 *Fasciola* tissue egg counts in relation to the worm burden and EPG

Animal #	*Fasciola* worm count	EPG count	Tissue egg counts				
			Liver	Lung	Mesenterium	Kidney	Spleen
1	1	6	0	0	0	0	0
2	5	2	0	0	0	0	0
3	51	27	325	25	17	1	0
4	0	0	0	0	0	0	0
5	0	3	0	0	0	0	0
6	5	0	0	1	0	0	0
7	11	0	3	1	0	0	0
8	96	41	200	3	11	0	1
9	45	18	5	22	1	0	0
10	26	2	254	37	0	3	2
11	64	95	414	8	1	2	5
12	35	5	40	1	0	1	3
13	9	5	3	3	4	0	3
14	176	27	106	8	5	3	4
15	25	20	37	7	0	1	5
16	6	3	2	0	0	0	0
17	0	0	0	0	0	0	0

Appendix 6 continued

Animal	*Fasciola*		Tissue egg counts				
#	worm count	EPG count	Liver	Lung	Mesenterium	Kidney	Spleen
18	5	0	0	0	0	0	0
19	0	0	0	0	0	0	0
20	6	0	1	0	0	0	0
21	0	0	0	0	0	0	0
22	0	0	0	0	0	0	0