

**THE PREVALENCE AND FACTORS INFLUENCING  
OCCURRENCE OF BOVINE FASCIOSIS IN THE KAFUE  
AND ZAMBEZI RIVER BASINS**

**BY  
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*A dissertation submitted to the University of Zambia in fulfillment of the award of  
the Degree of Master of Science in Veterinary Parasitology*

**THE UNIVERSITY OF ZAMBIA  
SCHOOL OF VETERINARY MEDICINE  
DEPARTMENT OF CLINICAL STUDIES**

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## DECLARATION

I, **Andrew Malata Phiri**, do hereby declare that this dissertation represents my own work and that it has never been submitted before for the degree at this university or indeed any other university.

Signature: .....

*AP . P.*  
*Phiri*

Date: .....

*5/10/2004*

## DEDICATION


“A hundred times every day I remind myself that my inner and outer life depends on the labors of other men, living or dead, and that I must exert myself in order to give in the same measure as I have received and I am still receiving”- Albert Einstein. I, therefore, dedicate this thesis to my mother Margaret Zulu who has been a pillar and source of strength as well as a wealth of inspiration; my sisters Leah, Rachael and Miyanda for keeping the faith; and my wife, Clara Govea-Phiri, for always being there for me.

## APPROVAL

This dissertation of **Andrew Malata Phiri** is approved as fulfilling the requirements for the award of the Degree of Master of Science in Veterinary Helminthology of the University of Zambia.

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## ABSTRACT

In Zambia, very little is known on the prevalence and impact of fasciolosis especially in cattle of the poor traditional farmers most of whom live along the Kafue and Zambezi flood plains. The main aim of this study, consisting of four components, was to determine the prevalence and factors influencing the occurrence of bovine fasciolosis in the Kafue and Zambezi rivers basins. In the prevalence study carried out from March to December 2002, 841 cattle from selected abattoirs in Central, Southern and Western provinces were inspected and 677 faecal samples collected and analysed for fluke eggs. 53.3% of cattle were positive at meat inspection while 48.7% on coprological examination. Cumulative prevalence of 59.4% (n=677) was recorded using both methods. Significant differences in the prevalence were found among the slaughterhouses ( $p<0.001$ ), age groups ( $p<0.050$ ) and origin ( $p<0.001$ ) of the examined cattle. These results indicate that *Fasciola gigantica* infection is an important condition in cattle and that a high number of livers are condemned or trimmed because of this infection.

Seasonal pattern of bovine fasciolosis was determined by liver inspection and coprological examination of 288 cattle at slaughter from March 2002 to February 2003.

The post rainy season had the highest prevalence rate at liver inspection and coprological examination (41.3% and 45.0%, respectively) while the lowest were in the cold and dry season (24.8% on both methods). The distribution of fluke eggs was significantly different ( $p < 0.001$ ) among seasons with the rainy season having higher egg counts (median=146) than both the cold and dry season and the hot and dry season (median=118). Based on the results of faecal egg examination and slaughterhouse studies, it was found that even though *F. gigantica* is present throughout the year in cattle in Zambia, it is highest in the post-rainy season.

A total of 70 condemned and 32 non-condemned livers were collected at random and dissected to assess liver fluke burden. Significantly higher ( $t=19.33$ ,  $p < 0.001$ ) flukes were found in condemned livers (mean=1.70, SD=0.59) than in non-condemned livers (mean =0.07, SD =0.25). Liver flukes were found in 9.4 % (n=32) of the non-condemned livers. Fluke egg counts from animals with condemned livers were significantly higher ( $\chi^2=84.24$ ,  $p < 0.001$ ) than in animals with non-condemned livers. However, there was no correlation ( $r=0.003$ ,  $p=0.978$ ) found between egg counts and the number of flukes.

Between August and October 2003, freshwater snails representing six species were collected from the Kafue river basin. Out of the 984 snails collected and shed for infection, 135 were positive with larval trematodes representing 13.7% prevalence. Most trematode infections were recorded from *Lymnaea natalensis* (42.8 %) which harboured four of the five morphologically different cercariae found. *Gymnocephalous* (family

Fasciolidae) and *Longifurcate-pharyngeate distome* (LPD) (families Strigeidae and Diplostomatidae) were the commonest cercariae types recorded while *brevifurcate-apharyngeate distome* (BAD) (family Schistomatidae) was the least common. Critical determinants of this infection were found to be distance of settlements and/or cattle kraals as well as the number of animals in nearby homesteads and presence of *Lymnaea* snails.

This study strongly shows that fasciolosis caused by *F. gigantica* is a major constraint of cattle production in the Zambezi and Kafue river basins throughout the year and favourable factors are available to introduce and maintain the infection.

## CHAPTER ONE

### INTRODUCTION

There are different constraints to livestock production world wide which include, among other things, diseases (Ogborn, 1999; Ministry of Agriculture And Cooperatives [MAFF], 1999). One of these constraints in the tropics, particularly Zambia, is a disease known as fasciolosis caused by the giant liver fluke, *Fasciola gigantica*. Fasciolosis has a world-wide distribution, occurring throughout Africa, Asia and the Pacific (Losos, 1986; Dargie, 1986; Fabiyi, 1987) hampering productivity in ruminants in endemic areas (Vercruysse and Claerebout, 2001). It affects about 300 million cattle and 250 million sheep (Maurice, 1994). Although mostly a problem of young stock, the disease is a problem in older animals, too (Keyyu *et al.*, 2003a). Although it prefers animals, about 2 million people have become infected with *Fasciola* after eating contaminated watercress and other aquatic plants (Maurice, 1994) and thus the disease has assumed zoonotic importance. Apart from mortality, especially in young animals, the disease reduces live weight gains, poor carcass quality due to its effect on carcass composition, liver condemnations, lowered milk production, and abortions (Hope-Cawdery, 1984; Vassilev and Jooste, 1991). Additional costs occur from implementation of control measures including the cost of purchasing and application of fasciolicides (Hyera, 1984). The smaller size, anaemia and consequently, diminished capacity for draught work of infected animals also impacts on crop production, particularly in regions where animal draught power is required for land preparation and transport (Spithill *et al.*, 1999).

The prevalence rate of fasciolosis due to *F. gigantica* in many parts of Africa has been determined, mainly at slaughter, by several workers including Malek (1980) in Cameroon and Ethiopia (45% and 30 to 90% respectively). Schillhorn van Veen (1980) determined a 65% prevalence rate in Nigeria while Cheruiyot (1987) found 19.8% prevalence rate in Kenya. In Tanzania, Hammond (1965) and more recently Keyyu *et al.* (2003a) reported prevalence rates of between 41 and 50% as well as 28.4 and 63.8%, respectively. On the other hand, Chambers (1987) in Zimbabwe found a 50% prevalence rate. In Zambia, particularly Western province, Silangwa (1973) and Pandey and Ahmadu (1998) recorded prevalence of 89% and 51%, respectively. The usual information available about this disease is the percentage of livers condemned for fasciolosis at the slaughterhouse. Prevalence rates differ from region to region within the same country due to numerous factors which include those related to agro-ecological zones and intermediate host habitation (Over, 1982).

Trematode infections are known to be highly prevalent in Western and Southern provinces of Zambia especially in the Kafue and Zambezi wetlands (Silangwa, 1973; Pandey and Ahmadu, 1998). Estimation of economic loss due to fasciolosis at national or regional level is limited by lack of accurate estimates of the disease prevalence. Very little is known on the prevalence and impact of fasciolosis especially in cattle of the poor traditional farmers in Zambia in spite of the well-known economic importance of the disease in livestock and the fact that cattle remain the most important type of livestock in the country contributing 60% of the total meat and milk consumed (MAFF, 1999). The few reports on the prevalence of

bovine fasciolosis in the country only focused on the prevalence in Western province and only used liver inspection methods.

Studies on the epidemiology of *F. hepatica* by Cameron (1934) as well as Boray and Enigk (1964) found a direct relationship between the rate of development of the parasite and the environmental temperature of the host. Dinnik and Dinnik (1963a) working on *F. gigantica* suggested the same. Subsequent studies carried out on bovine fasciolosis around the world have established that there is a relationship between transmission and climatic factors (Mzembe and Chaundhry, 1979; Bhatia *et al.*, 1989; Pandit *et al.*, 1989; Manga-Gonzalez *et al.*, 1990; Njau *et al.*, 1990). Information concerning the seasonality of fasciolosis in Zambia and the relationship between transmission and climatic factors is either limited or lacking. Since cattle in the wetlands change pastures at various periods of the year according to the flood water line, it is desirable to identify when and where the cattle are at risk of picking up the infective metacercariae on pastures.

Many studies have been carried out on the pathology of fasciolosis in ruminants (Hammond, 1970; Uzoukwu and Ikeme, 1978; Singh and Parihar, 1988; Phiri, 1997). However, the assumption that there is an association between the number of eggs per gram of faeces and the total number of worms has been questioned. This is because egg counts are known to be influenced by many factors including varying fecundity of species of parasites, ingesta volume, age of worms and host resistance (Tarazona, 1986).

In Zambia, conditions that favour the existence and infection of snails with larval trematodes are present. However, studies undertaken on these larval trematode

infections in the country have been few and far between (Dinnik, 1961; Wright, 1966, Wright *et al.*, 1979). Most of this work was restricted to the *Bulinus* snails. Large water surface areas found in and around ox-bow lakes, marshes, swamps, lagoons, ponds and canals in the flood plains of Zambia accompanied by high temperatures make them suitable areas for the existence and breeding of the snail *Lymnaea natalensis*. 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 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. Zambezi and the Kafue river basins, which are massive wetlands in Zambia, are the major traditional cattle rearing areas. They are extensive grasslands covering about 7,100 square metres or about 2% of the total area of Zambia. These basins are low lying, generally flat and the rivers that meander through them have created over the years numerous lagoons, ox-bow lakes and marshy areas with permanent water. These wetlands are subject to annual flooding from December/January to June/July. These areas are ideal for the existence of Lymnaeid snails, the intermediate hosts of liver fluke. As early as 1930, fasciolosis was widespread and enzootic in the Barotse cattle of the Zambezi flood plain. This observation was reaffirmed by Le Roux (1957). The main human activities in these river basins are cattle rearing and fishing. Silangwa (1972) estimated that about 75% of the Barotse cattle in the Upper Zambezi basin utilize the wetland pastures and about 40% of the Tonga-Ila cattle in the Central Kafue river basin utilize the wetland pastures during June/July to November/December.

The general objective of the study was to determine the prevalence and factors influencing occurrence of bovine fasciolosis in the Kafue and Zambezi river basins.

Specific objectives were

(a) To collect baseline data on the prevalence of fasciolosis in the Kafue and Zambezi river basins

(b) To assess factors affecting infection the occurrence of fasciolosis in the two basins.

## CHAPTER TWO

### LITERATURE REVIEW

#### 2.1 Parasite

Fasciola is a parasite that causes a condition known as fasciolosis. Fasciolosis is a helminthosis of mammals caused by Fasciolidae trematodes of the genus *Fasciola* which migrate in the hepatic parenchyma, and finally establish and develop in the bile ducts. These parasites are dorso-ventrally flattened, unsegmented, leaf-shaped, hermaphrodite, greyish-brown in colour and usually possess a spiny integument (Soulsby, 1982; Hansen and Perry, 1994). As seen in figure 2.1, the adult ones have two suckers for attachment; the oral sucker being at the anterior end surrounding the mouth while the ventral sucker is located at the level of the 'shoulders' of the fluke (Urquhart *et al.*, 1987). The internal organs, which are branched, lie in a parenchyma as there is no body cavity.

According to Kendall (1965), the trematode genus *Fasciola* probably comprises only two true species, *Fasciola hepatica* (Linnaeus, 1758) and *F. gigantica* (Cobbold, 1855) both of which occur in Africa. Two other species of *Fasciola* have been described from Africa, *F. nyanzae*, (Leiper, 1910) of the hippopotamus (*Hippopotamus amphibious*) and *F. tragelaphi* (Pike and Condy, 1966) known only from an antelope, the Sitatunga (*Tragelaphus spekei*).

**A****B**

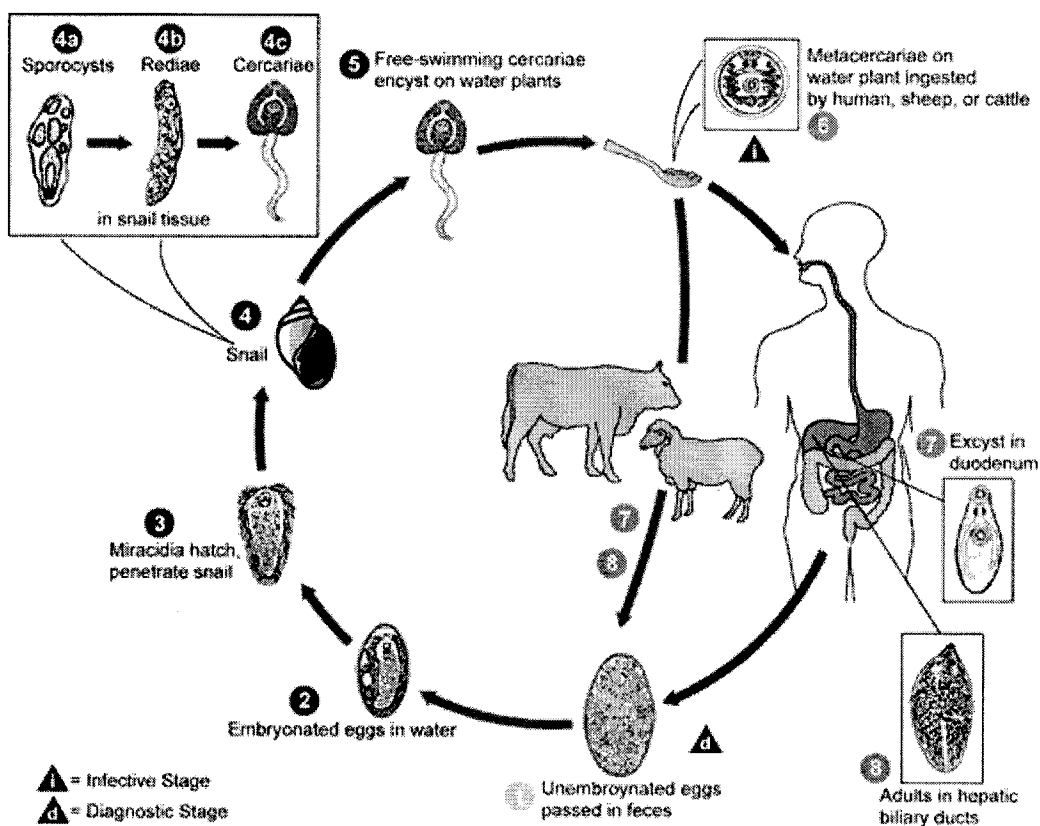
**Figure 2.1** Adult *Fasciola gigantica* with flattened, unsegmented, leaf-like body (A) and its eggs (B).

*Fasciola hepatica* (30 x 12mm) is distributed worldwide and is common in the temperate regions. It is also found in the highlands of Ethiopia, Kenya, South Africa and Lesotho. *Fasciola gigantica* (about 75x 12 mm), on the other hand, is found in warmer climates of Asia and is the commonest and widely distributed liver fluke in Africa (Kendall, 1965; Soulsby, 1982). The preferred snail host of *F. hepatica* is *Lymnaea truncatula* which is adapted to the cooler areas of the Africa's eastern highlands, whereas *L. natalensis* is the host for *F. gigantica* and occurs throughout tropical and subtropical Africa. In some regions (e.g. in Pakistan), the two overlap in their distribution (Kendall, 1954).

## 2.2 Life cycle

*Fasciola hepatica* (Linnaeus, 1758) and *F. gigantica* (Cobbold, 1885) have an indirect life cycle with the snail as an intermediate host (Figure 2.2). The eggs laid by adult flukes are passed into the duodenum together with the bile, and leave the definitive host in the faeces. Development and hatching of eggs are controlled by light, temperature and salinity of the water (Hansen and Perry, 1994). Fluke eggs will hatch throughout the year if the temperature is above 10° C (FAO, 1994). Eggs hatch into miracidia, the first juvenile stage. For further development, miracidia require intermediate snail hosts of the genus *Lymnaea*. The miracidium actively penetrates the snail casting off its ciliate covering and develops into sporocyst (undifferentiated cell mass). Each sporocyst gives rise to five and up to eight rediae. These in turn develop into one or more generation of rediae. Daughter rediae give rise to cercariae. These cercariae leave the snail swimming in the water and within a few minutes to two hours have to settle on blades of grass or other plant forms just below water-level. After casting off the tails, cercariae secrete coverings to encyst themselves. At this time, the encysted cercariae now called metacercariae are infective and can remain so for up to four months (Soulsby, 1982). A single miracidium hatched from the fluke egg can produce up to 4 000 metacercariae attached to the herbage (FAO, 1994). The final host picks up the metacercariae that are found as small white cysts with the grass and water when grazing and drinking, respectively. Development in the vertebrate host follows the ingestion of metacercariae (Hammond and Sewell, 1974; Losos, 1986). These excyst in the duodenum and within twenty-four hours of infection the majority of immature

flukes occur in the abdominal cavity (Georgi, 1985; Urquhart *et al.*, 1987). Within a week, the majority of flukes will have penetrated the liver capsule and be migrating in the liver parenchyma. Migration occurs for weeks after which they enter the main bile ducts. They increase in number and reach sexual maturity soon afterwards. From then onwards, the flukes start laying eggs that are found in the bile and subsequently faeces. It takes at least 8 weeks for *F. hepatica* to develop from infection to a mature fluke in the bile ducts, while *F. gigantica* takes a minimum of 11 weeks for the same process (Schumacher, 1938; Hammond and Sewell, 1974).



**Figure 2.2** Life cycle of *Fasciola* species (source: Centers for Disease Control, Atlanta, USA website).

### **2.3 The Economic Importance of fasciolosis**

More than 85% of tropical livestock are owned by smallholder mixed farmers or by other traditional groups. In sub-Saharan Africa, livestock products contribute over 25% of the Gross Domestic Product (GDP) (Ogborn, 1999). When the contribution made by manure and animal draught power is added, the figure rises sharply. Draught power is used across 52% of the developing countries' cropping lands, with households owning at least 1–2 heads. Draught animals used for both ploughing and transport are very important components of many farming systems.

The measurement and evaluation of economic effects of diseases including fasciolosis were considered Bitakaramire and Okao (1975) as well as Morris and Meek (1980). Ogunrinade and Ogunrinade (1980) reported that the assessment of losses due to parasitic diseases was of great relevance to many tropical countries where economic realities often determine the type and scope of control measures envisaged. Those losses due to mortality and chronic ill effects (e.g. decrease in milk yield, reduced weight gain, decreased fertility, poor carcass conformation, cost of treatment, susceptibility to other infections, reduced draught power) are probably the most difficult to assess. Therefore, the financial loss due to liver condemnations alone is often used in assessing losses due to fasciolosis (Hammond and Sewell, 1974; Malone *et al.*, 1982; Losos, 1986). Similarly, Hope-Cawdery (1984) reported that the usual estimate of prevalence has been based on liver condemnation rates.

### 2.3.1 Liver condemnations

Economic losses due to fasciolosis are a global concern. Livers are condemned when they are visually unpleasant (aesthetic) and are unpalatable to eat, diseased or have severe lesions. The usual information available is the percentage of livers condemned for fasciolosis at the abattoir. The figure given usually bears little association to the actual number of animals infected as the livers are often not totally condemned in light infections but only the affected parts are removed. Additionally, it is also easy to miss very light infections (Froyd, 1959) and the efficiency of detection will vary with the training and experience of the meat inspector.

The United Kingdom and Republic of Ireland recorded a ten-year reduction (up to 1987) in condemnations from 35% to 6% and 75% to 38% respectively (Dargie, 1986). Malone *et al.* (1982) indicated that about 1.4 million livers were condemned in 1981 alone in the Gulf Coast States of the U.S.A. amounting to an estimated US \$7.2 million loss. These condemned livers represented 22.4% of cattle livers that were condemned for all causes (6,184, 679) or 4.4% of all cattle slaughtered (32, 898, 968). Africa, on the other hand, seems to have had the brunt of the disease effects as shown in Table 2.1. In a two-year study carried out in a rural abattoir in Nigeria, Schillhorn van Veen (1980) showed that 65% of cattle, 40.8% of sheep and 17.6% of goat livers were condemned because of fluke infection. Still in Nigeria, Ogunrinade and Ogunrinade (1980) estimated an annual financial loss of US \$1.03 million as a direct result of mortality due to fasciolosis. In Kenya, an annual average of 11.5% in cattle, 2.5% in sheep and 2.7% in goats of all livers from slaughtered animals were condemned due to flukes (Anon, 1986). In the Ethiopian highlands,

about 51% of all livers were condemned because of *Fasciola spp* (Ngategize *et al.*, 1993). They further estimated a loss of US \$10.9 million because of small ruminant mortality from *F. gigantica* infection. Similarly, in Zimbabwe, Vassilev and Jooste (1991) reported that 30 Zimbabwean dollars (Z \$30) per head of cattle were lost because of deaths caused by *F. gigantica*. A total of 43.3% of the rejected livers in 1989 alone were as a result of *F. gigantica*. The same authors noted that the 1986 national incidence of *Fasciola spp.* as determined from condemnation figures at slaughter in Zimbabwe was 46.3%. This amounted to 527 tons of liver meat either condemned or trimmed. According to Chambers (1987), the loss incurred per head slaughtered was Z\$3.45. Alves (1970) found out that in Mozambique, a 50% rate of all slaughtered animals harboured liver flukes.

The survey by Silangwa (1972) was the first comprehensive study in Western province of Zambia to attempt to assess the incidence rate of this parasite and its economic significance in the rural livestock economy of the flood plains. The results confirmed that bovine fasciolosis was highly enzootic among the cattle on these flood plains. The 98.4% incidence rate obtained from actual examination of a sample of 192 livers of cattle from the Zambezi flood plain was much higher than the 89.6% incidence rate based on records of condemned livers due to fasciolosis at commercial meat inspections during the three decades before 1972.

The records of the Department of Veterinary Services in Zambia showed that between 1944 and 1964 about 65,000 Barotse cattle from the upper Zambezi basin were slaughtered at Livingstone abattoir with an annual condemnation rate of 89%. Also an estimated 9,000 Barotse cattle livers were condemned due to fasciolosis per

year in abattoirs and licensed butcheries (Maclean, 1965). This average annual loss was estimated at 37,000 kilograms of liver meat (Silangwa, 1972). In Zambia's Western province, condemnation rates of between 33% and 73% of bovine livers were due to fasciolosis alone according to abattoir records of 1980 to 1994 (Pandey and Ahmadu, 1998). In the same period, 95, 018 cattle were slaughtered at Mongu abattoir and 48,692 livers (51%) were totally condemned because of fasciolosis. As a result of these condemnations, the province was estimated to have lost up to US\$ 228, 244 of income during this period. This figure does not include losses in animals that went out of the province for slaughter or were slaughtered at local butcheries and villages. Neither did it include partial condemnations from trimmings, mixed infections predominated by other infections nor unnoticeable fasciolosis infection. Bovine fasciolosis has had a great impact in Western province to an extent that no bovine liver could go without being at least partially trimmed at meat inspection (Banda, 2002; personal communication). The national losses from fasciolosis in cattle are reported to be tremendous and figures from meat inspection and condemnation of livers indicate that the losses are continually on the increase (Pandey and Ahmadu, 1998).

### **2.3.2 Other effects of bovine fasciolosis**

Accurate assessment of the effects of *Fasciola spp.* infestation is difficult due to concomitant infestation in the field situation (Dargie, 1986). Fasciolosis has considerable economic and social consequences, but it is still uncertain to what extent these effects represent real reductions in production efficiency. Vassilev and Jooste (1991) noted that potential losses appear to be greatest where cattle are

subject to liver fluke, gastrointestinal nematodes and nutritional stresses during the

~~season. The disease significantly depresses weight gain and affects body~~

composition in cattle (Sewell, 1966; Hope-Cawdery, 1984; Malone *et al.*, 1982).

Fasciolosis can affect the reproductive performance of farm animals by way of reducing the potential breeding performance, by impairing the growth rate of young stocks, puberty in heifers and prolongation of oestrus in mature animals (Kumar and Sharma, 1991). Of 110 rural cows in their study, over 36% normal cyclic, 50% anoestrus and 46.42% repeater cows had chronic *Fasciola* infection. Further results indicated that *Fasciola* infection might be one of the contributing factors responsible for infertility due to anoestrus and repeat breeding conditions in rural cows where the animals are loosely kept and raised under grazing conditions.

## **2.4 Transmission and the intermediate host**

### **2.4.1 Transmission**

Studies carried out on bovine fasciolosis around the world have determined that there exists a relationship between transmission and climatic factors (Ollenrenshaw, 1971 in England; Manga-Gonzalez *et al.*, 1990 in Spain; Bhatia *et al.*, 1989 and Pandit *et al.*, 1989 in India; Mzembe and Chaundhry, 1979 in Malawi; Njau *et al.*, 1990 in Ethiopia; Amato *et al.*, 1986 in Brazil and Malone *et al.*, 1985 in the USA). Bovine fasciolosis has long been recognised to be associated with cattle of the wetlands (Urquhart *et al.*, 1987). According to Troncy (1989), transmission depends on several factors related to the biology of the vector, the biology of the parasite and livestock management. In many tropical and subtropical regions, the scarcity of feed and water forces herdsmen to move their livestock long distances in search of the

commodities. Inevitably, this leads to overcrowding at water holes or grazing areas. This in turn exposes the animals to high levels of infection by metacercariae (Hammond and Sewell, 1990; Losos, 1986). In Australia, where conditions are dry and hot, infections are usually due to recent contamination of the environment (Meek and Morris, 1979), while on the other hand, in temperate regions such as the United Kingdom and most of Europe, massive levels of metacercariae can accumulate over a period of time (Ross *et al.*, 1967).

The snail hosts of *Fasciola* are confined to the family Lymnaeidae. The transmission of *Fasciola spp.* is complicated by the involvement of two species of *Lymnaea* snails, one amphibious i.e. *L. truncatula* with worldwide distribution (Urquhart *et al.*, 1987) and the other aquatic, *L. natalensis*, found in the tropical countries (Hammond and Sewell, 1990; Losos, 1986). The important *Lymnaea* species involved in the transmission of fasciolosis vary in their geographical distribution in the world and so do their habitats. Those for *F. hepatica* are amphibious snails living near edges of slow moving or stagnant water. Those that transmit *F. gigantica* live in deeper water and are aquatic snails in their behaviour (Hansen and Perry, 1994). Kendall (1954) reported that in Pakistan, *Lymnaea auricular rufescens* nearly always lives in bodies of water which are permanent throughout the year and which contain abundant vegetation. Clean fresh water seems to be essential for the snail's survival and that it is usual to find a close association with aquatic water plants such as *Elodea*. For *F. gigantica*, the snail hosts belong to the superspecies *L. auricularia*, distributed throughout Europe, Asia and Africa. In Africa and parts of Asia it is represented by *L. natalensis*. This species is also the intermediate host for *F.*

*nyanzae* of the hippopotamus (Dinnik and Dinnik, 1961). *Fasciola hepatica* is transmitted usually by *L. truncatula* in Africa, Europe and South West Asia (Kendall, 1954; Boray, 1969). In West Pakistan, *F. hepatica* replaces *F. gigantica* above an altitude of 1200 m (4000 feet) in relation to the distributions of their different snail hosts, and the parasites do not share an intermediate host (Kendall, 1954). An additional potential intermediate host for *Fasciola* in some parts of Africa is *Lymnaea columella*, an introduced snail of American origin. It is known to have become a host in some other parts of the world where it has been introduced including New Zealand (Pullan and Whitten, 1972). *Lymnaea columella* is also widely distributed in South Africa, Zimbabwe and Zambia, but any contribution it may make to transmission of fasciolosis has not yet been evaluated (Brown, 1994). The semi-amphibious habits of this snail would favour the encystment of metacercariae on the marginal vegetation likely to be eaten by grazing animals. Although the two *Fasciola* spp. appear to be specific in their intermediate hosts, Boray (1969) was able to show that *L. tomentosa* is capable of being a vector for *F. gigantica*.

Kendall (1965) observed that snails of all ages might be susceptible to infection but that the maturation of cercariae was dependent on sufficient time and supplies of food. In his work on *F. gigantica*, he found out that it seemed that with the preferred host, individuals of all ages and sizes were susceptible. However, the immature snail species were quite resistant as compared to the adults that could frequently be infected and the infection proceed to maturity. To some extent, that susceptibility

may be varied by the environmental conditions of the snail at the time of exposure to miracidia.

#### **2.4.1.1 Development of the parasite**

Some factors could affect the development of the parasite within the snail. These include temperature on rate of development, temperature variation on formation of daughter rediae and cercariae as well as the effect on the parasite by changes in the nutrition of the host (Kendall, 1965). A direct relationship between the rate of development of the parasite and the environmental temperature of the host had been determined for *F. hepatica* (Cameron, 1934; Boray, 1963). Dinnik and Dinnik (1963a) working on *F. gigantica* found similar findings as those authors on *F. hepatica*. If the temperature is above 10° C, Lymnaeid snails can produce eggs that may hatch throughout the year. On average these eggs can reach 3 000 per month and one generation of snails from egg to egg takes only about one month to complete under optimum conditions (FAO, 1994).

The conditions present in the wetlands in Zambia are suitable for the existence and breeding of the snail *L. natalensis*, which is the intermediate host of *F. gigantica*. The Kafue and Zambezi river basins support such a larger cattle population because of their large wetlands and good pastures during the dry season. Fasciolosis is, therefore, the major helminthic disease of cattle in these areas and plays an important role in the economy of the livestock owners. Studies by Silangwa (1972) on the occurrence of liver fluke metacercariae on pastures found that the water pastures on the wetlands are the main habitats of *L. natalensis* and that cattle pick up the metacercariae while grazing on these pastures during the dry season. During this

time, conditions are ideal for *F. gigantica* transmission. Snail and cattle populations aggregate in the flooded areas at a time when temperature is ideal for the development of eggs and larval stages in the snail host.

## **2.4.2 Snails and their habitats**

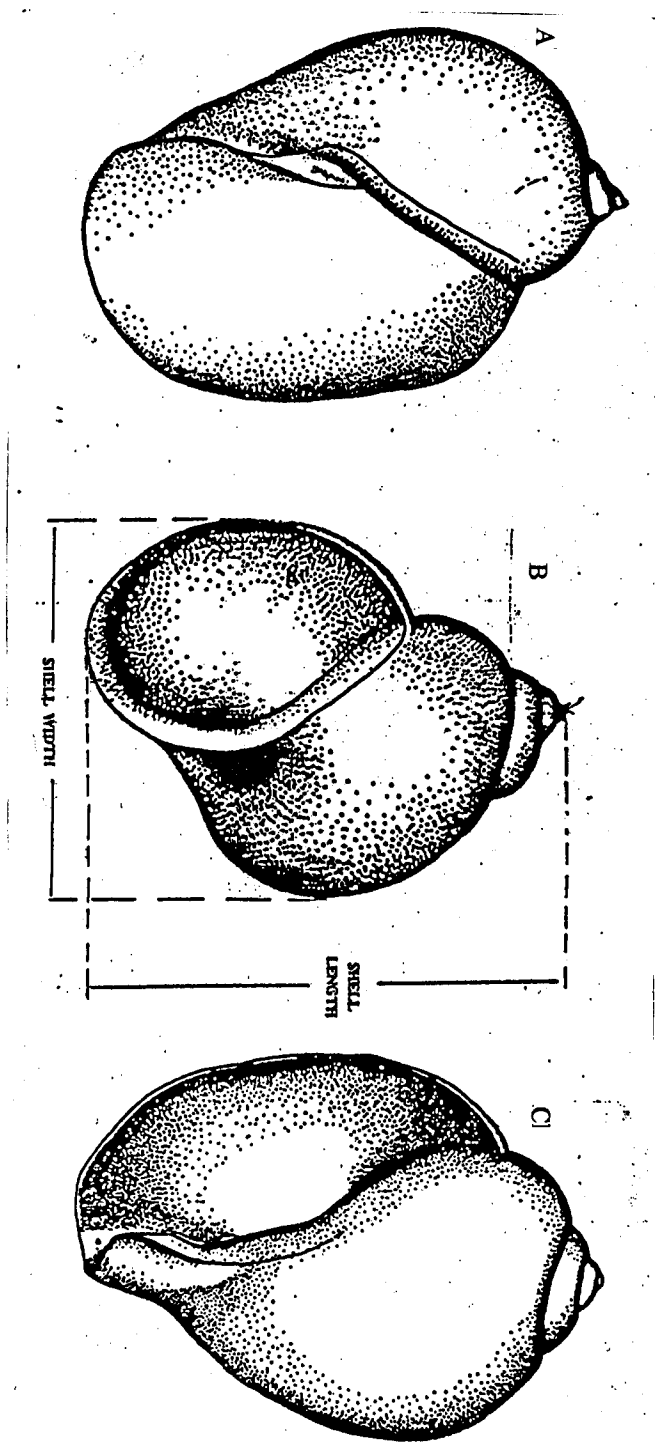
### **2.4.2.1 Snail Identification**

Certain species of African freshwater snails are exceedingly important both from a medical and veterinary viewpoint, for which reason, all African countries are working with them [Brown, 1994; Danish Bilharziasis Laboratory (DBL), 1998]. According to DBL (1998), the majority of about 60, 000 recent species of shell-bearing snails and all of the approximately 200, 000 described fossil species can be recognised by the shell alone. This makes the shell features important in the identification of snails to genus or species level. These include the form, shape and number of whorls, direction of coiling as well as sculpture and colour as shown in Figure 2.3.

Unlike the pulmonate snail (which include *Lymnaea* and *Bulinus spp.*), a prosobranch snail bends its foot when it draws itself into the shell. With a few exceptions, all snails of medical or veterinary importance live in freshwater (Brown, 1994). Almost all snail families living in fresh water are from a geological point of view old families as most of them are known from Mesozoic (Jurassic or Cretaceous) era. Owing to their great age, several families have an almost worldwide distribution (Brown, 1994; DBL, 1998). Unlike the determination of genus and species which is often a very difficult problem, freshwater snails are generally easy to identify to families. This is so because almost all members of one and the same family of

freshwater snails are often to a great extent alike, no matter from where in the world they descend (Brown, 1994).

Figure 2.3 The shell morphology of *Lymnaea natalensis* (A), *Bulinus tropicus* (B) and *Bulinus globosus* (C).



#### 2.4.2.2 Snail habitat

Most freshwater snails prefer stagnant or slowly running water. There are few, if any, pulmonates on the exposed shores of big lakes and in fast flowing rivers whereas prosobranchs and bivalves may be present. Freshwater snails are usually lacking in very acid or alkaline water, but can be found in all types of fresh water bodies from the greatest lakes, ponds and sluggish streams (DBL, 1998). Brown (1994) noted that the greatest species-diversities, though not necessarily the densest populations, of freshwater snails are usually associated with aquatic or subaquatic (emergent) leafy plants (macrophytes). Plants provide snails with shelter from solar radiation and the water current as well as act as sources of food and egg-laying sites. For example, the finding of egg capsules and juvenile snails indicated breeding at depths down to 4.5-5.0m by *L. natalensis*, *Biomphalaria pfeifferi*, *Bulinus natalensis* and *Burnupia* species (Brown, 1994).

Snail-plant associations differ among species and localities. These associations can be positive or negative. Several plants have been reported to affect snail populations in different ways. In Nigeria, for example, *B. pfeifferi* was positively associated with *Commelina* in a pond and with *Nymphaea*, *Lemna* and *Ceratophyllum* in a lake, but negatively associated with *Salvinia* (Thomas and Tait, 1984). Although snails are attracted to aquatic plants, they do not readily consume fresh tissues. They feed mainly on decaying tissues, epiphytic organisms and adhering detritus. The generally negative associations between snails and floating plants such as *Salvinia*, which densely cover the surface, as reviewed by Thomas and Tait (1984) may be due to the effect of shade in excluding submerged plants and decreasing the

oxygenation of the water. On the other hand, areas covered by water hyacinth on the Kafue river in Zambia (from Kafue Gorge to Nakambala water intake point) have been known to harbour relatively more snail populations and larval stages of insects than areas without the afore mentioned aquatic plant (Chota, 2003; personal communication).

#### **2.4.3 Snail shedding and identification of cercariae**

Harvesting/shedding of cercariae can be achieved basically by having the snail crushed or by natural emergence (Frandsen and Christensen, 1984). Of the two methods, the recommended one was natural emergence because it is contended that crushing the snail may result in the release of immature cercariae which are unsuitable for identification. It is important to note that non-cercarial organisms (e.g. leeches, crustaceans, nematodes and chaetogaster) living on the snail mantle or in the tissue of the snail may be recovered in attempting to shed cercariae and this happens mostly when using the crushing method (Frandsen and Christensen, 1984). Shedding by natural emergence of most types of cercariae (including those of all trematode species of major medical and veterinary importance in Africa) is carried out by placing single or groups of snails under artificial illumination in containers with either pond water or dechlorinated tap water at a temperature between 20–25 °C for a period of 2-6 hours (Frandsen and Christensen, 1984). In some types of cercariae, especially those from *Lymnaea* snails, emergence occurs during darkness (Hammond, 1970; Salimov and Azimov, 1983; Makundi, 2001). Therefore, no lighting is provided. Additionally, Manipol (1936) observed that darkness irrespective of the time of day, favours the shedding of cercariae. However, earlier

work on emergence of *F. hepatica* from *L. truncatula* done by Kendall and McCullough (1951) as well as Kendall (1965) concluded that there seemed to be no association between light and darkness but that stimulation of the snail, particularly by moving it to fresh water, was the factor that induced emergence. This action was considered largely a passive process of the cercariae. Depending on the goal of the study, it is necessary to check for emergence of cercariae for a 24 hour period of 12 hours light and 12 hours darkness (Frandsen and Christensen, 1984) or overnight.

The range of variation in cercarial morphology is considerable. To this effect, a classification system was created by Lühe (1909) for the sole reason of grouping cercariae into various types. In this classification, gross morphological characteristics that include number and position of body suckers, shape and relative dimensions of the cercarial tail and whether there is presence or absence of various surface structures (e.g. stylet and spiny collar) provide a basis for grouping cercariae into different major types. Using these morphological characteristics alone, identification of cercariae to the family level and sometimes to the genus level is generally possible (Frandsen and Christensen, 1984). Identification of cercariae to the major type level may normally be done using living and unstained cercarial specimens transferred to slides. Minor as well as not easily recognizable differences between and within genera, however, warrant further identification on the genus and species level. This can be done by way of supplementing earlier observations on morphology with those on cercarial biology. The biological criteria include, for example, the cercariae-producing snail, cercarial swimming behaviour and its resting position as well as further cercarial developmental scheme (i.e. encystations on an

external object). When cercarial morphology and biology do not come up with proper identification, this can be achieved on the basis of adult worm and/or egg morphology produced following infection of a definitive host (Frandsen and Christensen, 1984). The metacercariae of *F. gigantica* are grey-white when fresh, becoming grey-brown with age (Hammond, 1970).

## 2.5 Prevalence of fasciolosis

The major definitive hosts of *F. gigantica* are domestic animals (cattle, goats, sheep and water buffaloes) while equines (horses, donkeys, mules) are less susceptible (Soulsby, 1982). The prevalences of fasciolosis caused by both *F. hepatica* and *F. gigantica* in cattle have been reported world wide. 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 Kenya, there were several surveys carried out. Froyd (1959) found that 35.4% of the 1,000 cattle at Kenya Meat Commission abattoir at Athi River were infected with *F. gigantica*. Froyd (1960) in another survey, found that out of 1,000 cattle, 34.1% were infected with *F. gigantica* or had lesions that were assumed to be due to the chronic form of the disease despite absence of the parasites. Bitakaramire (1968) found that 32.84% of livers were condemned in 1966. In Tanzania, Hammond (1965) conducted a survey in the mainland and found out that the proportion of cattle livers condemned for fasciolosis varied from 1% at Tabora abattoir to 46.8% at Iringa. Furthermore, liver condemnations at Tarime and Musoma abattoirs were reported as 38% and 37.2%, respectively. Thus, Tarime and Musoma were the two other areas with high rates of condemnations. These figures did not reflect those livers with light infections that were trimmed but not condemned. In Uganda, the incidence of fasciolosis was investigated by Coyle (1958, 1961). In these studies, only *F. gigantica* infections were recorded in which total condemnations of bovine livers accounted for from 14.7% to 46.9% from 1948 to 1952 at Kampala abattoir. From 1951 to 1956, a range of 7.5% to 44.7% was found at Jinja abattoir. Coyle (1961) stated a 100% incidence in Eastern province of Uganda as most grazing areas were composed of grass swamps containing good snail habitats. In Mbarara abattoir, a 40% rate was found while in Mengo district, the incidence in cattle was about 25% in some areas but very low in others. Some prevalence rates of fasciolosis caused by *F. gigantica* in parts of Africa are shown in Table 2.1.

**Table 2.1** The prevalence of *Fasciola gigantica* in selected countries of Eastern and Southern Africa.

Country	Prevalence (%)	References
Kenya	19.8	Cheruiyot (1987)
Mozambique	70	Alves (1970)
Tanzania	28.4–63.8	Keyyu <i>et al.</i> (2003a)
Zambia	51	Pandey and Ahmadu (1998)
Zimbabwe	50	Chambers (1987)

*Fasciola gigantica* has also been found in wildlife: hippopotamus (*Hippopotamus amphibious*), grey duiker (*Sylvicapra grimmia*), kudu (*Tragelaphus strepsiceros*), giraffe (*Giraffa camelopardalis*), eland (*Taurotragus oryx*), lechwe (*Kobus leche*) elephant (*Loxodonta africana*), wildebeest (*Connochaetes taurinus*), buffalo (*Syncerus caffer*) by several workers in different countries in Africa (Sachs and Sachs, 1968; Coyle, 1961). This fluke has also been found in the warthog. Moderate prevalences have been found in wild grazing animals, although infection rates are generally low (Hammond, 1972). This is unlike the infection in cattle and sheep which is heavier and can cause considerable economic loss. Munyeme (2003, unpublished data) found *F. gigantica* in eight (80%) of the ten Kafue lechwe that were slaughtered in the Lochnivar National Park of Zambia. This park lies in the Kafue river basin. Deaths of wild animals as a result of fasciolosis have been recorded in Zimbabwe (Brown, 1994). In addition, prevalences of about 50% in buffalo, hartebeest and kob in some areas of Uganda suggest that fasciolosis is maintained there in the absence of domestic livestock. *Fasciola nyanzae* which is

known only in hippopotamus causes prevalences that can reach almost 100% (Hammond, 1972).

## **2.6 Diagnosis**

### **2.6.1 Gross pathology**

Lesions produced by *F. gigantica* and *hepatica* are the most constant and important in the liver although, occasionally, the parasite may reach the lungs or other tissues where they are usually found within abscesses (Jones and Hunt, 1983). Taylor (1964), Losos (1986) and Burger (1992) noted that gross pathological lesions depend on the stage and severity of the infection. The liver pathology is characterized by hepatic fibrosis and hyperplastic cholangitis, irregular liver outline, pallor and firmness with the ventral lobe being most affected and reduced in size (Urquhart *et al.*, 1987). Urquhart *et al.* (1987) further observed that sometimes the fluke eggs provoke a granuloma-like reaction that can result in obliteration of the affected bile ducts. Chronic cholangitis and bile duct obstruction lead to ectasia and stenosis of the ducts, periductular fibrosis and thickening of the walls such that the ducts become increasingly prominent and sometimes calcified (Ross *et al.*, 1967; Simesen *et al.*, 1968). Though calcification is usually seen in cattle and not in sheep (Taylor, 1964; Phiri, 1997), hyperplasia of the bile ducts in both species begins long before the flukes arrive (Taylor, 1964; Simesen *et al.*, 1968; Boray, 1969; Burger, 1992;).

### 2.6.2 Histopathology

Variable histological lesions have been noted in livers affected by *Fasciola spp.* (Uzoukwu and Ikeme, 1978; Singh and Parihar, 1988; Phiri, 1997). Singh and Parihar (1988) examined 5,544 animals (1,164 sheep and 4,380 goats) slaughtered at Bareilly abattoir in India. Lesions found that were produced by migrating stages of liver flukes included haemorrhagic tracts of erythrocytes and degenerating hepatocytes mixed with variable numbers of eosinophils, polymorphs and mononuclear cells. The lesions in the bile ducts and periportal areas consisted of hyperplastic changes with variable increase in the periductular connective tissue. In advanced stages, pronounced changes were encountered in the periportal areas (Singh and Parihar, 1988). Different levels of bile duct dilation and desquamation were observed in that study. Some times, liver flukes were found in the lumina causing flattening and/or hyperplasia of the epithelial lining. Degenerating flukes and eggs usually attracted foreign body giant cells surrounded by macrophages, lymphocytes and fibrous tissue forming nodules of various sized. Uzoukwu and Ikeme (1978) studied 6,887 livers of Fulani Zebu cattle slaughtered at Nsukku abattoir in Nigeria between 1975 and 1977. Out of that number, 15 livers were totally condemned while 60 were partially condemned. Marked interlobular fibrosis, proliferation of bile ducts and peribiliary fibrosis were also found. Numerous micro-abscesses containing *Fasciola* larvae or eggs can be found accompanied by cellular reactions and calcification (Uzoukwu and Ikeme, 1978). Patches of black pigment appear due to haematin discharged by the fluke (Sinclair, 1964). Ross *et al.* (1967) carried out a comprehensive histological comparison of the lesions of fasciolosis in

pigs and other hosts from 1–10 weeks after infection. In that study, pigs were shown to exhibit mild lesions than those found in cattle, sheep and goats.

### **2.6.3 Coprological examination**

Fasciolosis is mainly diagnosed by clinical observation and detection of eggs in the faeces. Additionally, Urquhart *et al.* (1987) stated that diagnosis is based on clinical signs, seasonal occurrence, prevailing weather patterns and a previous history of fasciolosis on the farm or the identification of snail habitats. However, coprological analysis is still the routine method used in diagnosing patent fasciolosis. Coprological examinations alone are of limited value especially when applied to prepatent infections or low-level infections (Losos, 1986; Burger, 1992). Liver fluke infection is still an endemic disease in many parts of the world and that there is a need for simple, reliable and sensitive serological tests for use in epidemiological surveys, routine diagnosis of infected herds and monitoring the response to drug therapy (Levieux *et al.*, 1992).

Coyle (1958) noted that naturally infected cattle in Uganda had low egg counts and that there was no correlation between the egg counts and the fluke burden. Boray (1969) and Duwell and Reisenleiter (1984) reported finding neither uniformity nor consistency in low, medium and high worm burdens after faecal egg counts. Hammond (1970) in one of his experiments found that faecal egg counts of *F. gigantica* in cattle are low after about 7-8 months post infection over a wide range of infection levels. He further observed that because of large mass of faeces passed daily, even the low counts might be of importance in the epidemiology of *F. gigantica* infection. To elaborate this fact, Taylor (1964) estimated that the average

adult bovine animal passes about twenty-two kilograms of faeces a day so that even a count of 5 EPG from such an animal would mean 110,000 eggs passed daily. Fluke eggs accumulate in the gall bladder and are thereby expelled unevenly creating variations in egg counts in the faeces. In addition to the above, there are some pathological changes that are encountered which include peribiliary obstruction of the egg passage (Boray, 1969; Malek, 1980; Urquhart *et al.*, 1987).

In cattle that harbour patent infections of *Fasciola*, the recovery of eggs from their faeces remains a sure method of diagnosis of liver fluke infection. This is so in spite of the vast array of serodiagnostic techniques that have evolved and been described over the decades. According to Boray (1985), the diagnosis of infections of *Fasciola* species in ruminants has relied on the detection of *Fasciola* eggs in the faeces of infected animals. However, there are drawbacks to this method. Detection of relatively small numbers of eggs in relatively large amounts of faeces is determined by the size of the sample and constitutes a major difficulty for diagnosis (Boray, 1985). Dumenigo *et al.* (2000) noted that the microscopic examination of fecal samples for the presence of *F. gigantica* eggs is often difficult as they may not be found during the prepatent period. Sometimes, it is as difficult even during the patent period because of intermittent excretion of parasite eggs.

## **2.7 Control Measures**

Control of liver flukes can take two forms: control of the snail intermediate host and treatment of affected cattle (Simpson *et al.*, 1985). As a general principle, control measures must be cheap and as simple as possible and should require the absolute minimum of effort and supervision if they are to be effective (Hammond, 1965).

Knowledge of the ecology of the parasites holds the key to the success of the control measures (Over, 1982). On the other hand, the economic feasibility of any measure(s) taken or being considered is another major factor (Hammond and Sewell, 1990).

### **2.7.1 Treatment of infected animals**

Simpson *et al.* (1985) noted that overall, the economic analyses show that treating for flukes is definitely a recommended practice and that even under the most conservative estimates, returns exceed costs.

Triclabendazole (TCB) is the most effective fasciolicide (Losos, 1986; Troncy, 1989; Hammond and Sewell, 1990; Richards *et al.*, 1990). Torgerson and Claxton (1999) have reported that since in non-industrialized countries anthelmintics may be prohibitively expensive, the need of a safe and less expensive fasciolicide is required. At present, the *Fasciola* is becoming resistant to one of the most world-wide recommended fasciolicide, TCB (Thomas *et al.*, 2000; Gaasenbeek *et al.*, 2001). Therefore, development of new, cheaper and safer fasciolicides should be encouraged. Rafoxanide is another fasciolicide commonly used (Losos, 1986; Troncy, 1989). Other anthelmintics include closantel, nitroxylnil, hexachlorophene and carbon tetrachloride.

Phiri (1997) observed that the efficiency of a fasciolicide should ideally be measured against its ability to cure most important *Fasciola* species from immature to mature stages (i.e. having a widest therapeutic index), and its being non-toxic to most host animals. The form of these drugs should enable easy application to large numbers of

animals in the field so as not to interrupt the normal life of the host or cause reduction in its productivity. The mode of application should not seriously interrupt the normal operations on the farm, either. In the past, commonly used fasciolicides were mainly hydrocarbons. Owing to their many side effects, they are no longer widely used. These include carbon tetrachloride, hexachloroethane and hexachlorophene (Gibson, 1969; Losos, 1986).

It is generally advised to treat all animals at the beginning of the dry season and exposed animals, again at the beginning of the wet season (Schillhorn van Veen, 1979). The latter treatment may be of strategic importance as it reduces the pasture contamination at the time that the majority of snails acquire miracidial infections (Schillhorn van Veen, 1980).

### **2.7.2 Snail control**

Unlike the control of schistosome intermediate hosts, the control of *Lymnaea* has been undertaken in only a few places (Brown, 1994). As a concept, snail control is an accepted part of the integrated control strategies that are most likely to succeed (Chandiwana and Christensen, 1988; World Health Organisation, 1993). It must be noted that controlling transmission of trematodes through reducing snail populations must be thorough to be effective, for a moderate reduction of snail numbers may result in an increased proportion of the remaining snails becoming infected (Brown (1994). This principle follows the ability of miracidia to actively seek a snail host and is a density-dependent effect demonstrated by mathematical modelling (Woolhouse and Chandiwana, 1990).

Measures to control snails can be divided into three categories, namely: chemical, environmental and biological. Control is attempted most often in water bodies modified or entirely made by man, ranging from various small habitats (e.g. earth dams, small rice fields and ditches) to large irrigation systems and man-made lakes (Brown, 1994).

### 2.7.2.1 Chemical methods

Snail populations and reduction of *Fasciola* transmission can be greatly reduced using chemical methods alone, but the costs of chemicals and skilled labour are recurrent, as the programme needs to be maintained permanently if effective disease control is to be achieved.

Molluscicides can be an effective means of reducing snail populations, at least temporarily, and has indeed played an important part in schistosomiasis control, reviewed comprehensively by McCullough (1986, 1992). Nonetheless, the high cost and environment impact renders them unsuitable for use in many countries. Mollusciciding is particularly well suited to small seasonal transmission sites in semi-arid areas (Brown, 1994). However, special problems are posed by rivers, man-made lakes, large irrigation schemes and dams. The efficacy of four molluscicides, Sodium Pentachlorophenate (NaPCP), N-tritylmorpholine (Frescon), copper sulphate, Niclosamide (Bayluscide®) and Yurimin on *Lymnaea ollula* was tested and it was found that Bayluscide® was the most effective of them all (Harada, 1974). Complete eradication of snails is rarely achieved. Usually, molluscicides are applied in water bodies without much consideration for other aquatic fauna. In some instances, non-target organisms might be exposed to harmful effects by the drugs

thereby upsetting the balance of nature. The example of copper sulphate being toxic to both livestock and fish when applied as a molluscicide at 0.1-0.2ppm is stated by Soulsby (1982). Webbe and Jordan (1982) and Appleton (1985) recorded similar findings.

#### **2.7.2.2 Environmental control**

Much has been written about the theory and practice of modifying man-made and natural water-bodies so as to reduce or eliminate snail populations (Webbe and Jordan, 1982). Considerable success has been achieved in reducing habitats for *Oncomelania*, the amphibious intermediate host for *Schistosoma japonicum* in the far East, but such methods have not been applied to a greater extent in Africa where the snail hosts are truly aquatic (Brown, 1994). Environmental factors which influence snail distribution and which might be manipulated to achieve snail control in Africa were suggested by Thomas and Tait (1984) and include factors like light, water chemistry (especially oxygen tension), water flow, sediment type, seasonal drying, aquatic and subaquatic plants and other snail species. Thomas and Tait (1984) further discussed measures to eliminate persistent snail habitats with favourable aquatic vegetation using, for example, (i) drainage and canalisation of the source areas of streams (ii) dredging and straightening of lower courses and (iii) removal of favourable vegetation from water and stream banks. However, any proposals for interfering with natural watercourses by engineering should always be treated with great caution. Brown (1994) stressed the fact that likely consequences of removing vegetation and increasing rate of flow in streams are soil erosion and flooding, production of environmental damage which could outweigh any reduction

achieved in transmission of snail borne diseases. Snail control can also be associated with the efficient management of irrigation schemes, as removal of aquatic vegetation promotes free flow of water and also removes shelter and food sources for snails (Brown, 1994). Prevention of seepage eliminates marshy habitats in which snail hosts can thrive. Weed control is, however, costly and plants speedily recover, with only a small effect on snail populations (Hilali *et al.*, 1985).

#### **2.7.2.3 Biological control**

Biological methods for the control of freshwater snails were reviewed by Madsen (1990, 1992) who concluded that more emphasis should be put on searching for pathogens or microparasites as agents for control.

#### **2.7.2.4 Management**

Control by farm management and environmental manipulation of the snail environment is arguably the cheapest method of avoiding fluke infection in areas where the eco-system permits (Phiri, 1997). This can take the form of providing good drainage for low-lying areas, fencing off water swamps, construction of water troughs etc (Simpson *et al.*, 1985; Hyera, 1984). Schillhorn Van Veen (1979) noted that there is some indication that limiting flood plain grazing just before the rainy season, as this reduces the miracidial contamination and subsequently the snail population can prevent outbreaks.

## CHAPTER THREE

### GENERAL MATERIALS AND METHODS

#### 3.1 Study areas

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, Namibia, Botswana, Zimbabwe, Mozambique, Malawi, Tanzania, and The Democratic Republic of the Congo. It occupies an area of 75 million hectares or about 752 610 square km. The land constitutes a series of plateaux that undulate between 900 and 1600 metres above sea level. About 80% of the country is covered by miombo woodland.

According to temperature and rainfall, the year in Zambia can be divided into 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 range is 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 before the onset of the rains. There is no rainfall during the dry season and humidity is often below 20% for extended periods. The country has been experiencing fluctuating weather patterns and partial droughts for most parts of the last five years thereby drastically affecting agriculture.

The entire study was done in Lusaka, Central, Southern and Western provinces of Zambia where predominantly traditional cattle are kept (Figure 3.1). These

provinces as well as Eastern province altogether harbour about 95% of the country's cattle population (Appendix Table 1). Unlike commercial farmers, traditional farmers rarely practise any form of worm control in their livestock. Important geographical features in these areas are the Kafue and Zambezi rivers with their associated wetlands. Livestock in Zambia are integrated with and play a crucial role in the mixed cropping systems that support most livelihoods in rural settings. The greater areas of the wetlands are covered by grasses of nutritional value only when green at the beginning (November-December) and end (June-July) of the flood season when the lagoons and the marshy pastures are still submerged. However, this vegetation of the lagoons, ox-bow lakes and the marshy areas consists of grasses which provide good grazing during the drier period from August to December.

### **3.2 Study design**

The study animals belonged to several cattle breeds. Three breeds of cattle in the traditional sector were recognised as Tonga in Southern and Central provinces (medium horned Sanga type) and Barotse cattle in Western province (long-horned Sanga type).

This study involved four components. The first component was the prevalence study involving liver inspection and coprological examination (faecal egg count) while the second component was the seasonality study in cattle that also used both liver inspection and coproscopic examination. The third component involved collection and/or purchase of infected and non-infected livers from abattoirs for dissections. The fourth and last component involved identifying and examining snails for larval trematode infection in the water bodies along the Kafue river basin.



### **3.2.1 The prevalence surveys**

They were carried out from March to December, 2002 and involved liver inspections. They were conducted based on the size of the abattoir and the willingness of both the management and the livestock owners to be involved in the surveys. Liver inspections were done on randomly selected cattle and it was from these inspected animals that faeces were collected. The selected abattoirs and slaughterhouses that catered for the cattle catchment areas of Lusaka, Central, Southern and Western provinces of Zambia were at Chisamba, Kafue, Mongu and Senanga.

### **3.2.2 The seasonality study**

This was undertaken from March, 2002 to February, 2003 at Kafue slaughter slab. At this slab, only cattle from traditional farmers in the Southern province of Zambia are slaughtered. During the study period, 288 randomly selected cattle were examined at slaughter during the weekly or monthly visits by liver inspection and their faecal samples by coprological examination. Cattle from Gwembe and Siavonga districts along the Zambezi river as well as Mazabuka, Monze and Namwala districts along the Kafue river basin were sampled. On average, 24 cattle livers were sampled monthly with a range of 9 to 45.

The study animals were distributed according to sex and age as shown in Table 3.1. Significantly ( $p < 0.001$ ) more males (56.4%) among younger cattle were examined in the study than they were females (25.6%). There were more females (74.4%) among sampled adult cattle than there were males (43.6%).

**Table 3.1** Age and sex distribution in numbers and percentage (%) of the sampled cattle in the study.

Age (years)	<u>Male</u>		<u>Female</u>		<u>Total</u>	
	No. examined	(%)	No. examined	(%)	No. examined	(%)
1-3	92	25.6	92	25.6	124	(43.1)
≥4	71	74.4	71	74.4	164	(56.9)

### 3.2.3 Liver dissections for the determination of fluke burden

A total of 70 condemned and 32 non-condemned livers were collected at random from Chisamba, Senanga and Mongu abattoirs and Turnpike slaughter slab from March, 2002 to October, 2003. About five livers were collected per monthly visit and analysed in the laboratory for a precise assessment of liver fluke burden. Livers that were used in the study came from cattle of varying ages ranging from a year to ten years old. However, the mean age was  $5.61 \pm 2.24$ . A hanging scale was used to weigh the collected livers. Faecal samples were collected from the rectum of animals whose livers were also examined and analysed for fluke eggs.

### 3.2.4 The snail survey for larval trematodes

Areas for quantitative snail sampling within the parts of the Kafue river basin were selected on the basis of the ocular survey (i.e. systematic search for snails without estimating density, but looking for the presence or absence of snails) (Madsen, 2003). Nineteen snail habitats from six areas in the river basin were sampled. Each

Habitat was represented with at least three sampling sites where possible. In identifying sampling sites, points where cattle accessed water and grass were critical (Figures 3.2 and 3.3). An inspection of the presence of hoof prints, faeces and the actual presence of cattle at such designated points were undertaken. Also noted was whether an area was infested with aquatic weeds like water hyacinth or not. All these areas are located in the East Kafue river basin. The areas were situated at Chiyasa, Blue Lagoon and Kaleya. Others were at Shimungalu, Kafue Bridge and Shing'oma.



**Figure 3.2** Shing'oma habitat 2 used for drinking water for cattle and humans, watering vegetables, laundry and washing utensils.



**Figure 3.3** Blue Lagoon, Kafue flats. Lots of cattle graze these vast areas rich in nutritive grasses for about six months of each year when pastures and water are scarce.

### 3.3 Sample collection

Simple random sampling method was employed in collecting all the samples. On any sampling visit, every animal slaughtered from arrival in the abattoir and before noon was sampled. The liver inspections were conducted at random every month of the year. It was from inspected animals that faeces and some livers (condemned and not condemned) were collected. Individual livers were put in labelled plastic refuse

bags before dissections were done at the laboratory. Faecal samples were collected from the rectum of an individual animal after it was stunned with a stun gun and bled. Examination gloves that were appropriately labelled were used for collection and transportation of faecal samples to the laboratory for analysis.

### **3.4 Age estimation**

Age estimation of cattle was done by inspection of the incisor teeth according to Yeates and Schmidt (1974). This follows a principle that there is a temporary set of four pairs of incisors (milk teeth) which are replaced, one pair at a time, by permanent incisors at a certain fairly predictable interval during the first three to four years of the animal's life. These are the changes that were used to estimate age. From 1.5 to 2 years, two central temporary incisors are replaced by two much bigger and broader permanent teeth. At 2 to 2.5 years, the next pair is generally replaced. At about 3 year old, only one temporary tooth remains on each side. At 3 to 3.5 years, a full set of broad permanent teeth is generally in place. From then on, the degree of wear of the teeth and the formation of necks at their base due to the receding gum line, give some help in assessing age of an animal. In those cattle that had horns, the length of the horns and their associated rings was also useful. Those animals estimated to be less than 4 years old were considered as young cattle while those 4 years and above as adults.

### **3.5 Liver inspections**

Liver inspections were done on all slaughtered animals that were sampled. The carcass was inspected routinely. After removing the trachea, associated lungs,

diaphragm and liver as one structure from the thoracic and abdominal cavities, they were hanged on a hook ready for inspection. Emphasis in our study was given to the examination of the liver and its associated gall bladder for the presence of liver flukes. Visual observation and palpation was made on the liver before sharp incisions were made on the surface and through the major bile ducts into the parenchyma (Figure 3.4). The exposed bile ducts were squeezed and examined for the presence of any flukes. Lymph nodes draining the lungs and liver were incised and examined for any infection.



**Figure 3.4** Liver inspections (visualization, palpation and incisions) done within the abattoirs during the study.

### 3.6 Coprological examination

The faecal samples collected from the rectum of cattle in the abattoir were processed in the laboratory for the recovery and detection of *Fasciola* eggs. It was very

important, however, to distinguish between *Fasciola* and amphistome eggs. Their differences were adequately described by Dinnik (1958) and Dinnik and Dinnik (1963b). *Fasciola* eggs are oval, operculated and golden brown eggs while those of amphistome are large, clear, operculated and containing large granules.

### **3.6.1 Trematode egg counts**

A sieving technique with the glass beads layer for the detection and quantification of *Fasciola* eggs as described by Taira et al. (1983) (Figure 3.5) was used to quantify the fluke egg. The method is both qualitative and quantitative. The beads are of a special size 590-710 micrometer in diameter and 2.5 specific gravity. Centrifuge tubes with rounded bottoms and of 60 ml capacity, 30 mm inside diameter, and 100 mm in height were used. The process of the beads comprised three steps, namely, pre-treatment, selective sieving and sedimentation. During pre-treatment stage, centrifuge tube A received one-gram of sample mixed with about 10 ml of tap water. The suspension was transferred to tube B through the 60 mesh. The tube was filled up with water that was used to rinse tube A. Tube B was kept still for 5 minutes to allow for sedimentation. During selective sieving stage, tube B was placed into the rack of the electric rotator and rotated five times at a velocity of about 10 seconds per rotation. All the supernatant containing debris on the beads layer was siphoned off. The beads were then agitated and the eggs re-distributed in the suspension after pouring 50 ml of tap water in to the tube. In the sedimentation stage lasting about five minutes, the beads sunk to the bottom in a moment and the fluid was returned to tube A. The resultant supernatant was siphoned off leaving about 2 ml of the sediment. The sediment was then placed on a glass slide by a Pasteur pipette and

mixed with a drop of 1% methylene blue for staining. When examined under a compound microscope using 40 and/or 100 times magnification, *Fasciola* eggs appear bright yellow in contrast with the blue colour of the debris.

Taking note of the fact that faecal egg per gram (EPG) count may not truly or accurately reflect the number of flukes in an animal, we graded the degree of infection according to a method by Vassilev and Jooste (1991) with minor modifications. Therefore, 1-10 EPG was considered chronic subclinical disease (light infection), 11-25 EPG as chronic clinical disease (moderate infection), 26-50 EPG as serious chronic clinical disease (heavy infection) while greater than 50 EPG as severe infection.



**Figure. 3.5** Trematode coprological examination apparatus employed in the processing of the collected faecal samples.

### **3.7 Liver dissections**

The liver dissections were done according to a method described by Hansen and Perry (1994) with minor modifications. Livers were sliced to about 5mm thickness in order to examine the hepatic parenchyma as well as to locate flukes in the bile ducts. The flukes that were seen while dissecting the livers were collected and temporarily stored in normal saline before being counted. When the whole organ was sliced, all the liver parenchyma was covered with water in a tray. These pieces of liver were then squeezed as they were being removed from water. The remaining water and flukes that were extracted from these tissues were passed through a sieve so that the trapped flukes on the sieve would be collected. All the collected flukes were then placed in Petri dishes and counted. As some flukes were broken, only the anterior ends were counted.

Liver sections showing pathological lesions were stored in 10% formalin and processed as described by Bancroft and Stevens (1982) with minor modifications. 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. Tissue blocks were made after the following five major steps. These were dehydration, clearing, impregnation, embedding and blocking out. 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. Imbedded tissues were blocked out after cutting straight lines around each tissue or group of tissues before breaking them apart. Tissues were cut into sections using a microtome set at 3 microtons thickness dial gauge. Each section was transferred by floating it in boiled and cooled distilled water before mounting on to a slide. Haematoxylin and Eosin stain was used for staining the prepared sections. The completed stained sections were then mounted with a cover slide ready for examination and photographing. These sections were then examined and photographed. All the photographs of histopathological sections were taken using Fuji 200 asa film.

### **3.8 Sampling of snails at potential habitats**

Snails were sampled using a scoop made from a kitchen sieve mounted on a 1.5 m Long wooden handle as described by Coulibaly and Madsen (1990) (Figure 3.6). Scooping was undertaken for 15 minutes at each potential snail habitat and at the edges of the water body as seen in figure 3.7. Numerous snail species of different sizes were collected from habitats with varying ecological factors (Appendix Table 2). All habitats had different levels of coverage by aquatic vegetation. Similarly, shading was present in all the habitats sampled and was provided by various types of vegetation.

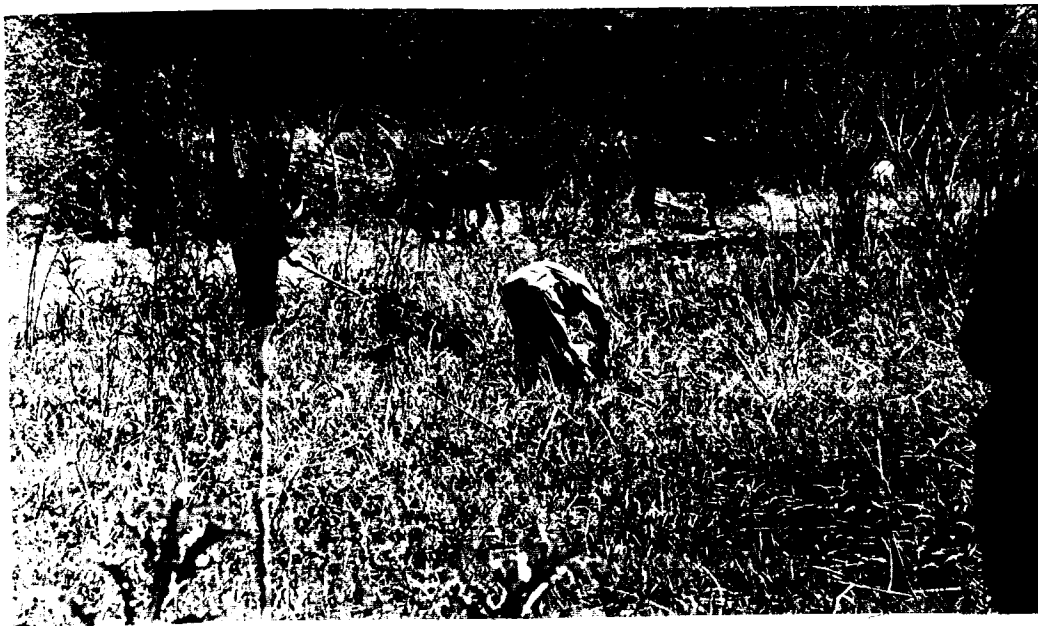
#### **3.8.1 Snail identification**

Snail species were identified according to the morphological features described by Brown and Kristensen (1989). The features noted were form, shape, number of whorls and direction of coiling, sculpture and colour of the shell. Cercariae-producing snail(s) were identified to species, or at least genus level as this was

important from the viewpoint of cercarial identification (Frandsen and Christensen, 1984).



**Figure 3.6** Snail sampling technique using a kitchen sieve tied to a wooden handle.



**Figure 3.7** Cattle grazing and drinking around pond as sampling of snails was in progress at Shing'oma.

### **3.8.2 Snail shedding/cercarial harvest**

Lymnaea snails over 6 mm in length were collected and examined for infection as it seemed unlikely that the infection would have developed enough to be easily seen in smaller ones. A strip of laboratory film, Parafilm "M"® (American National Can™, Chicago, Illinois), was stretched over a Petri dish to form a trough before an individual snail was put in. In order to prevent shell damage, a plastic forcep was used to lift a snail from the collection beaker and putting it in the trough created. Thereafter, about 10 ml of filtered pond water was deposited in the Petri dish using a 20 ml syringe. Based on findings of Da Costa et al. (1994) that shedding of cercariae from *L. natalensis* and their transformation into metacercariae occurred mainly at night, a cover was placed on the Petri dish containing an individually confined snail before putting it in the dark compartment overnight. This whole setup was left undisturbed in the laboratory under room temperature. Shedding of cercariae from other snail types was induced by exposing snails to artificial illumination for 2 hours as described by Frandsen and Christensen (1984).

### **3.8.3 Cercariae and metacercariae (cyst) identification and counting**

After shedding Lymnaea snails overnight, either cercariae or metacercariae were examined under a stereomicroscope using x 50–75 magnification. Similarly, cercariae from other snails were examined likewise. Additionally, a few cercariae from the emergence Petri dish were removed using a Pasteur pipette and transferred in a drop of water on to a glass slide. A cover slip was carefully placed on the glass slide and excess water was drawn using a strip of filter paper applied to one side of the cover slip. Removal of excess water gave rise to immobilization of the cercariae.

This in turn lead to slight flattening thereby making external structures of the cercaria or the cyst more easily observable. The prepared slide was then examined under a compound microscope at magnification of 160–400 times. Living and unstained cercariae were identified based on the basis of gross morphological characteristics, swimming behaviour, resting position and further cercarial development as described by Frandsen and Christensen (1984) and Schell (1985). In addition, cercariae belonging especially to amphistome and *Schistosoma* genus were identified in association with knowledge of their snail hosts.

Using a diamond pencil, grid lines were etched at the base of a Petri dish to aid in the counting and, where not possible, estimating numbers of cercariae/metacercariae. The grading of the level of infection in the snails was as follows: less than 200 were considered as mild infection; 200-1000 as moderate infection while that greater than 1000 as severe infection. Metacercariae/cysts of *Fasciola* were counted and handled while still adherent to the parafilm or floating on water, a method similar to that described by Urquhart (1954) with modifications.

#### **3.8.4 Coprological examination of cattle accessing snail habitats**

Faecal samples from 79 cattle that accessed the surveyed snail habitats were collected (Figures 7.2 and Fig 7.4) and analysed for the presence of liver fluke eggs. Some samples were collected from the rectum of the animals while many were collected from the ground just after the animals had defecated.

#### **3.9 Statistical analysis**

Analysis was performed using EPI-INFO 6.0 software (Centers for Disease Control, Atlanta, USA) and SPSS 11.0 for Windows (SPSS Institute, Chicago, USA).

### **3.9.1 Prevalence surveys**

The Pearson's chi-square test, Fisher's exact test and continuity corrected chi-square test were used to determine whether there were associations of origin, age and sex on the prevalence of fasciolosis. The Kappa test was used to measure the rate of agreement between meat inspection and coprological examination in the detection of *F. gigantica* infection.

### **3.9.2 Seasonality**

The Pearson's chi-square, Fisher's exact test and Yates corrected chi-square test were used to determine the associations of origin, age and sex of cattle on the prevalence of fasciolosis. Faecal egg count medians of males and females were compared by Mann-Whitney. Kruskal-Wallis (H) test was used to compare distributions of faecal egg counts among the different seasons.

### **3.9.3 Liver dissection for fluke burden**

Flukes were counted manually while their frequencies were calculated using SPSS. Students T-test was used to compare mean fluke egg counts from animals whose livers were condemned and those that were not condemned. Additionally, the test was used to examine whether there was any association between fluke egg counts and the number of flukes in livers.

## CHAPTER FOUR

### RESULTS

#### 4.1 Prevalence

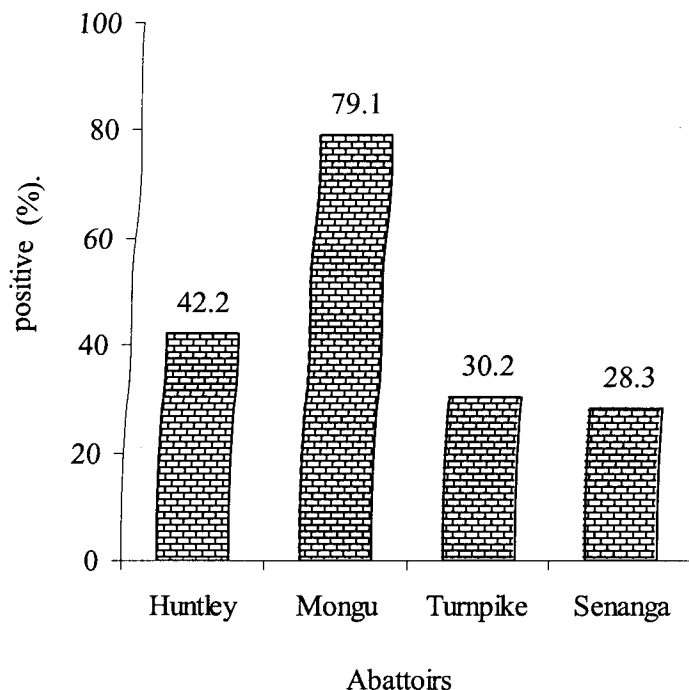
##### 4.1.2 Prevalence using liver inspection

A total of 841 cattle were inspected at slaughter. Out of the number examined, 439 cattle were found positive representing a prevalence rate of 53.3%. Figure 4.1 shows the extent of liver condemnations observed at one of the abattoirs during the survey.



**Figure 4.1** The extent of liver condemnation as a result of *F. gigantica* infection observed at Mongu abattoir.

Mongu abattoir had the highest prevalence (79.1%) while Senanga had the lowest (28.3%) (Figure 4.2). Significant differences ( $\chi^2=177.53$ ,  $p<0.001$ ) in the prevalences of flukes in cattle among the abattoirs and slaughterlabs were present.



**Figure 4.2** The prevalence (%) of *F. gigantica* in cattle according to surveyed abattoirs/slaughter slabs (Huntley, n=249; Mongu, n=344; Turnpike, n=202; Senanga, n=46).

The origin of cattle had a significant influence ( $p < 0.001$ ) on the prevalence. Mongu and Kalabo which lie along the Barotse flood plain had the highest prevalences of 82.6% and 55.7% respectively. Similarly, Mazabuka that lies along the Kafue wetlands had a prevalence of 51.6%, while Itezhi-tezhi had the lowest of 14.7% (Table 4.1).

**Table 4.1** Prevalence of *F. gigantica* in cattle according to various districts of origin within Southern (n=391), Western (n=390) and Central (n=60) provinces.

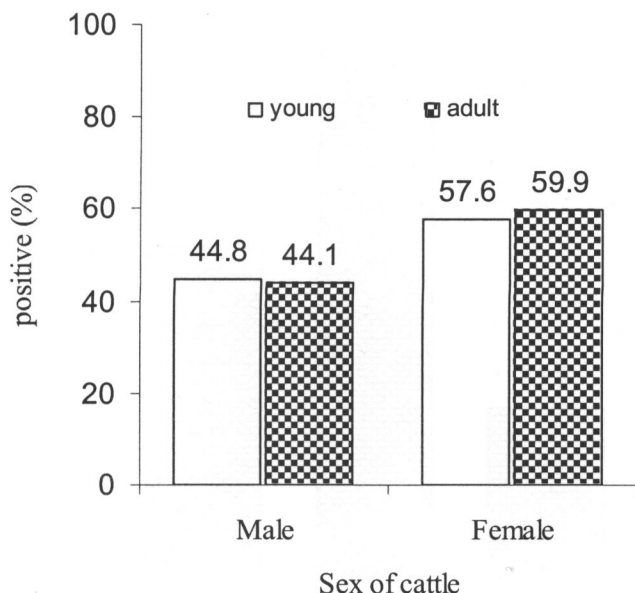
Origin	n	Positive	
		No.	%
Southern province			
Gwembe	119	36	30.3
Siavonga	64	19	29.7
Choma	79	47	47.0
Mazabuka	95	48	51.6
Itezhi-tezhi	34	5	14.7
Western province			
Kalabo	45	25	55.6
Mongu	299	247	82.6
Senanga	46	16	28.3
Central province			
Mumbwa	60	10	16.7

When the individual origins were grouped into provinces, Western province had the highest prevalence of 73.9% followed by Southern province with 39.6% while Central province had the least of 16.7%. Additionally, areas were assigned to either “closer to water” group or “far away from water” group depending on their distance to wetlands. Thus, Mongu, Mazabuka, Kalabo and Choma belonged to closer to water group while Gwembe, Siavonga, Itezhi-tezhi and Senanga (the origin considered was not near Zambezi flood plain) were put in the far away from water

group. Closer to water areas had significantly higher ( $\chi^2 = 8.97$ ,  $p < 0.001$ ) prevalence rate (66.0%) than those areas far away from water (34.4%).

Prevalence rates according to age groups were determined after examining 213 young and 628 adult cattle. Young and adult cattle showed prevalence rates of 47.4% and 55.7%, respectively. There was some evidence ( $\chi^2 = 3.83$ ,  $p = 0.050$ ) to suggest that adult cattle had a significantly higher prevalence of flukes than the younger cattle.

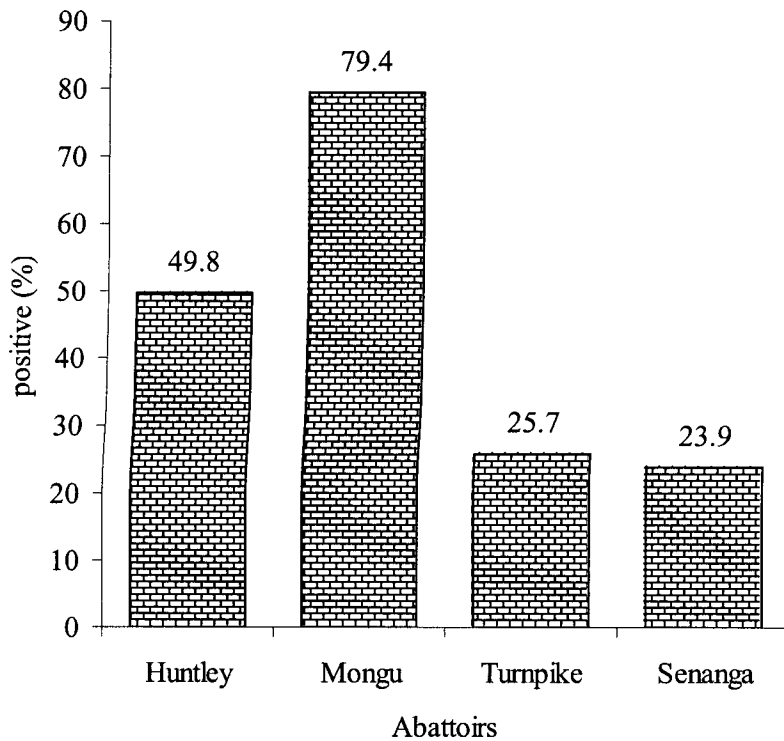
The prevalence rate for females (65.2%) was significantly higher ( $p < 0.001$ ) than that for males (36.3%). This was true even when the cattle were grouped according to age groups and sex. Prevalence rates in female young cattle (57.6%) and female adults (59.9%) was still higher than the male cattle whether young (46.0%) or adult (44.1%) (Figure 4.3).



**Figure 4.3** Prevalence of *F. gigantica* in young (n=209) and adult (n=632) cattle according to sex.

#### 4.1.3 Prevalence using coprological examination

The prevalence rate of liver fluke using fluke egg count (FEC) was found to be 48.7% (n=677). Egg count per gram (EPG) of faeces varied throughout the study period but ranged from 1 to 223 with a mean±SD of  $6.7 \pm 0.71$ . Variable prevalences were found at different slaughterhouses coprological examination (Figure 4.4). Mongu abattoir had the highest prevalence rate (79.4%) while Senanga had the lowest (23.9%). Significant differences ( $p < 0.001$ ) were found in cattle slaughtered among these slaughterhouses.



**Figure 4.4** Prevalence of *F. gigantica* in cattle according to abattoirs and slaughterslabs after coprological examination.

The origin of examined cattle had a significant influence ( $p < 0.001$ ) on the prevalence rate. Mongu that lies along the Barotse flood plain had the highest prevalence (80.6%). Similarly, Mazabuka that lies along the Kafue wetlands had a prevalence of 48.4%, while Itezhi-tezhi had the lowest of 20.6 % (Table 4.2).

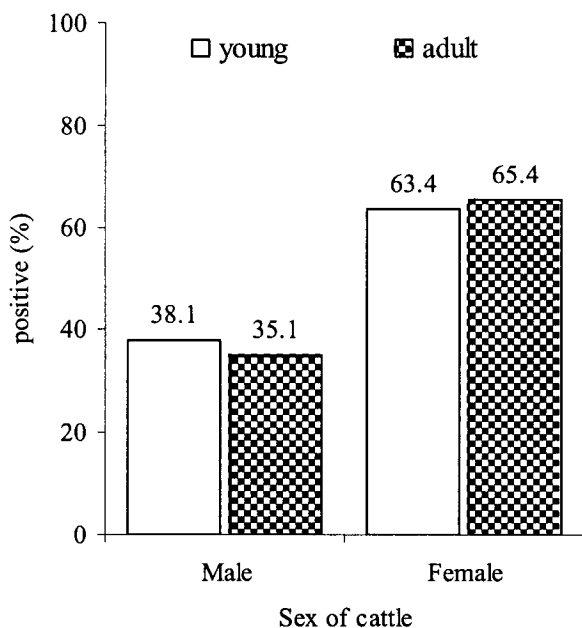
**Table 4.2** Prevalence of *F. gigantica* in cattle according to various districts of origin within Southern (n=391), Western (n=226) and Central (n=60) provinces.

Origin	n	Positive	
		No.	%
Southern province			
Gwembe	119	33	27.7
Siavonga	64	18	28.1
Choma	79	48	60.8
Mazabuka	95	46	48.4
Itezhi-tezhi	34	7	20.6
Western province			
Mongu	180	145	80.6
Senanga	46	11	23.9
Central province			
Mumbwa	60	23	38.3

When the origins were grouped into provinces, Western province had the highest prevalence of 69.0 %, followed by Southern province with 38.9% while the lowest was found in Central province (38.3%). Additionally, origins or areas were assigned to either “closer to water” group or “far away from water” group depending on their distance to wetlands. Thus, Mongu, Mazabuka and Choma belonged to closer to water group while Gwembe, Senanga, Siavonga and Itezhi-tezhi were put in far away from water group. Closer to water areas had significantly higher ( $\chi^2 = 101.41$ ,  $p < 0.001$ ) prevalence rate (67.5%) than those areas far away from water (28.5%).

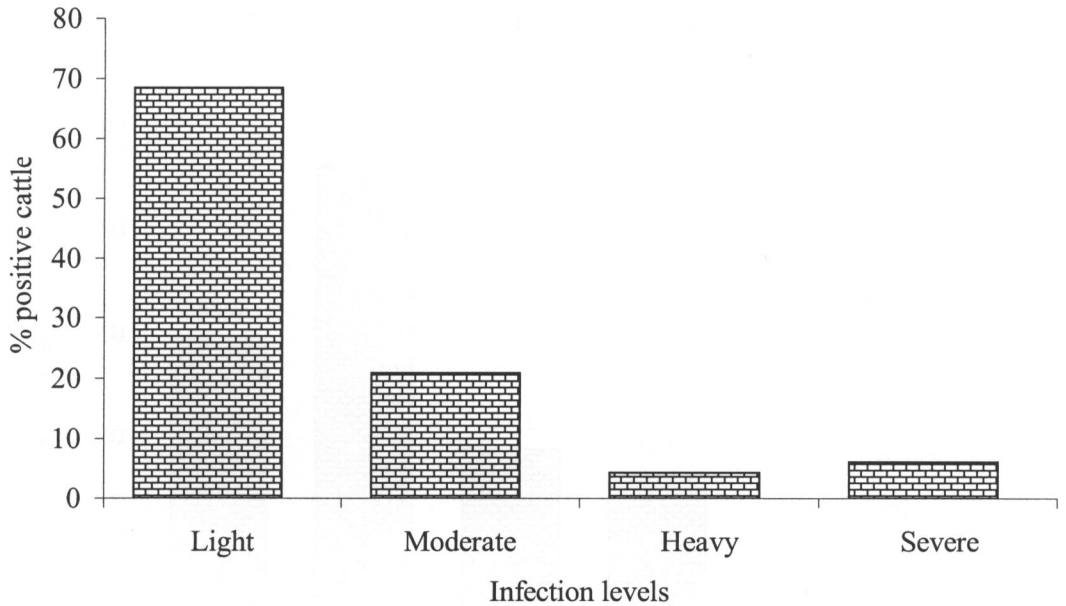
In order to determine prevalence of cattle according to their age groups, 158 young and 519 adults were examined. Even though the prevalence rate in adult cattle was higher (51.1%) than in young cattle (44.9%), the difference was not significant ( $\chi^2 = 1.58, p=0.209$ ).

The prevalence of fasciolosis for females (65.2%) was significantly higher ( $p < 0.001$ ) than that for males (36.3%) as shown in Figure 4.5. This was true even when the cattle were grouped according to age groups and sex; Prevalence rates for female young (63.4%) and female adults (65.4%) were higher than male young (38.1%) and male adults (35.1%).



**Figure 4.5** Prevalence of *F. gigantica* in young (n=59) and adult (n=518) cattle according to sex.

Based on the EPG classification, prevalence rates in the affected cattle were found as shown in Figure 4.6. More animals had light infection (68.5%) as compared to fewer animals that had either heavy or severe infection (4.4% and 6.2%, respectively).



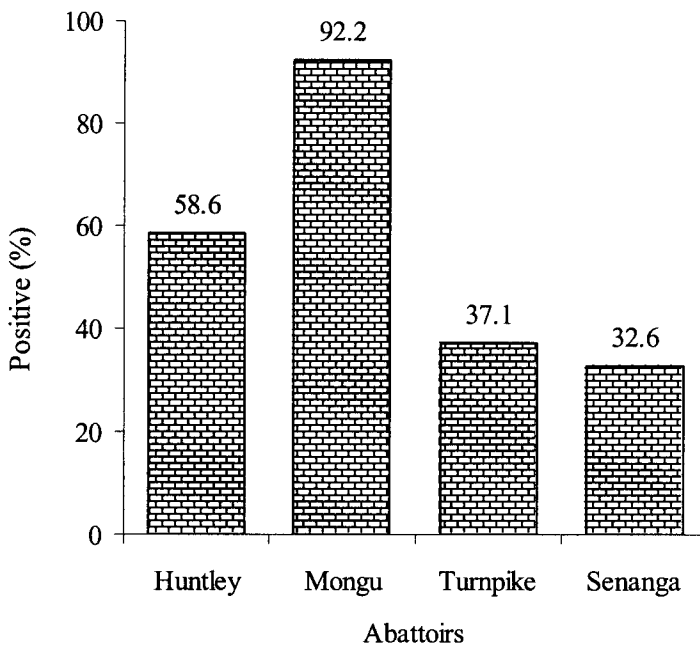
**Figure 4.6** Infection levels in affected cattle on egg per gram (EPG) classification [light (1-10 EPG), n=220; moderate (11-25 EPG), n=67; heavy (2-50 EPG), n=14; severe (>50 EPG), n=20].

#### **4.1.4 Prevalence using combined liver inspection and coprological examination**

Cumulative prevalence rate recorded from both liver inspection and coprological examination was 59.4% (n=677). Additionally, *F. gigantica* eggs were detected in

faeces of 64 (9.5%) cattle whose examined livers were passed as fit for human consumption. Associations of site of study, origin, age and sex of cattle to prevalence of *F. gigantica* infection were examined and are described below.

Variable prevalence rates were found at different slaughterhouses (Figure 4.7) after using both coprological examination and liver inspection. Mongu abattoir had the highest prevalence rate (92.2%) while Senanga had the lowest (32.6%). Significant differences ( $p < 0.001$ ) were found in cattle slaughtered among the slaughterhouses.



**Figure 4.7** Prevalence of *F. gigantica* in cattle according to surveyed abattoirs and slaughterslabs using both liver inspection and coprological examination (Huntley, n=249; Mongu, n=180; Turnpike, n=202; Senanga, n=46).

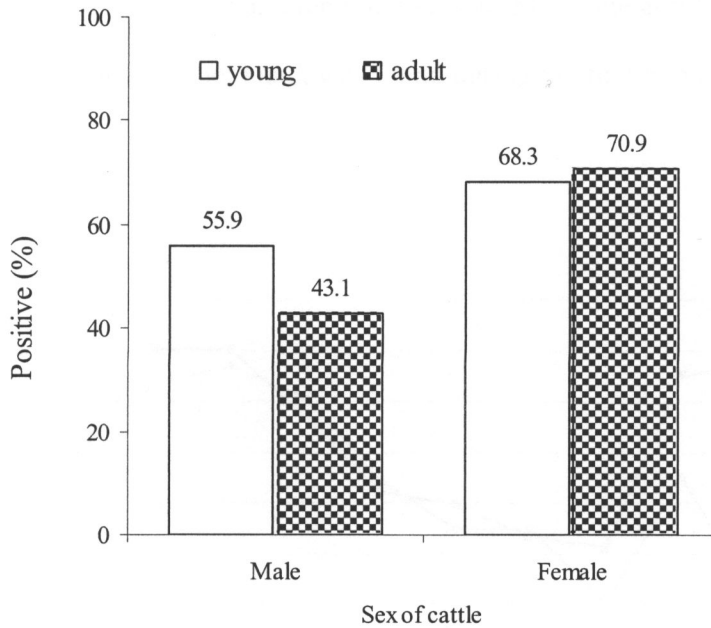
The origin of the examined cattle was found to have a significant influence ( $p < 0.001$ ) on the prevalence rate. Mongu that lies along the Barotse flood plain had the highest prevalence of 92.2%. Similarly, Mazabuka that lies along the Kafue wetlands had a prevalence of 68.4%, while Itezhi-tezhi had the lowest of 20.6%. Other origins of cattle had variable prevalence rates (Table 4.3). When the origins were grouped into provinces, Western province had the highest prevalence (80.1%) followed by Central province (51.7%) while Southern province had the least with 51.2%. Additionally, origins or areas were assigned to either “closer to water” group or “far away from water” group depending on their distance to waterways or systems. Thus, Mongu, Mazabuka and Choma belonged to closer to water group while Gwembe, Senanga, Siavonga and Itezhi-tezhi were put in far away from water group. Closer to water areas had significantly higher ( $\chi^2 = 151.48$ ,  $p < 0.001$ ) prevalence rate (83.1%) than those areas far away from water (63.5%).

The association of age of cattle on the prevalence of fasciolosis was determined after examining 158 young and 519 adult cattle. Adult cattle had a higher prevalence rate (61.7%) than in young cattle (53.2). There was evidence of this difference being significant ( $\chi^2 = 3.29$ ,  $p = 0.050$ ).

**Table 4.3** Prevalence of *F. gigantica* in cattle according to various districts of origin within Southern (n=391), Western (n =226) and Central (n=60) provinces using both liver inspection and coprological examination.

<b>Origin</b>	<b>n</b>	<b>No.</b>	<b><u>Positive</u> %</b>
<b>Southern province</b>			
Gwembe	119	40	33.6
Siavonga	64	25	39.0
Choma	79	63	79.7
Mazabuka	95	65	68.4
Itezhi-tezhi	34	7	20.6
<b>Western province</b>			
Mongu	180	166	92.2
Senanga	46	15	32.6
<b>Central province</b>			
Mumbwa	60	31	51.7

The prevalence of fasciolosis as shown in Figure 4.8 for females (70.7%) was significantly higher ( $p < 0.001$ ) than that for males (48.3%). When the cattle were grouped according to age groups, significant differences ( $p < 0.001$ ) were only noted among female adult cattle (70.9%) than the male cattle (43.1%).



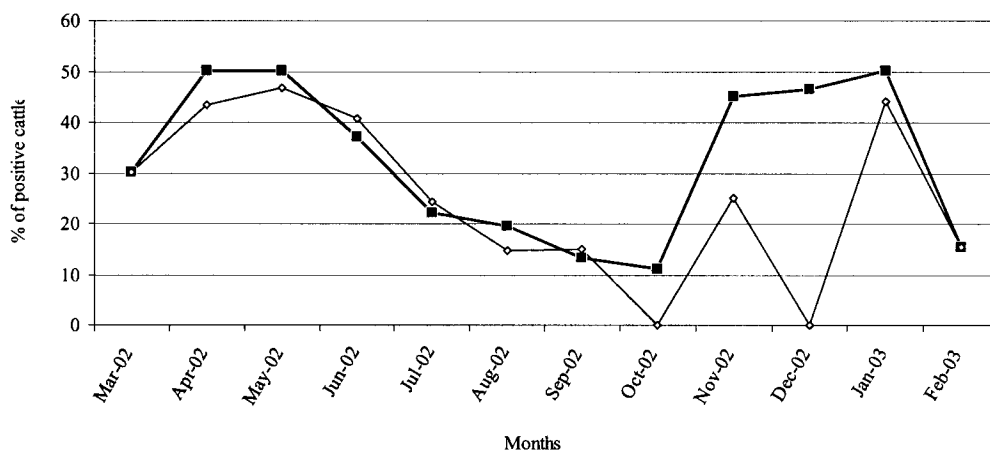
**Figure 4.8** Prevalence of *F. gigantica* in young (n=159) and adult (n=518) cattle according to sex at combined liver inspection and coprological examination.

## 4.2 Seasonality results

### 4.2.1 Seasonality using liver inspection

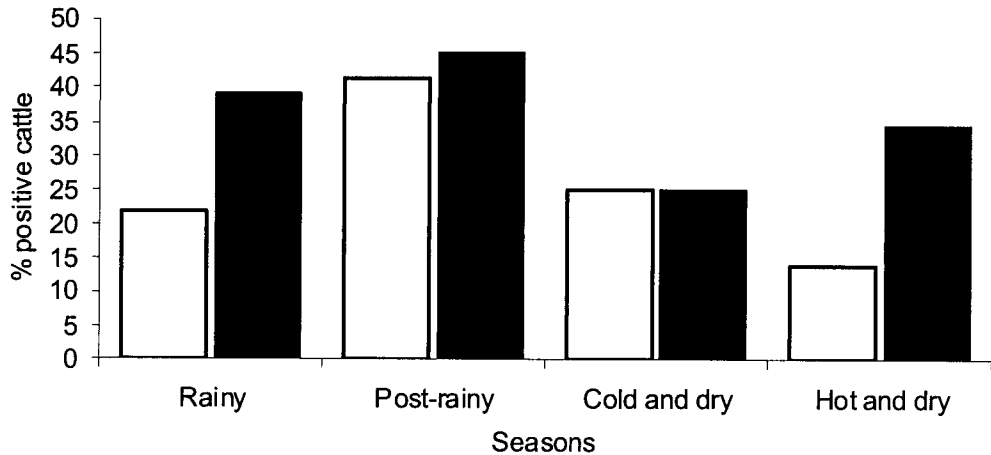
The highest prevalence rate was seen in May (46.7%) while there were no condemnations in October and December as shown in Figure 4.9. However, when months were grouped in to seasons, the seasonal pattern was as shown in Figure 4.10. The hot dry season (September–November) exhibited the lowest prevalence rate (13.8%); the rainy season (December–February) showed a gradual increase in prevalence rate (21.7%); the post rainy season (March–May) had the highest prevalence rate (41.3%) while the cold dry season (June to August) exhibited a 24.8% rate. Prevalence rates were observed to be relatively high at the beginning of

both the cold dry season and the rainy season (May/June and January, respectively). The lowest, on the other hand, was found during the first parts of the hot dry season in October.



**Figure 4.9** Monthly pattern of *F. gigantica* infection using coprological examination (■) and liver inspection (◇).

Fluke prevalence rates in the sampled cattle in the rainy (21.7%) and post rainy (41.3%) seasons were significantly higher ( $p=0.050$ ) than in those cattle sampled in the cold dry (24.8%) and hot dry (13.8%) seasons (Figure 4.10).



**Figure 4.10** Seasonal pattern of *F. gigantica* infection in the rainy (n=46), post rainy (n=80), cold dry (n=113) and hot dry (n=49) seasons using both liver inspection (□) and coprological examination (■).

Overall, there was no association observed between sex and prevalence ( $p= 0.630$ ) on the sampled cattle among the different seasons. However, differences within the same sex of cattle in different seasons were noted. Both male and female cattle had highest prevalence rates in the post rainy season (male: 41.8%, females: 40.0%) while the lowest prevalence rates were in the hot and dry season (males: 0.0%, females: 21.4%) (Table 4.4). There were significant differences ( $p= 0.050$ ) observed in fluke positivity among males in the different seasons unlike in females ( $p= 0.559$ ).

**Table 4.4** Seasonal pattern of *F. gigantica* infection according to sex of cattle using coprological examination.

Season	<u>Male</u>		<u>Female</u>	
	n	No. of positive %	n	No. of positive %
Rainy	27	4 14.8	19	6 31.6
Post-rainy	55	23 41.8	25	10 40
Cold dry	60	13 21.7	53	14 26.4
Hot dry	21	0 0	28	6 21.4

Table 4.5 shows the association of season, fluke prevalence and age of the sampled animals. All cattle, regardless of whether they were young or adult, had higher fluke prevalence rates in the post rainy season (39.1% and 42.1%, respectively) while the lowest prevalence rates were in the hot dry season (8.7% and 19.2% respectively). Adults had higher rates than young cattle in all the seasons. Even though there were differences in prevalences within males and females as well as between them, these differences were not significant.

**Table 4.5** Seasonal pattern of *F. gigantica* infection according to age of cattle using liver inspection.

Season	<u>1-3 years (young)</u>			<u>4 years (adult)</u>		
	n	No. of positive	%	n	No. of positive	%
Rainy	26	5	19.2	20	5	25
Post-rainy	23	9	39.1	57	24	42.1
Cold dry	52	9	17.3	61	19	31.1
Hot dry	23	2	33.3	26	5	19.2

### 5.2.2 Seasonality using coprological examination

On coprological examination, mean monthly and therefore seasonal fluke egg counts varied throughout the study period (Table 4.6). The highest prevalence rate was found in December (46.7%) and the lowest rate was observed in October (11.0%) (Figure 4.9). The months of January, March and April had mean egg counts of above 10 EPG. The highest prevalence rate was observed during the post rainy season (45.0%) and the lowest during the cold and dry season (24.8%). The seasonal pattern observed was that from November (end of dry season) to February/March (end of rains), more eggs were found. The post rainy season had the highest mean count as compared with the hot dry season which had the least mean counts. There was a marked reduction in egg counts as the rains subsided. During the cold dry and hot dry seasons, the counts were even lower.

**Table 4.6** Seasonal distribution of fluke prevalence and egg counts on coprological examination.

Season	Egg Positive		Mean egg	
	(%)	Egg range	counts	SEM
<b>Rainy</b>	39.1	1-97	9.21	3.76
<b>Post rainy</b>	45.0	1-155	11.53	4.93
<b>Cold dry</b>	24.8	1-12	0.74	0.26
<b>Hot dry</b>	24.9	1-4	0.38	0.19

The distribution of fluke eggs was significantly different ( $\chi^2=24.630$ ,  $p<0.001$ ) among seasons. The rainy season (median=146) had higher egg counts as compared with the cold dry season (median=118) and the hot dry season. The prevalence rates in the sampled cattle in the rainy (39.1%) and post rainy (45.0%) seasons were significantly higher ( $p=0.050$ ) than in those cattle sampled in the cold dry (24.8%) as well as hot dry (34.5%) seasons.

There were significant differences ( $p=0.040$ ) observed in fluke prevalence among females in the different seasons unlike in males ( $p=0.450$ ). In both male and female cattle, the highest prevalence rate was found in the post rainy season (male: 41.8%, females: 40.0%) while the lowest rate were in the cold dry season (males: 11.7%, females: 18.9%) (Table 4.7).

**Table 4.7** Seasonal pattern of *F. gigantica* infection according to sex using coprological examination.

Season	Male			Female		
	n	No. of positive	%	n	No. of positive	%
Rainy	27	7	25.9	19	8	42.1
Post-rainy	55	23	41.8 <sup>a</sup>	25	10	40.0
Cold dry	60	7	11.7 <sup>b</sup>	53	10	18.9
Hot dry	21	4	19	28	8	28.6

<sup>a, b</sup> figures for male cattle are significantly different at P<0.001.

No significant differences ( $p=0.751$ ) in the distribution of fluke eggs in the seasons according to the sex of the animal were present.

Table 4.8 shows the association of season, fluke prevalence and age of the sampled animals. All cattle, regardless of whether they were young or adult, had higher fluke positivity rates in the post rainy season (36.4% and 43.1%, respectively). The lowest prevalence rates in adult cattle were found in the hot dry season (16.0%) while in the young cattle the lowest prevalence rates were in the cold dry season (13.5%). Except for the hot dry season, adults had higher prevalence rates than young cattle. The association of age was only significant ( $p=0.003$ ) in adult cattle in the post rainy season as compared to adult cattle in the cold dry season. Cattle examined in the post rainy season irrespective of age were significantly ( $p<0.001$ ) more infected with fasciolosis than those in the cold dry season only.

### **4.3 Liver dissection results**

#### **4.3.1 Liver fluke burden**

Overall, condemned livers had higher weights (mean $\pm$ SD of  $4.45 \pm 0.16$ ) and fluke burden  $\pm$ SD ( $100 \pm 16.7$ ) than the non-condemned livers (mean weight  $\pm$ SD  $3.37 \pm 0.19$ ; fluke mean  $\pm$ SD  $0.66 \pm 0.49$ ) (Table 4.9). The number of flukes per condemned liver after dissection was high. For example, one liver was found to have harbour up to 912 flukes. All the condemned livers examined had flukes. On the other hand, 3 (9.5%) of the non- condemned livers ( $n =32$ ) had liver flukes and the burden was low (range of 1 to 15).

**Table 4.8** Seasonal pattern of *F. gigantica* infection according to age of cattle using coprological examination.

Season	<u>1-3 years (young)</u>			<u>4 years (adult)</u>		
	n	No. of positive	%	n	No. of positive	%
Rainy	26	7	26.9	20	8	40
Post-rainy	22	8	36.4	58	25	43.9 <sup>a</sup>
Cold dry	52	7	13.5	61	10	16.4
Hot dry	24	8	33.3	25	4	16.0 <sup>b</sup>

<sup>a, b</sup> figures for adult cattle are significantly different at P= 0.003.

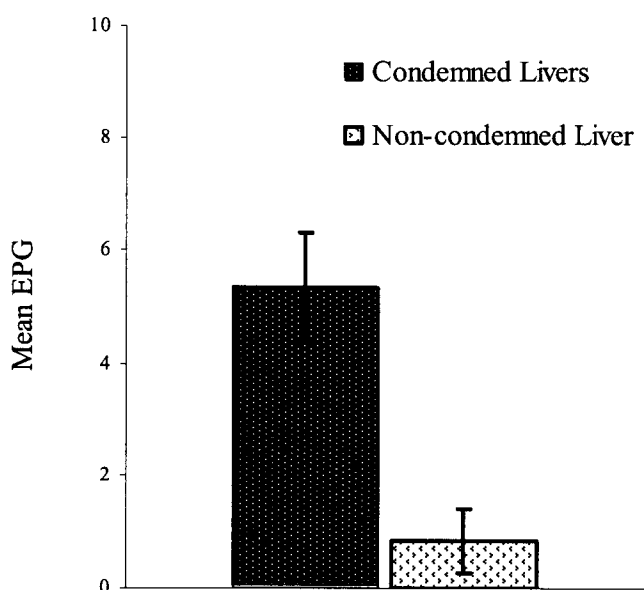
**Table 4.9** Weights of dissected livers and the number of flukes counted in the condemned (n=70) and non-condemned (n=32).

	<u>Condemned livers</u>		<u>Non-condemned livers</u>	
	Weight(Kg)	Number of flukes	Weight(Kg)	Number of flukes
Minimum	2.25	1	2.0	1
Maximum	8.1	912	5.0	15
Mean	4.45	100.6	3.37	0.66
SEM (±)	0.16	16.7	0.19	0.49

### 4.3.2 Fluke egg burden

Egg per gram (EPG) values of *F. gigantica* infection were higher in cattle with condemned livers (n=67) with a range of 1 to 35 than in those non-condemned livers (n=32) with a range of 1 to 13. The mean egg count in cattle with condemned livers was 5.36 as compared to those with non-condemned livers (0.84) (Figure 4.11).

Fluke egg counts from animals with condemned livers were significantly higher ( $\chi^2=84.24$ ,  $p<0.001$ ) than in the animals with non-condemned livers. Normalized data by mean logarithms (fluke number + 1) transformation were significantly higher ( $t=19.33$ ,  $p<0.001$ ) among the condemned livers (mean=1.70, SD=0.59) than in the non-condemned livers (mean =0.07, SD=0.25).



**Figure 4.11** Mean egg per gram (EPG) ( $\pm$  SEM) values of *F. gigantica* burden found on coprological examination from cattle whose livers were condemned (n=70) and non-condemned (n=32).

### **4.3.3 Correlation between liver flukes and egg per gram of faeces**

Only the flukes and EPGs of animals whose livers were condemned were included in the determination of correlation. There was no correlation found between fluke egg counts and the number of flukes ( $r=0.003$ ,  $p=0.978$ ).

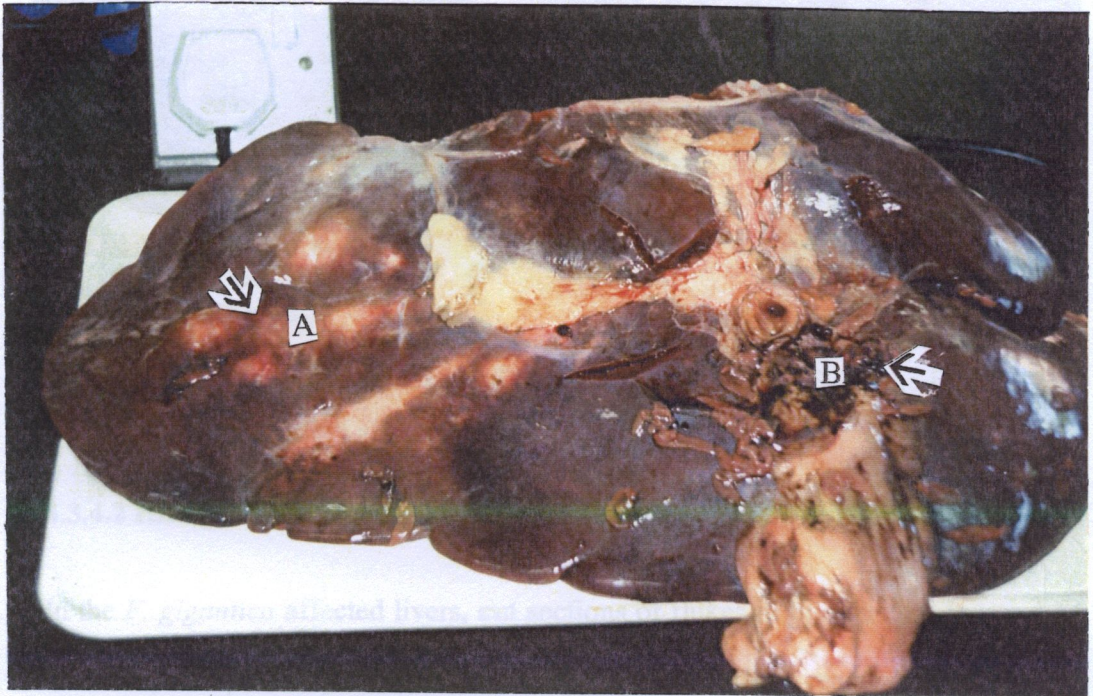
### **4.3.4 Liver pathology**

Gross and histopathological lesions were noticed especially with condemned livers than non-condemned ones after dissections were carried out.

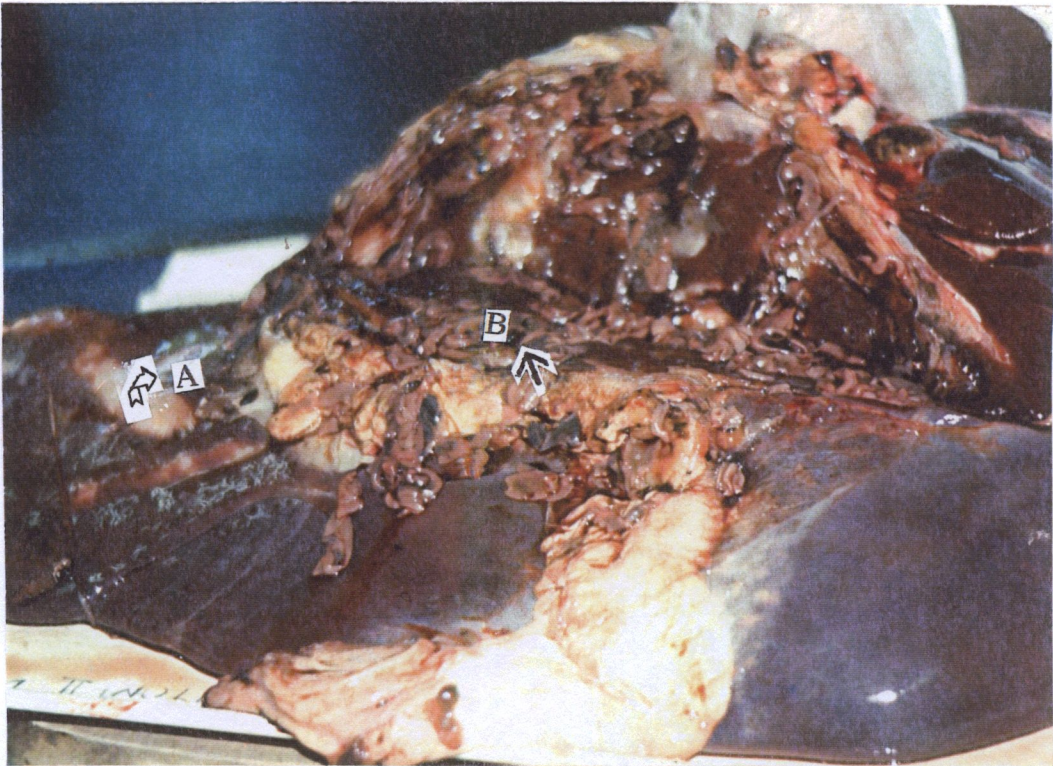
#### **4.3.4.1 Gross pathology**

Livers that had mild infestation of *F. gigantica* presented with apparently normal colour and appearance. Congested, enlarged, haemorrhagic and friable livers made up 15.7% (11/70) of the condemned livers. Immature flukes were occasionally seen mixed with dirty exudate in liver cavities. Bile duct enlargement was often localized, but in more advanced cases, the thickening of the bile ducts extended to all the major branches. Livers that had severe infestation accounted for 28.6% (20/70) and were either slightly reduced in size or enlarged with rounded borders (Figure 4.12). In these livers, parenchyma consistency was tougher than normal and therefore hard to cut. As a result of calcification, gritty sounds were also heard on cutting some severely affected livers. The liver tissues were often distinctly pale and hyperplastic. Hyperplasia was seen in all mature infections. Bluish-black and grayish white nodules were observed on the cut surface resembling abscesses. Some immature flukes that could not reach the bile ducts became enclosed in fibrous cysts in the liver parenchyma. Main bile ducts were distended with thickened walls while their lumina were filled with thickened yellowish-green exudate sometimes mixed with

flukes (Figure 4.13). Only two (6.3%) of the non-condemned livers showed pathology. This only affected the periphery of the organs; one liver had a single 1 x 0.5 cm fluid-filled cyst while the other had two pea-sized nodules containing encapsulated immature flukes.



**Figure 4.12** Liver affected with *F. gigantica* showing distended bile ducts (A) and purulent exudate mixed with liver flukes (B).



**Figure 4.13** Mucopurulent exudate (A) and mature flukes (B) in the bile ducts of a severely affected liver.

#### 4.3.4.2 Histopathology

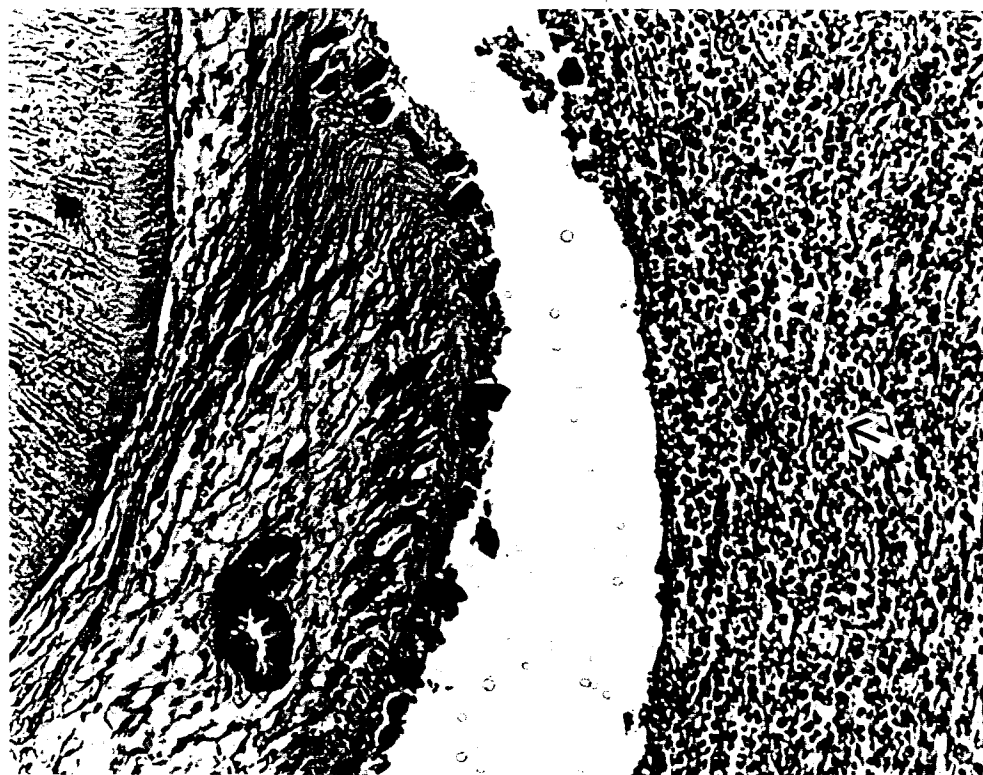
In the *F. gigantica* affected livers, **cut sections** of flukes in bile ducts (Figures 4.14 and 4.15) and parenchyma were **surrounded** by neutrophilic reaction with adjacent hepatocytes showing degenerative **changes**. Smaller bile ducts revealed mild to severe desquamation of the lining **epithelial** cells. In order to accommodate flukes with their cuticular spines, the **larger and** medium-sized bile ducts were dilated (Figure 4.15). The walls of these **bile ducts** were thickened and infiltrated with lymphocytes and plasma cells with **diffuse fibrosis** around them.



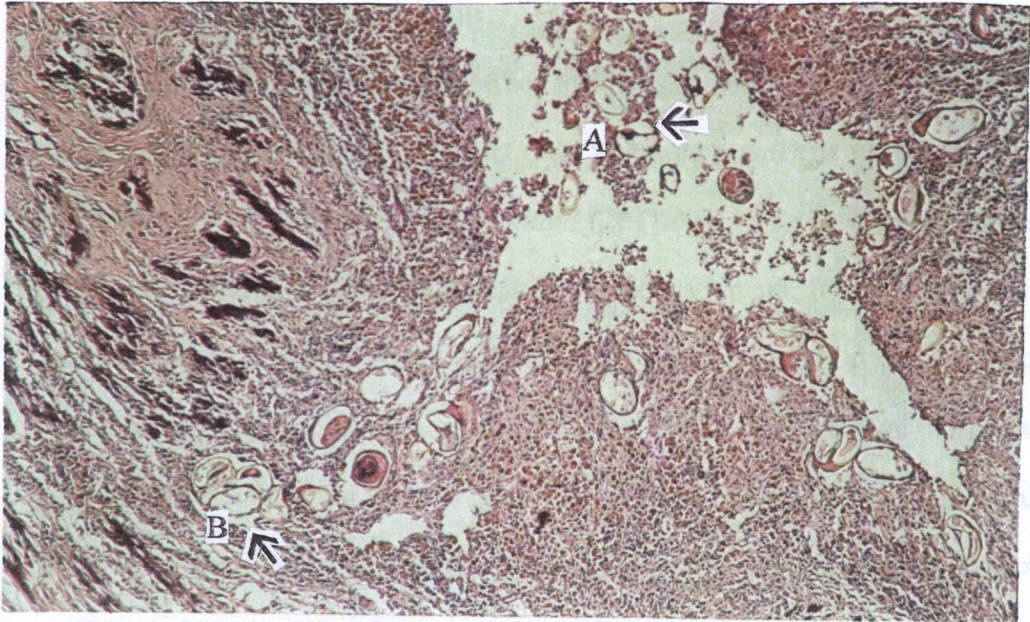
**Figure 4.14** Cross-sectional view of a mature *Fasciola gigantica* in the bile duct of an affected liver (x 40 magnification).

Degenerating flukes and fluke eggs attracted foreign body giant cells, macrophages, lymphocytes and fibrous tissue thereby forming various sized nodules. In some places, hepatocytes were completely replaced by fibroblasts and connective tissue, while in other places, they revealed various stages of cellular degeneration. Mature flukes were found lying within dilated bile ducts (Figure 4.14). In a few cases, fluke eggs having a yellow tint were recognized in the bile ducts (Figure 4.16). A few macrophages and kupffer cells containing yellowish brown pigment (hemosiderin)

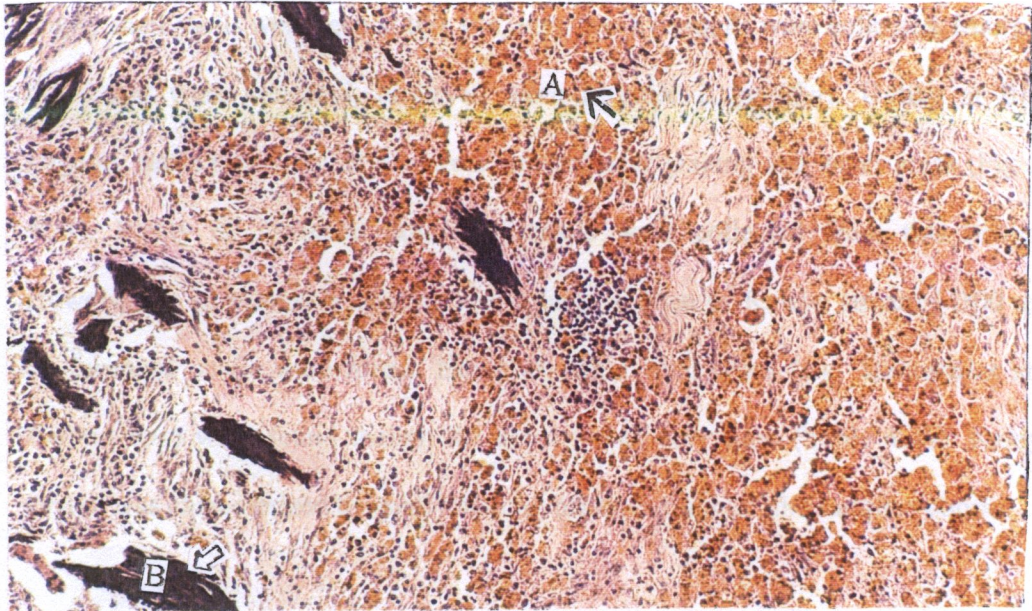
were also recognized (Figure 4.17). Calcium deposits were seen as bluish-black patches in some liver sections (Figures 4.16 and 4.17).



**Figure 4.15** Cross-sectional view of liver fluke with its prominent spines in the bile duct. Notice the massive cellular infiltration in the liver parenchyma on the right of microphotograph (x 100 magnification).



**Figure 4.16** *Fasciola gigantica* eggs seen in the bile ducts (A) and encapsulated in the liver (B) parenchyma (x 40 magnification).



**Figure 4.17** Hemosiderin (A) and calcium (B) deposits in *F. gigantica* affected livers (x 100 magnification).

#### 4.4 Snail study results

##### 4.4.1 Frequency of sampled snails

A total of 984 snail species were identified including *Bulinus tropicus* (350), *Bulinus globosus* (160), *Biomphalaria Pfeifferi* (38), *Lymnaea natalensis* (306), *Cleopatra nswendweensis* (78) and *Bellamyia capillata* (2) (Table 4.10). *Lymnaea natalensis* snails were found at all habitats except along Kaleya stream. It was to find cattle faeces immersed in water (Figure 4.18) in which snails were found on grasses and aquatic plants. Quite a high number of snails were observed attached on dead leaves and other organic debris in water (Figure 4.19). This was usually in close proximity to deposited cattle faeces. Snail density declined as one progressed sampling one meter away from the edge of a water body.

The habitats at Blue Lagoon harboured numerous wild bird species and the Kafue lechwe (Figure 4.20). Birds were seen eating insects and snails. Though quite many empty snail shells were seen at this site, very few live snails were collected. Many of those that were collected, however, were juvenile *B. pfeifferi*. Unlike at Blue Lagoon, cattle kraals at Chiyasa were situated near grazing and water areas (Figure 4.21). Plenty of *Lymnaea natalensis* snails were collected from this habitat. However, in an extensive search along a large section of Kaleya stream (~300 m), no *Lymnaea* snails were found. However, other snail types (e.g. *Melanoides* and *Bulinus spp*) were found though the density was very light (Table 4.10). Kaleya stream was characterized by gently sloping banks throughout the sampled course.

**Table 4.10** Frequency of collected snails according to sampled areas of the Kafue river basin

Area	Snail types									
	<i>Lymnaea</i>	<i>Bulinus</i>	<i>Melanooides</i>	<i>Bulinus</i>	<i>Physa</i>	<i>Biomphalaria</i>	<i>Bulinus</i>	<i>Bellamyia</i>	<i>Cleopatra</i>	
Shin'goma	133	278	0	138	0	1	0	0	0	0
Kafue	5	39	0	7	0	0	0	0	0	0
Bridge										
Chiyasa	121	27	0	15	0	6	0	0	0	51
Blue	2	0	0	0	0	24	4	0	0	0
Lagoon										
Kaleyia	0	4	9	0	7	0	0	0	0	0
Shimungalu	45	2	28	0	2	7	0	2	2	27
<b>Total</b>	<b>306</b>	<b>350</b>	<b>37</b>	<b>160</b>	<b>9</b>	<b>38</b>	<b>4</b>	<b>2</b>	<b>2</b>	<b>78</b>



Figure 4.18 Faeces passed in water as cattle grazed and drank around the habitats.

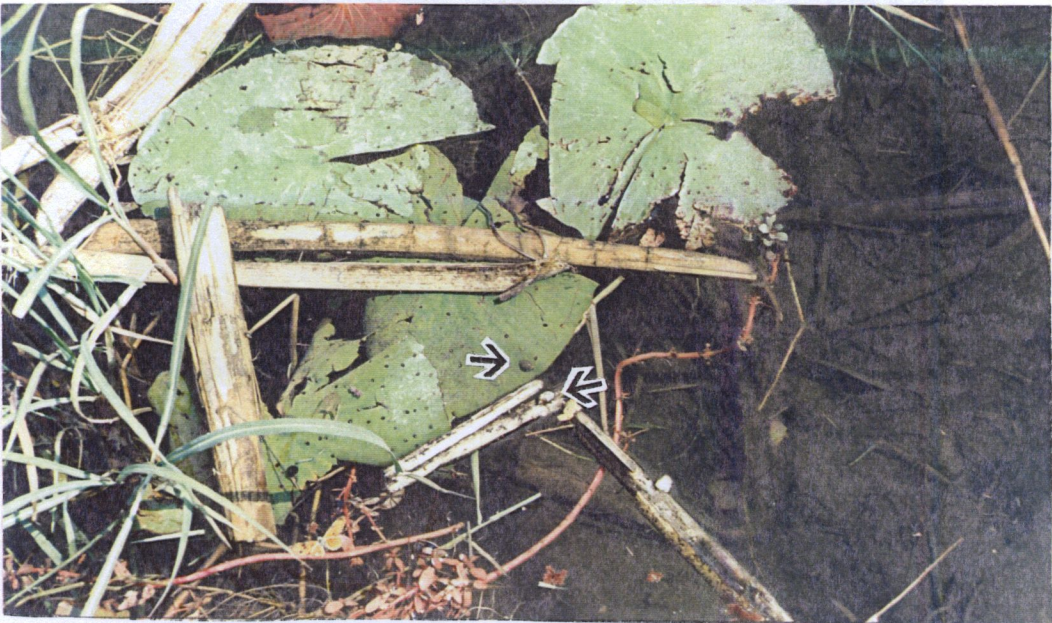
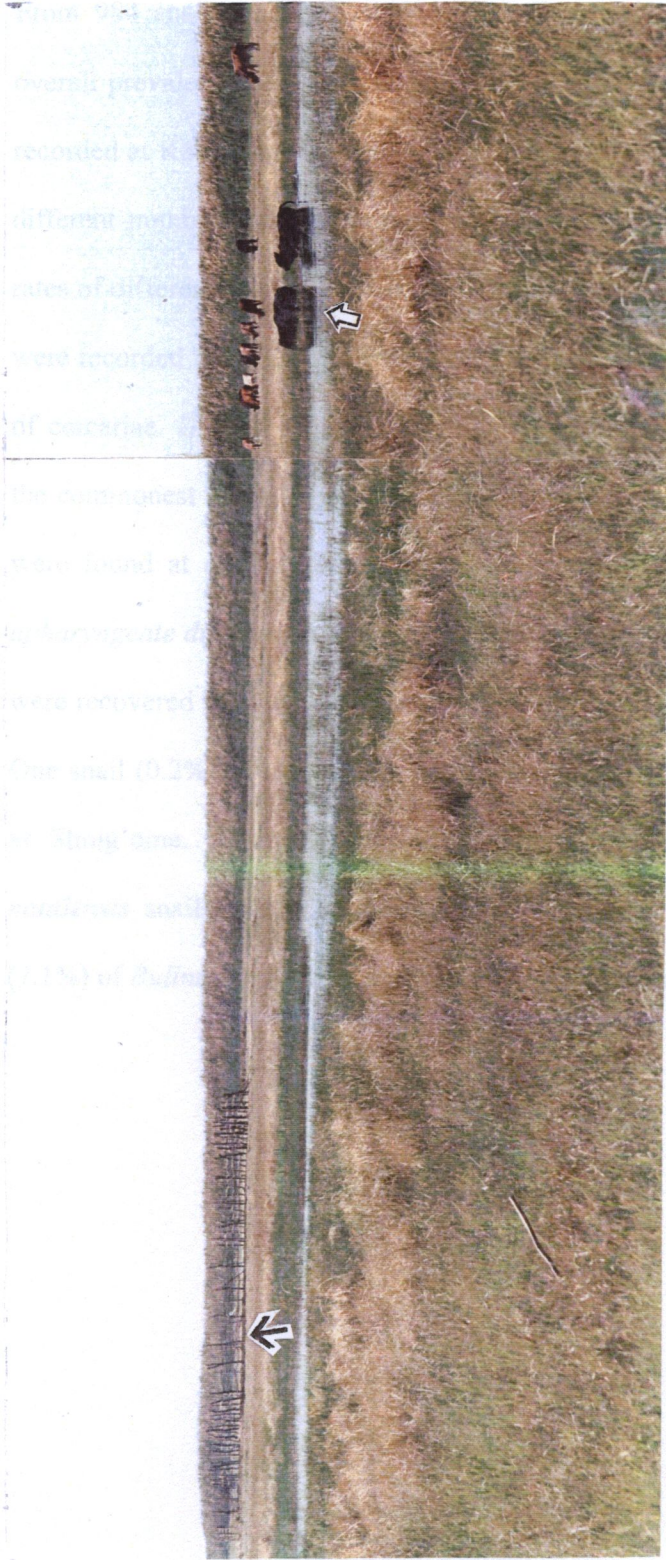


Figure 4.19 *Lymnaea natalensis* snails on a water lily and on floating dead stalks.

Abundant growth of different kinds of hydrophytes and plenty of organic debris from leaves fallen from surrounding trees were observed along the stream banks. There was quite a significant human contact at all habitats sampled, especially at sites that were used for laundry, fishing, watering and drinking purposes (Appendix Table 2).



**Figure 4.20** Blue Lagoon, Kafue flats. A suitable snail habitat lies in the foreground. Plenty of wild birds colonise the submerged pastures.



**Figure 4.21** Chiyasa, Kafue flats. Cattle grazing and drinking water at one of the sites. Their kraal stands very close to snail habitat as seen on the left of the figure. Access to water pastures is 100% for at least six months of the year.

#### 4.4.2 Cercarial/metacercarial harvest

From 984 snails collected, 135 were positive with larval trematodes making an overall prevalence of 13.7% (Table 4.11). There was no larval trematode infection recorded at Kaleya stream while the highest rate was found at Chiyasa (33.2%). The different numbers of *L. natalensis* snails collected at different sites and shedding rates of different cercarial types are shown in Table 4.12. Most trematode infections were recorded from *L. natalensis* (42.8%). *Lymnaea natalensis* harboured four types of cercariae. *Gymnocephalous* and *Longifurcate-pharyngeate distome* (LPD) were the commonest types of cercariae harvested from snails. *Gymnocephalous* cercariae were found at all sites where *Lymnaea* snails were collected. The *Brevifurcate-apharyngeate distome* (BAD) was the least common cercariae found. No trematodes were recovered from *B. capillata*, *B. pfeifferi*, *M. tuberculata* and *C. nswendweensis*. One snail (0.2%) of the 416 *Bulinus* snails had avian *Schistosoma* cercariae (BAD) at Shing'oma. Avian *Schistosoma* cercariae (BAD) were shed from eight *L. natalensis* snails; 5 (11%) from Shimungalu and 3 (2.5%) from Chiyasa. Three (7.1%) of *Bulinus* spp. snails shed amphistome cercariae only at Chiyasa.

**Table 4.11** The overall prevalence (%) of trematode infections in snails collected from the six study areas of the Kafue river basin.

Area	Number (%) infected snails	Habitat type	District	by people and cattle collected	Nature of use of site	
					No. of	with trematodes
Shing'oma	River/pond	Kafue	D, F, B, L, W	550	36 (6.5)	
Kafue Bridge	River	Kafue	D	51	5 (9.8)	
Chiyasa	Lagoon	Mumbwa	D, F, B, L	220	73 (33.2)	
Blue Lagoon	Plain	Mumbwa	D, F, B, L	30	1 (3.3)	
Kaleya	Stream	Mazabuka	D, B, L, W	20	0 (0)	
Shimangalu	Lagoon	Mazabuka	F, B, L	113	20 (17.7)	
<b>Total</b>				<b>984</b>	<b>135 (13.7)</b>	

D, drinking site for cattle and/people; F, fishing; B, bathing; L, laundry; W, watering vegetables

**Table 4.12** The prevalence (%) of infection with larval trematodes and the level of shedding (<200, mild; 200-1000, moderate; >1000, severe) in affected *L. natalensis* from six areas of Kafue river basin of Zambia.

Area	No. of snails collected	cercarial type	No. of snails infected (%)	Shedding levels		
				Mild (%)	Moderate (%)	Severe (%)
Blue Lagoon	2	Gym	1 (50)	1 (50)	0	0
Chiyasa	121	BAM, Gym, LPD, BAD	70 (57.9)	13 (10.75)	28 (22.3)	30 (24.8)
Kaleya	0	0	0	0	0	0
Shimungalu	45	Gym, LPD, BAD	20 (44.4)	14 (31.1)	3 (6.66)	3 (6.66)
Kafue Bridge	5	BAM, LPD, Gym	5 (100)	4 (80)	1 (20)	0
Shing'oma	133	BAM, Gym, LPD	35 (26.3)	12 (9)	8 (6)	15 (11.3)
Total	306		131 (42.8)	44 (14.4)	40 (13.1)	48 (15.7)

BAD, brevifurcate-apharyngate distome cercaria (species of either Spirorchidae or Schistomatidae families);

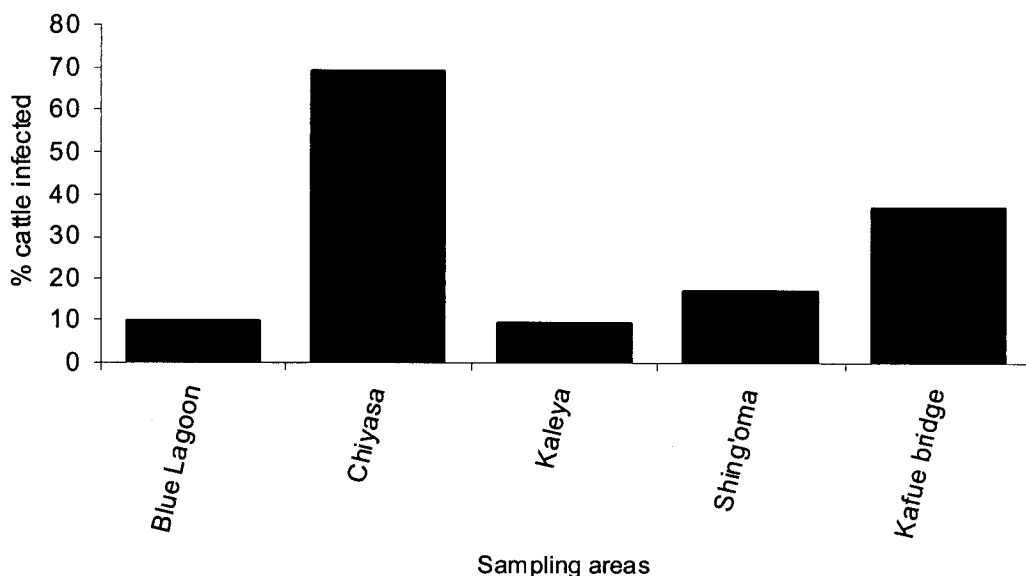
BAM, brevifurcate-apharyngate monostome cercaria (species of family Clinostomatidae);

LPD, longifurcate pharyngate distome cercaria (species of families Strigeidae and Diplostomatidae);

Gym, gymnocephalous cercaria (species of family Fasciolidae).

#### 4.4.3 Coprological examination of cattle accessing snail habitats

Different prevalence rates were found from examined cattle grazing from each habitat on coprological examination (Figure. 4.22). Cattle at Chiyasa had the highest rate (68.8%) while those at Kaleya and Blue Lagoon had the lowest (9.1 and 9.5%, respectively). Even though no *L. natalensis* snails were found along Kaleya stream, 9.1% (2/22) of the cattle sampled exhibited *Fasciola* infection (Figure 4.22). In the Blue Lagoon National Park, Shing'oma and Chiyasa, *Fasciola* infection was recorded in both cattle and snails. In these places, the infection was high in Lymnaea snails than in cattle.



**Figure 4.22** Proportion of cattle infected with *F. gigantica* in selected parts of Kafue river basin (Blue Lagoon, n=21; Chiyasa, n=16; Shing'oma, n=12; Kafue Bridge, n=30).

## CHAPTER FIVE

### DISCUSSION

#### 5.1 Prevalence survey

The prevalence of *F. gigantica* infection in cattle recorded in our study shows that coprological examination (48.7%) detected infected animals much lower than liver inspection (53.3%). However, the cumulative prevalence by both tests was higher (59.4%) than the individual methods. These findings suggest that one method alone was unable to detect all the infected animals.

The prevalence rates as reported by Pandey and Ahmadu (1998) (51.0%) and Silangwa (1973) (89.0%) were also higher, but in their studies mainly abattoir records of liver inspection were used. Apart from that, these findings were restricted to only one major cattle area of Zambia (i.e. Western province). Therefore, the picture given was not representative of the situation countrywide. Our present study was done in all the major cattle keeping areas of the country except Eastern province thereby recording prevalence from a representative sample. However, prevalence for Western province alone (73.9%) is similar to Silangwa (1973) but very different from Pandey and Ahmadu (1998). The use of both liver inspection and coprological examination for our study made it possible to depict a realistic picture of fasciolosis. Both mature and immature fluke infections could be detected to a greater extent than if only liver inspection was performed. The survey by Silangwa (1972) was the first comprehensive study in Zambia to attempt to assess the incidence rate of *F.*

*gigantica* and its economic significance in the rural livestock economy of the flood plains. The 79.1% prevalence found in the Barotse cattle at Mongu abattoir in our present study was lower than the finding of Silangwa (1972) (89.6%) but higher than that found by Pandey and Ahmadu (1998) (51.0%). In one of his studies, Silangwa (1972) reported 98.4% incidence rate after examining 192 livers of cattle from the Zambezi flood plain. This rate was much higher than the 89.6% prevalence rate that he previously found after examining records of condemned livers due to fasciolosis at commercial liver inspections during the three decades before 1972. In Zambia's Western province, condemnation rates of between 33.0% and 73.0% of bovine livers were due to fasciolosis alone according to abattoir records of 1980 to 1994 (Pandey and Ahmadu, 1998). In this period, 95,018 cattle were slaughtered at Mongu abattoir and 48,692 livers (51.0%) were totally condemned because of the infection. Results from the present study confirm that bovine fasciolosis is highly enzootic among the cattle on the Barotse flood plain and therefore agree findings of Silangwa (1972) as well as Pandey and Ahmadu (1998).

The prevalence rate found at liver inspection in this study (53.3%) ranks among the highest in Africa. When liver inspection is as thoroughly done, even light and/or immature infections could be detected. Only Schillhorn van Veen (1980) in Nigeria, Alves (1970) in Mozambique, Malek (1980) in Ethiopia and Keyyu *et al.* (2003a) in Tanzania reported higher prevalence rates of 65.0%, 70.0%, 30.0 to 90.0% and 28.4 to 63.8% respectively than we found in this study. This may be due to most of their studies being undertaken a long time ago at single abattoirs and in smaller regions of their countries. The areas of their studies, therefore, could have had conditions more

favourable for *Fasciola* than other parts that were not studied. When such results are postulated to represent prevalence of *Fasciola* in a country, there is a tendency to distort the real picture.

Coprological examination (Faecal egg count) is a common method used in the diagnosis of most helminthosis at antemortem. In cattle that harbour patent infections of *Fasciola*, the recovery of eggs from their faeces remains one of the surest methods of diagnosis of liver fluke infection. *Fasciola gigantica* eggs were detected in 9.5% (n=677) of animals whose livers were passed as fit for human consumption. We noted therefore that immature or light fluke infection detected at coprological examination could not be detected with the use of liver inspection alone. At the same time, we recorded a considerably higher faecal egg count in cattle as compared to Vassilev and Jooste (1991). Vassilev and Jooste (1991) collected 14,668 cattle faeces for monitoring the level of *F. gigantica* infection from February 1988 to May 1990 in Zimbabwe. During this period, 26.3% of faeces were found positive with egg count per gram of faeces (EPG) range of 1 to 86. Infection levels found in our study using epg classification showed that 68.5% of infected cattle had light infection, 20.9 % moderate infection, 4.4% heavy while 6.2% were severely infected. This pattern agrees with findings of Vassilev and Jooste (1991) who reported light (42.3%), moderate (37.8%) and heavy (19.9%) infections.

Generally, numbers of cattle found positive on coprological examination were lower than those on liver inspection. Different sampling periods throughout the year could have had an influence on this finding with some animals exhibiting immature or prepatent infections. Other reasons could include collection of only one faecal

sample per animal and the relatively low sensitivity of the test that could have missed some light infections. Boray (1985) reported that the diagnosis of infections of *Fasciola* species in ruminants has relied on the detection of *Fasciola* eggs in the faeces of infected animals. However, the method's drawbacks mainly related to the detection of infection were highlighted by Dumenigo *et al.* (2000). The major difficulty for diagnosis of eggs in faeces arises when there is a need to detect relatively small numbers occurring in relatively large amounts of faeces. Therefore, detection of these relatively small numbers of eggs is determined by the size of the sample. In agreement, Dumenigo *et al.* (2000) reported that the microscopic examination of faecal samples for the presence of *F. gigantica* eggs is often difficult as they may not be found during the prepatent period. Sometimes it is as difficult even during the patent period because of intermittent excretion of parasite eggs. Despite limitations to coprological examination, we can still conclude from the 76.0% agreement with liver inspection that it can be relied upon in establishing the extent of the infections in the cattle population under field conditions where they exist. The difference in prevalences found between liver inspection and coprological examination could have been influenced by many factors including ingesta volume and host resistance (Barth *et al.*, 1981), however, we also assumed that the chances of finding fluke eggs in a given faecal sample depended on the adult fluke burden in the liver. Therefore, the lower positivity rate we obtained in our study could have been largely due to presence of very light infections and the fact that light infections can not easily be detected by examination of only one sample from each animal (Morris and Shaw, 1942; Coyle, 1958). Hammond (1970) suggested that the length of infection has an influence on the fecundity of the fluke and that because of large

mass of faeces passed daily, even the low faecal egg counts might be of importance in the epidemiology of *F. gigantica* infection. In our study, we came across fluke eggs accumulated in the gall bladders of some livers and these eggs can be expelled unevenly creating variations in egg counts in the faeces.

Cumulative prevalence (59.4%) recorded by considering positive cattle on both liver inspection (53.3%) and coprological examination (48.7%) was higher than that found on either test. This was so because some animals were positive at liver inspection while being negative on coprological examination and vice versa. Any animal found positive on either test was considered infected with *F. gigantica*. Prevalence rates in the individual provinces of Western, Southern and Central were greater than 50.0%. This result confirms that *F. gigantica* infection is not only prevalent in Western province as earlier reported but also in other major cattle keeping areas of Zambia except Eastern province.

The association of age of cattle to prevalence of *F. gigantica* infection was examined and showed that adult animals had the highest prevalence rate (61.7%). These cattle contributed the greatest to overall prevalence than the young ones (53.2%). Keyyu *et al.* (2003a) working in the southern highlands of Tanzania also found that traditional farms had the highest prevalence of *F. gigantica* (63.8%) and the prevalence of *F. gigantica* was higher in adults (59.75%) than it was in yearlings (52.0%) and calves (25.7%). Similarly, Pfukenyi and Mukaratirwa (2001) in Zimbabwe found that unlike calves, adults had the highest prevalence of *F. gigantica* infection. Though DeChaneet and Dunsmore (1988) did not find any significant association between the presence of a fluke and the age of the animals, our findings found an association.

This agrees with the report of Negesse (1994). By the time cattle are usually sold, they would have had a longer exposure to infection as well as possibilities of travelling long distances to access metacercariae- and Lymnaea-infested grazing areas and watering holes.

The association of sex was examined and showed that the sex of cattle had an influence on the prevalence of fasciolosis. Female cattle (70.7%) exhibited a significantly higher prevalence rate than males (48.3%). This is contrary to Dechaneet and Dunsmore (1988) who reported that there was no significant association between the presence of a helminth and the sex of the animal. The differences observed in the prevalence of flukes might be due to differences of exposure of the animals to the intermediate stages of the flukes, the tendency of keeping weak and small animals at pastures near home and therefore, away from marshy areas or due to variable resistance to the worms. After selecting a few male cattle for breeding and draught power purposes, it is a common and acceptable trend among livestock farmers to sell off unwanted young male animals. However, they retain most or all of the female animals for breeding purposes and milk production. Thus, female animals live longer than their male counterparts and are only tendered for slaughter after culling. To support our observation, several workers (Perry *et al.*, 1984; Nadaraja *et al.*, 1984; Simuunza, 2001) found that the proportion of female to male animals (especially cows to oxen) is high and attributed this trend partly to the reasons advanced above.

This study is the first one of its kind that employed coprological examination as well as liver inspection and covered a large part of the country. The present work shows

that bovine fasciolosis is highly prevalent in cattle in Zambia. Prevalence figures generated did not include liver condemnation losses in most of the animals that went out of the studied provinces for slaughter or were slaughtered at local butcheries and villages. However, this survey still provides important baseline data for future monitoring of the fluke infection and the evaluation of economic losses.

## 5.2 Seasonality study

Our study determined the seasonal pattern in the four seasons and also examined the associations of age, sex and origin of cattle using coprological examination and liver inspection. The seasonal trends found using both methods were similar. More cattle were found positive during the post-rainy season using both methods than in any other season. Coprological examination, however, picked up more positive cattle during the hot dry (24.9%), the rainy (39.1%) and the post-rainy (45.0%) seasons than liver inspection in the same periods (13.8%, 21.7% and 41.3%, respectively). Such disparities can arise as a result of light and/or immature infections. The post-rainy and rainy seasons were identified as periods of highest prevalence rates at both liver inspection and coprological examination. During these periods, two peaks were identified as being in April/May and November/December. However, fasciolosis has been determined to occur throughout the year. This agrees with findings of similar work in Zimbabwe by Vassilev and Jooste (1991).

In agreement to our findings, previous studies (Mahlau, 1970; Silangwa, 1973) reported *F. gigantica* infection rate in cattle as being high during the dry season and low during the rainy season while the peak transmission of trematodes as occurring at the end of the rainy season (Makundi, 2001; Ngategize *et al.*, 1993). Schillhorn

van Veen *et al.* (1980) observed that in Nigeria most infections are acquired during the second half of the dry season. Since *Fasciola* can live for more than one year in cattle producing eggs constantly, this complicates the determination of the major risk periods (Rangel-Ruiz *et al.*, 1999). After examining 2730 condemned livers of cattle from March 1989 to February 1992, they reported two infection periods; the first being during the dry season and the second during the rainy season. However, they considered the one that occurs during the dry season as the most important.

The high prevalence rate found in our study using liver inspection in the post rainy season (41.3%) unlike the lowest during the hot dry season (13.8%) indicates that more mature liver flukes were present in the bile ducts. This finding is expected during this time as the slaughtered cattle would have been infected or reinfected in the previous hot and dry season. This agrees with Lima *et al.* (2001) who reported that the highest level of parasite contamination of pasture occurs during the dry season. A low prevalence in the hot dry season could have arisen because of ongoing infection or harbouring of immature and/or light infections. Light infection can have prominence as it may be easily missed (Froyd, 1959) and the efficiency of detecting it will vary with the training and experience of the meat inspector. The findings of Hyera (1984), Pandey and Ahmadu (1998) as well as Negesse (1994) also support the seasonal pattern found in our study. Hyera (1984) as well as Pandey and Ahmadu (1998) also found that *F. gigantica* prevalence rates were high during the wet season and low during the dry season. However, they did not include livers that were trimmed as a result of fluke infection.

In the post-rainy season, higher mean egg counts ( $11.5 \pm 4.9\text{SEM}$ ) and cattle found positive at coprological examination were recorded as compared to any other season with the highest EPG of 155. As the rains ended and drier conditions set in, egg counts got lower in some cattle in the cold dry season and the hot dry season in which mean egg counts of less than 1 EPG (egg range 1–12 and 1–4 EPG, respectively) were found. Such disparities can arise as a result of light and/or immature infections. The fluke EPG variation among the months and seasons is in support of Morril and Shaw (1942) and Coyle (1958) who found that liver fluke egg counts fluctuate in more than one faecal sample collected even from the same animal but at different times.

Since most of the time cattle of all ages access the same pasture zones, infection is picked up without regard to age. Although calves are kept near settlements and away from heavily contaminated pastures, they get infected almost immediately they are introduced to full grazing because of their naivety and the heavy pasture contamination around the watering holes. The finding of positive cattle among the young agrees with Lima *et al.* (2001) who demonstrated that infection in calves can be picked up from as early as two months old. Using coprological examination, the association of age was noticed only between two of the four seasons, namely, post-rainy season and cold dry season in which adult cattle had a significantly higher prevalence than the young cattle. This is in agreement with Negesse (1994). Adult animals are known to move long distances in search of scarce pastures and water thereby increasing their chances of infecting as well as getting infected at overcrowded watering holes. This follows a principle of repeated infections and

dose-dependent effect. Alternatively, older animals would have built up resistance to the parasite over the years due to repeated challenge and therefore only exhibit none or low infection. To this effect, FAO (1994) in agreement reported that spontaneous recovery does occur in chronically infected cattle and that previously infected animals partially resist re-infection.

Only Negesse (1994) examined the associations of sex, age and origin of cattle, but even he used coprological examination alone. The association of sex reported in our study was only significant on coprological examination where female cattle had a higher prevalence rate than in male cattle despite both groups grazing together in the communal pastures. There was, however, no significant difference between male and female cattle in the fluke egg distribution throughout the year.

Since *F. gigantica* infection has been determined to occur throughout the year, a study on strategic anthelmintic treatment schemes applicable to different climatic conditions and cattle production systems is suggested. Treating all the animals at the beginning of the dry season (October) and to exposed animals, repeating such treatments at the beginning of the post-rainy season (April) season is desirable. Another treatment is necessary (e.g. in June or July) in areas like the Kafue and Zambezi flood plains where development of larval stages may be continuous as a result of favourable climatic and environmental conditions. There is the need to do other seasonality studies for different geographical regions of the country to come up with a representative pattern. At the same time, it would be imperative to determine seasonality pattern in the field where egg counts in animals would be done throughout the year.

### 5.3 Liver dissection study

No correlation was established between the number of flukes found after dissecting 70 condemned livers and 32 non-condemned livers and fluke egg counts. This is in agreement with Coyle (1958), Hammond (1970) as well as Duwel and Reisenleiter (1984). Coyle (1958) found that naturally infected cattle in Uganda had low egg counts and that there was no correlation between the egg counts and the fluke burden. Boray (1969) as well as Duwel and Reisenleiter (1984) reported finding neither uniformity nor consistency in low, medium and high fluke burdens after examining faecal egg counts. It has been reported by Bisset *et al.* (1996) that egg counts are positively correlated to the worm burden in young animals especially those that are in their first grazing period. This relationship, however, slowly fades away as the animals gain immunity against the worms (Ploeger *et al.*, 1994; Bisset *et al.*, 1996). Acquisition of immunity affects the positive correlation in that egg production by female worms is depressed and low egg counts may be recorded even though significant numbers of adult worms are present in the host (Winks *et al.*, 1983). Hammond (1970) found that over a wide range of infection levels, faecal counts of *F. gigantica* eggs in cattle were low after about 7 to 8 months post infection. Even these low counts might be of importance in the epidemiology of *F. gigantica* infection because of the large mass of faeces passed daily. To elaborate this fact, Taylor (1964) estimated that an average adult bovine animal passes about twenty-two kilograms of faeces a day so that even a count of 5 EPG from such an animal would mean 110,000 eggs passed daily. Fluke eggs can also accumulate in

the gall bladder and thereby lead to their uneven expulsion. As a result, variations in egg counts in the faeces may be created.

According to Boray (1985), the diagnosis of infections of *Fasciola* species in ruminants has relied on the detection of *Fasciola* eggs in the faeces of infected animals. In older animals, faecal egg counts can be used to indicate the extent and intensity of parasitic infections in the flock or herd and thus may act as an indicator of the level of pasture contamination (Murrell *et al.*, 1989). However, in agreement with Roberts and Swan (1981), we caution against the use of faecal egg counts to predict individual animal's parasite burden, but state that the technique provides information with satisfactory degree of precision. It may also aid rational decision-making in the diagnosis and control in herds.

Damage caused by *F. gigantica* and *F. hepatica* varies. The lesions produced are the most constant and important in the liver although occasionally the parasite may reach the lungs or other tissues where they are usually found within abscesses (Jones and Hunt, 1983). Taylor (1964), Losos (1986) and Burger (1992) found that gross pathological lesions depend on the stage and severity of the infection. The gross and histopathological findings in this study closely resembled those described by Singh and Parihar (1988). In agreement with Urquhart *et al.* (1987), these findings were also characterized by hepatic fibrosis, hyperplastic cholangitis, irregular liver outline, pallor and firmness. The ventral lobe of the liver was the most affected and reduced in size. *Fasciola gigantica* is known to cause necrosis and consequent fibrosis of the liver. The basic principle at play essentially consists of replacement fibrosis of necrotic tissue. Fibrosis was found heaviest in the vicinity of the bile ducts, but in

severe cases it was noticeable throughout the liver tissue. Urquhart *et al.* (1987) reported that sometimes the fluke eggs provoke a granuloma-like reaction that can result in obliteration of the affected bile ducts. Chronic cholangitis and bile duct obstruction lead to ectasia and stenosis of the ducts, periductular fibrosis and thickening of the duct walls so that the ducts become increasingly prominent and sometimes calcified (Ross *et al.*, 1967; Simesen *et al.*, 1968). Though calcification is usually seen in cattle and not in sheep (Taylor, 1964), hyperplasia of the bile ducts in both species begins long before the flukes arrive (Taylor, 1964; Simesen *et al.*, 1968; Burger, 1992).

On histopathological sections, various lesions were also noted. Numerous micro-abscesses containing immature *Fasciola* or eggs were found accompanied by cellular reactions and calcification. Similar findings were reported by Uzoukwu and Ikeme (1978) and Singh and Parihar (1988). Patches of brown pigment as described by Sinclair (1964) and arising from haematin discharged by the fluke were prominent in some sections. Chronic infestations were characterized by marked liver cirrhosis. Cirrhosis arises from repair processes after damage caused by migrating immature flukes and chronic inflammation induced by them. Age, infestation experience, breed of the animal, nutritional status and other intercurrent factors might have a role in modifying the pathological pictures reported (Uzoukwu and Ikeme, 1978). One important aspect of *Fasciola* infection is that under field conditions, it can be continuous and therefore the animals affected suffer from the disease in its chronic form.

Nonetheless, it is apparent from previous studies by Hope-Cawdery *et al.* (1984) that the sooner the parasite can be destroyed, the smaller will be the damage, but greater the economic efficacy of the therapeutic drug both in sheep and cattle. The severe damage exhibited in the examined condemned livers and subsequently to gluconeogenesis may explain why traditional cattle do not achieve growth rates and levels of body condition experienced in the commercial animals.

#### **5.4 Snail larval trematode survey**

Overall larval trematode infection in *L. natalensis* in the present study was high, particularly at Chiyasa (57.9%). This could be attributed to high parasite pressure, making contact between miracidia and snail a common occurrence. Snails at Chiyasa had 57.9% infection as compared to 68.8% in cattle. Except for this finding, prevalence rate of *Fasciola* infection in cattle by coprological examination from other sampled areas was much lower than that observed in snails. This could partly be a result of sampling snails and cattle mostly during the dry season. This is when most habitats were easily accessible by both humans and their cattle. In addition, majority of *Fasciola* infections in cattle have been demonstrated to occur in the second half of the dry season (Schillhorn van Veen, 1979). Schillhorn van Veen (1980) also reported that redial infections in snails are common in the wet season and beginning of the dry season. Shedding of cercariae would then mainly occur during the middle of the dry season when the infection rate in the snails is declining (Schillhorn van Veen, 1979). Metacercariae are known to be acquired by grazing animals mainly during the middle of the dry season. The resulting patent infections are present at the very end of the dry season or beginning of the wet season.

However, the timing may vary depending on the length of the rainy season and on the grazing habits of the animals. Similar conclusions have been drawn from the fact that output of *Fasciola* eggs in cattle is also highest during the beginning of the wet season (Schillhorn van Veen, 1980).

The Kafue river basin is home to large herds of cattle which are known to be predisposed to infection with *Fasciola* as they graze pastures in wet areas for at least half of each year. The risky periods to infection by trematodes begin in June/July when wetland waters recede remarkably up to November/December when the rains begin. During this period, cattle have unlimited access to potentially metacercariae-infested pastures. Sichoongo (2003, personal communication), other cattle farmers and traders talked to confirmed that cattle coming from the Kafue river basin, regardless of age, had liver fluke infestation. As a result of this situation many livers have been condemned at slaughterhouses.

Five morphologically different types of larval trematodes infecting the sampled freshwater snails were recorded in the study. *Lymnaea natalensis* harboured four of the identified cercariae. These infections in snails did not occur in isolation but concurrently as confirmed by finding amphistome and *Fasciola* eggs in faeces of cattle grazing the same pasture zones. Overall, results of trematode larval infections were low. Different workers have also found low larval trematode infections (Loker *et al.*, 1981; Mattison *et al.*, 1995; Kigadye, 1998; Chingwena *et al.*, 2002). Several reasons for low infections have been advanced including the following: direct consequence of high rates of mortality induced by parasites (Sousa, 1992; Wright,

1966) and hosts developing acquired resistance to infection resulting from host-parasite co-evolution (Begon *et al.*, 1990).

It is apparent that while some snails can support more than one species of trematodes, others are more selective. In this study, however, we did not record any mixed infections. Though natural infections with amphistomes in *B. pfeifferi* (Dinnik, 1965) and *B. forskalii* (Dinnik, 1961; Wright *et al.*, 1979) have been recorded in Zambia, we did not find any infection in the above mentioned species. Only a small percentage (0.2%) of *B. tropicus* was found shedding amphistome cercariae. This could have arisen as a result of collecting few snail numbers and that most of the collected *B. pfeifferi* and *B. forskalii* were juvenile. Anderson and May (1979) and Ewers (1964) have reported that larval trematode infections are dependent on numbers of snails. This low prevalence finding agrees with findings of other authors (Brown *et al.*, 1988; Chao *et al.*, 1993; Schmidt and Fried, 1997). Even though it may occur, concurrent infection with more than one parasite species is comparatively uncommon (Kendall, 1964). Some theories have been advanced as why this is so. Double infections in snails could be more pathogenic and cause higher mortality rates than could single species infections (Sousa, 1992); antagonism among species could lead to scarcity of double infections (Sousa, 1993; Lafferty *et al.*, 1994) and temporal as well as spatial variation in the abundance of eggs and miracidia of different trematode species in relation to how often a snail can be infected by two or more species at the same time (Williams and Esch, 1991; Sousa, 1993).

Observations of Agersborg (1924) have indicated that different larval stages of a trematode may co-exist in a single snail. As such, all the cercariae do not mature at

the same time. These cercariae may leave the snail during an extended period of time as groups of parasites tend to leave the snail at intervals (Kendall, 1964). It is unknown as to what extent this phenomenon contributed to cercarial harvest and consequently prevalence in snails in our study.

Kendall (1964) reported that with a preferred host of *F. hepatica*, *L. truncuntula*, a complete loss of infection has never been demonstrated. A similar outcome might be reached when comparing the preferred host for *F. gigantica*, *L. natalensis*. *Lymnaea* snails from Shimungalu, Mazabuka district, exhibited infection even in the absence of recent direct cattle contact. This finding could have come about because cercariae and infected snails floated and drifted downstream from nearby cattle access points to Shimungalu sampling site. Even though they are not likely to outlive the infection, Kendall (1964) reported that under special conditions an individual snail was kept until as many as 4 000 mature cercariae had emerged before it died.

Factors affecting the increase in larval trematode infections in Zimbabwe during the dry season also applied in our study (Chingwena *et al.*, 2002). *Lymnaea natalensis* was easier to find during the dry season when the snails were found concentrated in the numerous marshy areas, the marginal shallow water of the main river, the lagoons and the ox-bow lakes than during the wet season. In the savannah, the snails are most abundant during the beginning of the dry season (McCullough, 1965; Schillhorn van Veen, 1980). However, the population decreases towards the end of the dry season when most streams and pools dry up. Many of the young and developing snails are washed away in torrential streams after rains during the wet season. Important determinants of snail abundance could include density of

hydrophytes and amount of aquatic vegetation as well as organic debris. On the other hand, critical determinants of trematode infections in snails could be distance of settlements and/or cattle kraals as well as the number of animals in nearby homesteads. There was quite a significant human contact at all habitats sampled, especially at sites that were used for laundry, fishing, watering and drinking purposes. Typical examples were noted along Kaleya stream in Mazabuka district and Chiyasa, Mumbwa district where human settlements and cattle kraals were present very near water bodies.

Reduced water volumes in the habitats during the dry season, and compounded by increased levels of contact with livestock favours accumulation of amphistome and *Fasciola* eggs in close proximity to potential snail habitats. Livestock aggregate around water bodies as a result of scarcity of pasture elsewhere and the increased grazing available around these wet areas. During this time, ideal conditions for parasite transmission are fulfilled. Snail and cattle populations aggregate in these wetland areas at a time when water temperature is also ideal for the development of *F. gigantica* eggs and larval stages in the snail host. These factors lead to increased frequency of contact between miracidia and intermediate snail hosts hence increasing the prevalence of infection in snails (Chingwena *et al.*, 2002). Standen (1963) found that *L. natalensis* were susceptible to infection with *F. gigantica* at all ages but that half-grown specimens were the most suitable. However, Dinnik and Dinnik (1959) stated that young *L. natalensis* could be infected much more easily than older specimens would. Local overcrowding of animals has particular importance in the epidemiology of trematode infections, firstly in the heavy contamination of snail

habitats with ova and secondly, in the ingestion of metacercariae. On the other hand, overstocking forces cattle to graze closer to the ground and therefore close to faeces resulting in the consumption of higher number of helminth eggs and metacercariae (Negesse, 1994).

In conclusion, the results from this study seem to imply that the risk of infection with liver fluke is high where vector snails occur. Studies by Cheruiyot and Wamae (1990) have shown that cercariae of *F. gigantica* shed from experimentally and naturally infected *L. natalensis* encyst to varying degrees on different objects. In nature, cercariae of *Fasciola* having been released in the dark (Salimov and Azimov, 1983) normally encyst on suitable surfaces, preferably grass or other vegetation and await their host. Cercariae can also encyst on other objects such as floating leaves particularly so when they float horizontally (Azimov, 1985). Sometimes, as noticed in our study, cercariae encyst on the surface of water where they may float indefinitely or sink to the bottom. While the surface of water as an encystment site would probably be negligible as a major source of infection to hosts, it may provide a source of infection to livestock, even man, if contaminated water is drunk raw (Cheruiyot and Wamae, 1990).

Water hyacinth (*Eichornia crassipes*) and other aquatic plants were seen to harbour and promote proliferation of different snails. Different amounts of algae and detritus were common on stalks and other parts of these plants and thus providing food sources for snails. The various aquatic plants provided shelter of snails from radiation and strong water concurrent. They also acted as suitable sites for laying their egg masses. Presence or absence of suitable food, mainly unicellular plants, is

one of the factors that influence snail populations (Boray, 1963). Boray (1963) further found that although snails are not able to ingest live plants, except for very soft tissues such as the leaves of the common watercress or duckweed, *L. tomentosa* (intermediate host of *Fasciola hepatica*) is capable of digesting cellulose. Seasonal influences on egg laying are apparent because the food of the snail consists predominantly of algae, the growth of which is conditioned by temperature. Soon after hatching the young snails leave the egg mass and commence active feeding. The type of bottom in the water governs sources of food and provides protection for the snails (Boray, 1963). The snails may prefer a muddy, alluvial bottom covered by moderate or sparse vegetation or no plants at all where unrestricted sunshine helps the growth of suitable algae. Sufficient light is necessary for normal reproduction. From our own observations and experiences of farmers, it was not elucidated whether cattle were able to graze these weeds or not.

## CHAPTER SIX

### CONCLUSIONS AND RECOMMENDATIONS

Eastern province was not included in the prevalence survey. There is need, therefore, for work on fasciolosis to be carried out in cattle from this area where none has been reported. This province is one of the major cattle keeping areas in Zambia but it differs from the rest of them by not having significant wetlands. After showing the magnitude of this disease in cattle of Western, Southern and Central provinces, the government must come up with a clear policy on its management country wide that will include offering extension services and remedial measures especially to poor traditional farmers whose cattle are hardest hit.

If losses due to bovine fasciolosis are to be minimized, it is important that different aspects of the disease be studied in different ecological regions of Zambia. This would enable formulation of appropriate control strategies. After undertaking the liver dissection study, several recommendations can be made. The sample size for non-condemned livers (n=32) was not the same as in the condemned livers (n=70). Therefore, in future, similar but larger sample sizes must be considered. Additionally, a study to examine the relationship between the amount of metacercariae ingested on the grass and liver fluke burden is suggested. From the present study where 9.4% of non-condemned livers were found to harbour liver flukes, it has been demonstrated that abattoir records are not a true reflection of *F. gigantica* infection in the field. This finding did not include those flukes that could have found in partially trimmed livers. In the routine inspection of livers, the meat inspector avoids mutilating the liver by not making cuts which reduce the sale of the

organ. As a result, flukes found outside the area of inspection may not be detected. It was for this reason that liver flukes were found in 9.4% (n=32) of the dissected non-condemned livers. This finding is similar to and supports the result in the prevalence survey where 9.5% (n=677) of faecal samples from animals whose livers were not condemned had fluke eggs. In spite of these figures, liver inspection is still a reliable method used to detect *F. gigantica* infections at slaughter.

The snail study only investigated the presence of larval trematodes during the dry season. Therefore, in order to improve the understanding of the distribution of the infection in Zambia, seasonality of *Fasciola* among other trematode infections, among other things, should be determined from different regions throughout the year. When such information is made available, it can be used together with the knowledge of the seasonal pattern of trematodes of cattle in general and *Fasciola* in particular to formulate efficient control programmes. Physical factors such as flooding and drought play an important role in the distribution of snails. Flooding helps in the dissemination of snails over a large surface area. If such snails may be infected with larval trematodes, then this infection is likely to be spread to other cattle rearing areas where the disease was not initially present. Drought conditions are able to bring about the destruction of many snails through desiccation. Snail populations can be wiped out when drought conditions persist for long periods. However, as shown elsewhere by Kendall (1964) and Bitakiramire (1968), *L. truncatula* and *L. natalensis* are capable of surviving for much extended periods in a state of drought-induced dormancy.

Since seasonality study was undertaken at one slaughterhouse catering for cattle from Southern province, there is also the need to do other seasonality studies for different geographical regions of the country. Such studies would define a representative pattern for the country. In doing so, the sample size to be examined should be much larger than the one we considered. In addition to the seasonality studies at the slaughterhouses, it is imperative also to determine this pattern in the field where EPGs in animals should be performed throughout the year.

To come up with efficient control programmes of fasciolosis, their design must include information on weather and seasonal surveying of the definitive and intermediate hosts. These control programmes can be three faceted. Firstly, strategic anthelmintic applications aiming at eliminating the parasite from the definitive hosts at periods of highest risk in order to effectively prevent or reduce pasture contamination. One treatment done in April and another in October can be both curative and prophylactic because they would reduce the fluke burden acquired from ingestion of surviving metacercariae. Additional treatment (s) in June/July may be necessary in areas like the Kafue and Zambezi flood plains where development of larval stages is continuous as a result of favourable climatic conditions. Secondly, a control program must aim at reduction of the number of snail intermediate hosts through drainage especially in areas with canals and marshes. Thirdly, reduction of the chances of infection in livestock may be achieved by efficiently managing their grazing.

In the use of anthelmintics, there must be some form of synchronized deworming that should be carried out in all animals in an area within a reasonable short-time. In

addition, community organization and adequate drug supply remain the hallmarks of such a successful campaign. Important to note, however, is the ability of an anthelmintic to effectively work against both mature and immature flukes. Since it was common to record concurrent *Fasciola* and amphistome infections in our study, drugs to be used should also be effective against these parasites. Worm control programmes should be coupled with a significant improvement in the nutrition and management of livestock to achieve meaningful economic benefits especially at the farm level (Keyyu *et al.*, 2003b).

Application of molluscicides would be ineffective in many parts of the wetlands as these *Lymnaea* habitats are topographically unsuitable and difficult to cover effectively. In such instances, regular application would be desirable since these snails rapidly repopulate within 3–6 months after effective treatment (Brown, 1994; FAO, 1994). In most situations, non-target organisms like fish might be exposed to the harmful effects of the drugs thereby upsetting the balance of nature (Webbe and Jordan, 1982; Appleton, 1985). Even though molluscicide application may not necessarily offer a practical and viable option for control of fasciolosis in Zambia, combination with other suitable methods could be useful for snail control in smaller isolated habitats like farms, irrigation canals, marshes, ponds, etc. In these habitats, regular clearing of vegetation from drainage and irrigation canals (as it is done sometimes in Western province) reduces silting and blockages. Removal of aquatic vegetation promotes free flow of water as well as removes shelter and food sources for snails, while preventing seepage (Brown, 1994). However, weed control is costly and plants speedily recover, with only a small effect that can be achieved against

snail populations (Hilali *et al.*, 1985). Silting and blockages of these waterways normally extends suitable snail habitats by creating marshy areas, through seepage, where contaminated herbage is most accessible to livestock (FAO, 1994). In as much as removal of vegetation helps in the control of fasciolosis, Brown (1994) argued that soil erosion and flooding resulting from such an action may produce environmental damage which could outweigh any reduction achieved in transmission of snail-borne diseases.

The increase in the human population in many of the habitable areas of Zambia means that more land is needed for agricultural crops among other things. This is often acquired at the expense of traditional grazing areas. When the pressure on available grazing increases, livestock are forced to use less suitable areas which may include those infested with *Lymnaea* and contaminated with metacercariae of *F. gigantica*. In areas adjacent to game parks and reserves, pressure on the available grazing means that livestock encroach more and more on to the land used by wild mammals. This aspect may be important in the epidemiology of fasciolosis in these areas.

After many modes of control have been tried elsewhere (e.g. anthelmintic treatment of cattle at the beginning of the dry season and repeated treatments to exposed animals at the beginning of the wet season- Schillhorn van Veen, 1979), there is some indication that outbreaks of fasciolosis can be prevented by limiting wetland grazing just before the rainy season. This strategy reduces the miracidial contamination and subsequently snail infection. If practical, especially in communal lands, keeping livestock away from potentially contaminated pastures during the dry

seasons may be the most rewarding system to be encouraged. However, this would bring in the problem of supplemental feeding as most cattle survive on the lush pastures found in the wetlands.

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**APPENDIX**

**Appendix Table 1** Year 2002 estimates of cattle population in Zambia according to provinces, husbandry system and age (Source, Ministry of Agriculture and Co-operatives).

Province	Traditional			Semi-commercial			Commercial			Overall Totals
	Young	Adult	Total	Young	Adult	Total	Young	Adult	Total	
Southern	92259	362668	454927	5231	20563	25794	18452	72533	90985	571706
Western	184000	481876	665876	0	0	0	0	0	0	665876
Central	21760	147509	169269	4352	29502	33854	5440	36877	42317	245440
Lusaka	25820	60280	86100	5164	12056	17220	6455	15070	21525	124845
Eastern	27762	112149	139911	5552	22430	27982	6941	28037	34978	202871
Northern	10127	51938	62065	2025	12985	15010	0	0	0	77075
Luapula	1642	5665	7307	0	0	0	0	0	0	7307
N/Western	14118	89626	103744	0	0	0	0	0	0	103744
Copperbelt	6462	23860	30322	1292	4772	6064	323	1193	1516	37902

Appendix Table 2 Variation in the ecological factors\* observed at different snail sampling areas.

Area	Aquatic vegetation	Type of shading provided	Nature of substratum	Slope of bank	Distance to houses/number of animals
Blue Lagoon	50-75% S, E, F	grasses	mud and leaves	Flat	Settlements within 100m. Hundreds of cattle and lechwe. Thousands of wild birds
Chiyasa	> 75% S, E, F	Azola, Water lilies, grasses, Kariba weed	mud and leaves	Flat	Settlements within 50-200m. Hundreds of cattle. Kraals near pasture zones.
Kaleya	<5% S, F	grasses	mud and leaves	Sleep	Settlements & kraals within 20-200m.
Shimungalu	> 75% S, F	water hyacinth, Kariba weed	mud	Flat	Cattle access area > 100m away.
Kafue bridge	> 75% S	grasses	mud & leaves	Flat	Settlements within 500m. Hundred cattle access area.
Shing'oma	25-50% S, E, F	grasses, water lilies, Water hyacinth	mud and leaves	Flat	Settlements within 200m. About thirty cattle.

S, submerged; E, emergent; F, floating

\*Ecological factors are those defined by Madsen (2003)