EPIDEMIOLOGICAL STUDIES ON BOVINE FASCIOLOSIS IN BOTSWANA

By

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DECLARATION

I declare that this thesis is my own account of my research and contains as its main content work which has not previously been submitted for a degree at any tertiary education institution

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ABSTRACT

Fasciolosis, commonly known as liver fluke disease, is a disease of the liver of domestic livestock, principally ruminants, caused by pathogenic trematodes of the genus *Fasciola*, which comprises two species, *F. hepatica* and *F. gigantica*. *Fasciola hepatica* is the more common and important of the two, with a worldwide distribution, whereas *F. gigantica* is more restricted, found primarily in warmer parts of the world where it causes tropical fasciolosis in cattle, sheep and goats.

Fasciola gigantica infection in cattle is potentially one of the most important parasitoses affecting the productivity of herds in many developing countries by being an impediment to reproduction and growth, causing damage to livers, which can occasionally become inedible for humans and, in some cases, can result in the death of affected animals. The economic importance of fasciolosis is mainly due to direct losses from condemnation of infected livers during meat inspection at abattoirs. The disease is also a zoonosis, however, it is rarely diagnosed in humans. Prior to the study reported in this thesis, little was known on the epidemiology of this parasitic disease in Botswana. Therefore, the main aim for undertaking this study was to determine the prevalence and estimate the economic significance of fasciolosis in cattle, as well as to determine the geographical distribution of the intermediate host snail, *Lymnaea natalensis*.

Lymnaea natalensis is an aquatic snail that has generally been accepted as the intermediate host that plays an essential role in the epidemiology of *F. gigantica*

infection in Africa, even though a miscellany of other Lymnaeid snails can be involved in the transmission of the fluke.

The present study determined the prevalence and assessed the economic importance of *F. gigantica* infections in cattle through retrospective and prospective studies, by acquisition of data from meat inspection records and regular visitation to inspect livers of slaughtered cattle at selected abattoirs, respectively. In addition, a cross-sectional survey of fasciolosis was carried out through a coprological examination of live animals to determine the prevalence in live cattle from six districts in Botswana. This information will be used as the basis for future epidemiological surveillance of this important parasitic disease of ruminants in Botswana. An understanding of the epidemiology of fasciolosis and distribution of the intermediate host would assist in the design of appropriate control programmes in Botswana.

The results from the present study have indicated that *F. gigantica* infection is present in cattle in Botswana, but the prevalence is very low (0.74%; 95% CI: 0.57, 0.94%) and not widespread as previously anticipated. The disease was present in only one (Central) of the six districts covered by this study, and was localized within the Tuli Block area, in Machaneng village, at the eastern margin of the country. Although the exact geographical origins of some of the fasciolosis-positive cattle was occasionally difficult to ascertain in abattoirs in the south of the country, it was highly likely that they originated from the northern part of the country and were already infected before being moved to the south, where they were eventually sent to the abattoirs.

The prevalence reported in this study rank among the lowest, not just in Africa, but the world as a whole, in terms of prevalence, infection intensity and economic impact in cattle. The study also revealed that the only species of liver fluke found in Botswana is *F*. *gigantica*. The results of the financial losses demonstrated a low financial burden as a consequence of condemnation of *Fasciola*-infected livers during the twelve-year period under consideration. These findings suggest that bovine fasciolosis is neither a major cause of liver condemnations at abattoirs nor a significant cause of reduced productivity in cattle in Botswana.

In spite of the low prevalence of *F. gigantica* infections in cattle, the disease showed a significantly higher prevalence in adult animals than weaners and calves. Gender differences in susceptibility were observed, with females demonstrating a significantly higher infection than males. Also of note was an indication of breed differences in susceptibility to infection. The Brahman and Brahman crosses exhibited a higher prevalence whereas the Nguni cattle showed no infection at all. These findings imply varying levels of immunity in these breeds, with a higher resilience and resistance shown by the Nguni native breed.

The population dynamics of the intermediate host snail, *L. natalensis*, was not determined since no snails were detected from the potential habitats investigated. The failure to detect snails was most likely linked to the drought that prevailed in Botswana during the two years this research was carried out.

The present study was able to reveal the existence of concurrent natural infection of cattle with liver fluke, *F. gigantica*, and the stomach fluke, amphistome, in Botswana. There was a significant positive association between the two trematode infections, as has also been reported in other parts of Africa. However, the prevalence of co-infection was low (0.16%; 95% CI: 0.09, 0.27%) and this could be attributable to the absence of *F. gigantica* infection in the other five districts of study.

It is concluded that infection of cattle from Botswana, with *F. gigantica*, is low and the distribution of the fluke is linked to suitable environmental and climatic conditions for the intermediate host.

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Mochankana, M.E. and Robertson, I. D. (2012) A retrospective study of bovine fasciolosis in abattoirs in Botswana. Annual Poster Day for year 2012. School of Veterinary and Life Sciences

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COMMONLY USED ABBREVIATIONS

ALP – Alkaline phosphatase

ANOVA – Analysis of variance

AST – Aspartate amino transferase

AUD – Australian Dollar

BMC – Botswana Meat Commission

BWP – Botswana Pula (Currency)

CI – Confidence Interval

DMS – Department of Meteorological Services (Botswana)

DVS – Department of Veterinary Services (Botswana)

EPG – Egg per gram

EU – European Union

FAO – Food and Agricultural Organization

FMD – Foot and Mouth Disease

GGT – Gamma glutamyl transferase

GLDH – Glutamate dehydrogenase

LDH – Lactate dehydrogenase

LOG_{10} – Logarithm 10

MITI – Meat Inspection Training Institute

MoA – Ministry of Agriculture (Botswana)

MSA – Multi species abattoir

OR – Odds ratio

PCV – Packed cell volume

SEM – Standard error of the mean

Spp – Species

SPSS – Statistical Programme for the Social Sciences

UK – United Kingdom

USA – United States of America

USD – United States Dollar

WHO – World Health Organization

χ^2 – Chi square

CHAPTER ONE

1 GENERAL INTRODUCTION

Botswana is essentially an agricultural country despite progressive structural transformation in its economy since independence in 1966. The agricultural sector, which comprises the pastoral and arable sub-sectors, has declined in recent years and contributes approximately only 2% of the total gross domestic product (GDP), of which 70% is derived from beef production, the most important agricultural enterprise in Botswana (Statistics Botswana, 2010). The livestock sub-sector is dominated by cattle, and crop production is dominated by the four major crops of sorghum, maize, millet and beans (Statistics Botswana, 2010). In spite of the decline in the agricultural sector, the sector still remains of strategic importance to the country's economy, and is driven largely by international beef exports to the European Union (EU) and South African (SA) markets. Therefore, there is the necessity for continued rural development to improve agricultural productivity despite the poor performance of the sector, particularly given the fact that the majority of people in rural areas derive their livelihood from agriculture (Statistics Botswana, 2010).

Livestock production in Botswana, as in many developing countries, plays many roles in the socio-economic lives of the Botswana people, including the provision of food, income, employment and draught power. However, the performance of the beef industry is currently unsatisfactory due to the presence of diseases, in particular foot and mouth disease (FMD), which have had a negative impact on the productivity of livestock in the country (Department of Veterinary Services, 2008). Fasciolosis, commonly known as liver fluke disease, is potentially one of the most important diseases resulting in poor livestock productivity in the country, and its prevalence and therefore economic significance, could be higher than currently envisaged. However, as fasciolosis manifests predominantly as a subclinical or inapparent parasitic infection, and rarely results in severe clinical signs in cattle, it is currently regarded as a disease of lesser importance and consequently little work has been conducted on the disease in the country.

Fasciolosis is a disease of the liver of domestic livestock caused by infestation with flukes of the genus *Fasciola*. It is a worldwide parasitic disease common in ruminants (Marques and Scroferneker, 2003), and is widely distributed in tropical and subtropical areas of Africa and Asia where it has a major impact on the productivity of domestic ruminants (Keyyu *et al*., 2005b). It is regarded as one of the most important parasitic diseases of cattle, sheep, goats and buffaloes (Marques and Scroferneker, 2003). Its major impact is through lowered productivity of herds and flocks by inhibition of growth, reduction in milk and meat production, damage to livers so that they are unsuitable for human consumption and death of livestock, and also results in significant expenditure on treatment and control (Saleha, 1991; Coelho and Lima, 2003).

Lymnaeid snails, the intermediate hosts of *Fasciola* species, play a very essential role in the epidemiology of fasciolosis (Coelho and Lima, 2003). Therefore, fasciolosis occurs throughout the world predominantly in areas where climatic and environmental conditions exist, which favour the survival and proliferation of the intermediate host snail (McGavin and Zachary, 2007). In contrast, the disease is absent in areas where conditions are not suitable for the development of the intermediate hosts (Torgerson and Claxton, 1999). The distribution of *Fasciola* spp in the environment is variable, and in spite of this variability, the parasite requires a constant set of suitable conditions of moisture and temperature for the reproduction of the intermediate host snail and the subsequent development of the intra-molluscan larval stages (Torgerson and Claxton, 1999).

The epidemiology of fasciolosis in ruminants has previously not been investigated in Botswana. The purpose of the study reported in this thesis was to investigate the epidemiology of fasciolosis in cattle in Botswana and to design a suitable strategic control programme for the disease. In the following chapter, the key literature on fasciolosis is discussed.

CHAPTER TWO

2 LITERATURE REVIEW

2.1 Introduction

Liver fluke is a general name for the digenean trematodes (Phylum Platyhelminthes - Class Trematoda – Subclass Digenea) (Borgsteede, 2002). Fasciolid flukes are among the largest and best known digenetic trematodes and have considerable veterinary significance (Lotfy *et al*., 2008). In veterinary science, there are two families of importance namely, the Fasciolidae with the genera *Fasciola* and *Fasciolides*, and the Dicrocoelidae with the genus *Dicrocoelium*. Of major importance to domesticated animals, particularly ruminants, is the genus *Fasciola* with two species, *Fasciola hepatica*, the common temperate liver fluke, and *Fasciola gigantica*, the tropical large liver fluke (Spithill and Dalton, 1998; Borgsteede, 2002; Jones *et al.*, 2006).

Liver flukes are significant pathogenic parasites resulting in fasciolosis, which is sometimes called liver fluke disease, liver rot or hepatic distomiasis (Jones *et al*., 2006). *Fasciola hepatica* has a cosmopolitan geographical distribution, occurring worldwide, whereas *F. gigantica* is more limited, being restricted to tropical countries in Africa, the Middle East, Eastern Europe and southern and eastern Asia (Torgerson and Claxton, 1999; Keyyu *et al*., 2005b; Taylor *et al*., 2007). The wide distribution of *F. hepatica* is believed to be, in part, caused by its adaptability to different Lymnaeid snails (Lotfy *et al*., 2008).

Fasciola hepatica was officially classified in 1758 by Linnaeus (Soulsby, 1982; Faria *et al.*, 2005; McKay, 2007; Lotfy *et al*., 2008). However, it wasn't until a century later (Cobbold, 1856) that *F. gigantica* was classified (Soulsby, 1982; Lotfy *et al.*, 2008). However, the origins, patterns of diversification and biogeography of fasciolids are poorly understood, although they are assumed to have originated in Africa and migrated to Eurasia, with secondary colonization of Africa (Lotfy *et al*., 2008). These hepatic trematodes have an indirect life cycle, using several species of amphibious snails of the genus *Lymnaea* as intermediate hosts.

2.2 The parasite

The parasite, *Fasciola*, affects the liver of animals, in particular ruminants. *Fasciola gigantica* is a large liver fluke that causes tropical fasciolosis, and is regarded as one of the most important platyhelminth parasites of ruminants. It is restricted only to the warmer regions of the world including parts of Africa and Asia (Radostits *et al.*, 2007; Soliman, 2008), the Pacific Islands, eastern Europe, southern United States of America (USA), Russia, and the Middle East (Soulsby, 1982; Jones *et al.*, 2006). However, *F. hepatica* is considered the more common and important liver fluke that is widely distributed throughout the world (Soulsby, 1982; Urquhart *et al.*, 1996; Radostits *et al*., 2007; Taylor *et al.*, 2007; Khan *et al*., 2009). *Fasciola gigantica* is better adapted to cattle than sheep (or goats) in that it is more infective and lives for a longer time in cattle (Suhardono and Copeman, 2008) than other domestic ruminants. Wild herbivores are also susceptible to the parasite, whereas laboratory animals are not readily infected. Human infection may occur in areas where the parasite is endemic in domestic ruminants (Suhardono and Copeman, 2008).

Flukes in the Fasciolidae family are well known for their large body size (Lotfy *et al.*, 2008). The two species of *Fasciola* are hermaphroditic, bilaterally symmetrical and leaf-shaped digenetic trematodes which require a snail of the genus *Lymnaea* as an intermediate host. *Fasciola hepatica* is greyish-brown with a conical front part that is marked off by distinct shoulder-like broadening and a pointed rear end. Adult *F. hepatica* measure approximately 30 mm long (FAO, 1994; Taylor *et al*., 2007). *Fasciola gigantica* resembles *F. hepatica* in most respects, but are larger in size, with adults measuring up to 75 mm in length. The body is more transparent, with the shoulders comparatively less discernible, and the tail being more rounded (Soulsby, 1982; Jones *et al.*, 2006; Radostits *et al.*, 2007; Taylor *et al.*, 2007).

2.2.1 The Life Cycle of *Fasciola*

Fasciola hepatica was the first fluke for which a complete life cycle was discovered (Lotfy *et al.*, 2008) with that of *F. gigantica* being discovered subsequently and being similar in most respects. Adult flukes live in the bile ducts, mainly of ruminants, where they produce eggs which are transported from the common bile duct into the duodenum and subsequently into the hosts' faeces (McKay, 2007).

The eggs are passed out in faeces undeveloped and subsequently separate from the faecal material and undergo embryonation, hatching into miracidia under optimal conditions of temperature and moisture (Andrews, 1999; Kusiluka and Kambarage, 2006; Taylor *et al.*, 2007). The rate of development is dependent upon temperature, therefore at a moderate ambient temperature of 22 to 26˚C, *F. hepatica* eggs hatch in 9 to 12 days. In contrast, eggs of *F. gigantica* take slightly longer to hatch in approximately 17 days, with little development occurring below 10˚C (Soulsby, 1982; Taylor *et al*., 2007). The liberated miracidia have a short lifespan and must find and invade a suitable intermediate host (snail) within 3 to 24 hours of hatching (McKay, 2007; Radostits *et al*., 2007; Taylor *et al*., 2007). The finding and recognition of the host snail in aquatic environments is assisted by mucus glycol-conjugates, which are essential signal molecules excreted by snails (Kalbe *et al.*, 2000).

When the fully developed miracidia leave the eggs, they swim actively to penetrate the intermediate host (Molina, 2005). Once inside the snail, development proceeds through sporocyst, redia and cercarial stages (Kusiluka and Kambarage, 2006; Taylor *et al.*, 2007), in 25 to 100 days during the warm season (Molina, 2005), but this is extended to 175 days during the cold season (Soulsby, 1982). The cercariae then leave the snails, as motile forms, and swim until they find herbage where they encyst to form metacercariae, which are the infective stages of the fluke. The metacercariae are ingested by grazing ruminants, the definitive hosts, with infected aquatic plants or water to complete the life cycle (Kusiluka and Kambarage, 2006; Hutchinson and Love, 2007; McKay, 2007).

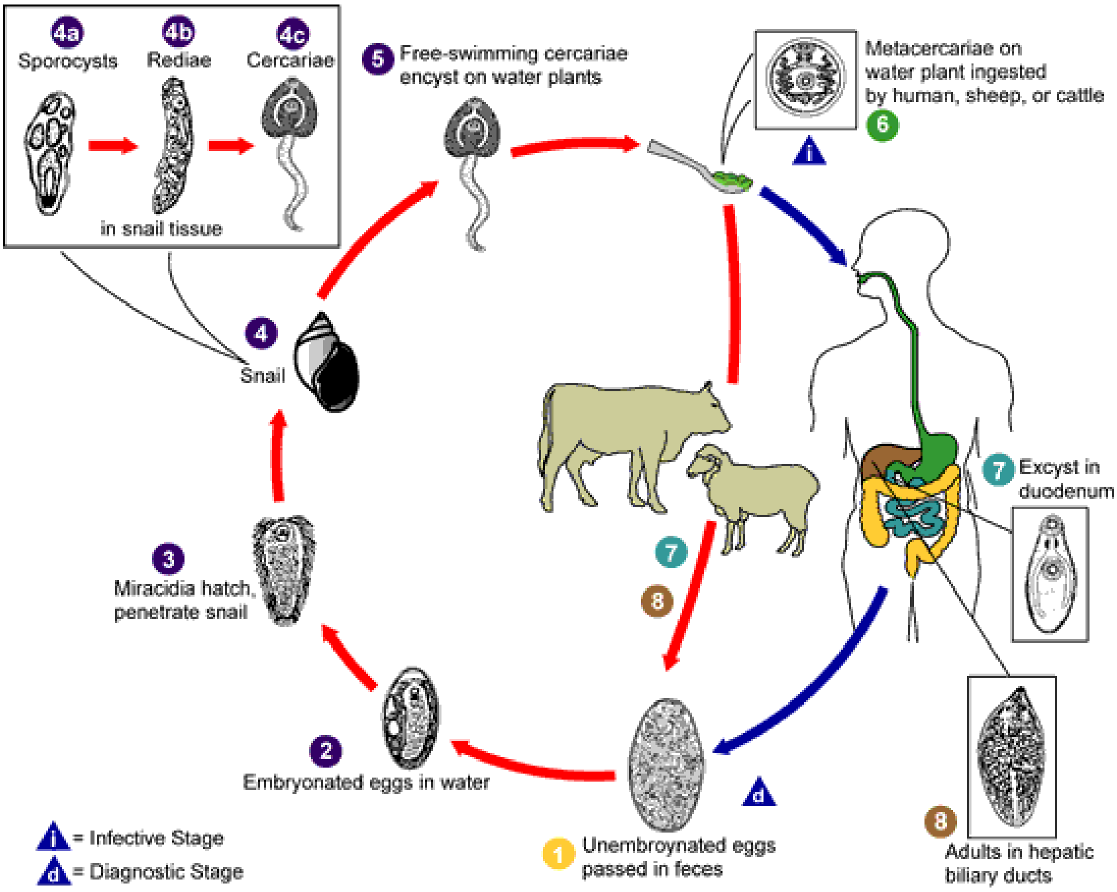


Figure 2.1 *Fasciola gigantica* life cycle (Source: Centre for Disease Control)

The development from miracidium to metacercaria can take a minimum of 6 to 7 weeks under favourable field conditions, although in unfavourable conditions, a period of several months may be required (Taylor *et al*., 2007). Following ingestion by the definitive host, metacercariae exsheath in the small intestine from where young flukes migrate across the peritoneal cavity, into the liver. The young flukes wander through the hepatic parenchyma for 4 to 8 weeks (Hutchinson and Love, 2007; Radostits *et al.*, 2007), growing from 0.1 to 10 mm, before entering the bile ducts where they mature, and commence egg laying in approximately 9 to 12 weeks post infection (Soulsby, 1982; Radostits *et al.*, 2007; Taylor *et al.*, 2007). (The life cycle is outlined in Figure 2.1).

2.2.2 Intermediate hosts of *Fasciola*

Fasciola flukes develop in suitable intermediate hosts, belonging to the phylum Mollusca; class Gastropoda; subclass Euthyneura and Pulmonata (Torgerson and Claxton, 1999) and these are often specific to a particular area. The freshwater snails of the genus *Lymnaea* (synonym, *Galba* or *Radix*) are considered to be most important in the transmission of *Fasciola* species: In African countries where *F. gigantic*a is prevalent, *Lymnaea natalensis* (Krauss, 1848) is the most important intermediate host. In contrast, the synonymous *Radix natalensis*, together with *Galba truncatula,* are believed to play a role in the transmission of the parasite in Egypt (Soliman, 2008). *Lymnaea rubiginosa* and *L. auricularia* serve as intermediate hosts in Asia, including Indonesia, Malaysia, and the Philippines whilst *L. rufescens* and *L. acuminata* are the host snails in India and other tropical parts of Asia (Soulsby, 1982; Molina, 2005; Soliman, 2008).

In countries where *F. hepatica* is a problem, *L. tomentosa* is the most important intermediate host. In Australia, *L. tomentosa* is an indigenous fresh water snail, however, the introduced snails, *L. columella* and *L. viridis*, can also act as intermediate hosts. *Lymnaea columella* appears to be the most important intermediate host in New Zealand, although *L. tomentosa* and *L. truncatula* can also play a role. In contrast, *L. (Galba) bulimoides* is the most important snail for *F. hepatica* in the USA and the Caribbean, *L. truncatula* is the principal intermediate host in the United Kingdom and other European countries, as well as in Africa, and *L. columella* has been identified as the intermediate host in Canada, central and south America (Soulsby, 1982; Boray *et al*., 1985; Amato *et al*., 1986; Graczyk and Fried, 1999; Hutchinson and Love, 2007; McKay, 2007; Radostits *et al*., 2007; Taylor *et al*., 2007). *Lymnaea cailliaudi* is regarded as the main intermediate host of *F. hepatica* (but can also act as the intermediate host for *F. gigantica*) in Egypt, and has also been reported in east Africa (Soliman, 2008).

Experimental challenges have indicated that some species of *Fasciola* can adapt to new intermediate hosts. It has been shown that the Australian intermediate host snail of fasciolosis, *L. tomentosa*, is also receptive to the miracidia of *F. gigantica* from east Africa, Malaysia and Indonesia (Soliman, 2008).

2.2.3 Ecology of the Lymnaeid snail

In general, snails survive well and multiply rapidly where wet or damp conditions exist (Urquhart *et al*., 1996). Soulsby (1982) noted that lymnaeid snails have a preference for poorly drained land, drainage channels and areas of seepages from springs or broken drains. They also prefer non-acidic, low-lying swampy areas with slow moving water, however, land with small streams, springs, spillages and leakages from sources such as water troughs or which has been irrigated may also facilitate their survival (Boray, 1964; Radostits *et al.*, 2007). Also, any work that alters the drainage of land or the application of lime to improve pasture may create an environment suitable for the survival of the snail and hence the transmission of *Fasciola* spp (Radostits *et al.*, 2007).

Snails vary in their aquatic requirements, with some having a more aquatic life (Radostits *et al.*, 2007), while others are mainly amphibious (Dunkel *et al.*, 1996). For example, *L. elodes* is aquatic and is mostly found fully submerged (Dunkel *et al*., 1996), *L. truncatula* prefers wet mud to free water (Taylor *et al*., 2007), whereas other species are tolerant of dry conditions and are found in the fringes of water and rarely in the middle of standing water (Dunkel *et al.*, 1996). However, all snails are restricted to damp or wet environments (Radostits *et al*., 2007). Although they have a preference for aquatic or at least amphibious environments, lymnaeid snails do have the ability to withstand periods of drought and conditions of low temperature by undergoing aestivation or hibernation for several months, respectively, deep in the mud (Soulsby, 1982; Taylor *et al*., 2007). This ability, together with their extreme rapid rate of reproduction, ensures infection of snails and completion of the life cycle (Andrews, 1999).

Under field conditions, some snails may survive for several months in dry mud and on the return of moist conditions they rapidly grow to maturity, with simultaneous rapid development of the developmental stages of the fluke. Consequently, within a short time, large numbers of cercariae may accumulate on herbage when moist conditions return to the snail habitat (Soulsby, 1982). In contrast, one study in Indonesia reported that snails died during the dry season due to a lack of water, with no evidence of aestivation, with the surviving snails being mainly those located in streams, rivers or springs (Suhardono and Copeman, 2008).

Snail habitats may be permanent or temporary, with the latter habitats increasing or decreasing in size depending on water availability (Radostits *et al*., 2007). Temporary habitats, which are often created following heavy rainfall or flooding, may include muddy gateways, vehicle-wheel ruts, hoof prints, springs, mud along the banks of slow-moving banks rivers, or streams and areas near drinking troughs (Soulsby, 1982; Taylor *et al*., 2007). Permanent habitats may include the banks of (irrigation) ditches or deep streams, large dams, lakes, swamps and the edges of ponds, lakes or dams (Boray, 1964; Hurtrez-Boussès *et al.*, 2005; Taylor *et al*., 2007). Although permanent water sources may provide indefinite security for the maintenance of snails, such habitats have been found to have lower numbers of snails than habitats containing temporary water sources (Boray, 1964).

Even though there are many ecological factors affecting snail populations including the vegetation, depth of water, water current, light and competition by other snails (Soliman, 2008), the two most important factors that determine the development, time of occurrence and the severity of fasciolosis are temperature and rainfall (Ollerenshaw, 1970; Amato *et al*., 1986; Radostits *et al*., 2007). It is well established that a minimum temperature of 10˚C is required before snails can breed (Radostits *et al*., 2007; Taylor *et al*., 2007) whilst temperatures above 35˚C can result in the death of larvae. The average temperature should be higher than 10˚C in order to facilitate reproduction (Amato *et al*., 1986), as it is only when the temperature rises to 15˚C and is maintained above that level, that significant multiplication of snails and larval fluke stages occurs (Taylor *et al*., 2007). The optimum temperature range for the survival and development of *L. natalensis* and *L. tomentosa* ranges from 15 to 26˚C (Boray, 1964; Kusiluka and Kambarage, 2006).

The requirements for embryonation and hatching of *Fasciola* eggs include suitable temperature (20-25˚C), availability of oxygen and sufficient light (Boray, 1964; Kusiluka and Kambarage, 2006) and suitable moisture conditions for snail breeding. The development of *Fasciola* within the snail are facilitated when rainfall exceeds transpiration, and field saturation is attained (Taylor *et al*., 2007). Such conditions are also necessary for the development of fluke eggs, miracidia searching for snails and for the dispersal of cercariae being shed from the snails (Taylor *et al*., 2007). These requirements are reported to be provided by the humid environments, which prevail in most sub-Saharan countries, and favour the embryonation and hatching of eggs in the region throughout the year (Kusiluka and Kambarage, 2006).

The findings of a recent field study in Indonesia showed that a temperature of 20˚C and a relative humidity as high as 95%, provide the most favourable conditions for the survival of metacercariae. In contrast, as the temperature increases and relative humidity decreases, the duration of survival and proportion of metacercariae surviving, decreases (Suhardono *et al*., 2006b). Similarly, metacercariae immersed in water survived for a longer time than those exposed to direct sunlight due to desiccation (Suhardono and Copeman, 2008).

Some studies have shown that in a suitable environment of moderate temperature and wet pastures, a population of up to 3300 snails/m^2 may develop (Soulsby, 1982). Similarly, a field study in Indonesia found that the population dynamics of *L. rubiginosa* in paddy fields varied with the cropping practices adopted, with the population being very high during the wet season (Suhardono and Copeman, 2008). Therefore, temperature and rainfall have a large effect on both the spatial and temporal abundance of snails and the rate of development of fluke eggs and larvae (Radostits *et al*., 2007). Research has also determined that metacercariae are not likely to persist for long periods under pasture conditions even though they can survive for a long time under laboratory conditions (Soulsby, 1982). Metacercariae can also survive for some months in moist hay but are unlikely to survive normal hay/silage-making for extended periods due to desiccation or the heat of summer when these activities are usually undertaken (Soulsby, 1982).

The findings from earlier research also demonstrated that there is a relationship between the size of the snail and the number of developmental stages, with larger snails having more larval stages than smaller snails (Ollerenshaw, 1970; Soulsby, 1982). These findings were confirmed by the results of a recent study in Indonesia which failed to detect infection in snails smaller than 10 mm long. In contrast, infections were detected in larger snails measuring between 10 and 20 mm in length (Suhardono and

Copeman, 2008). Consequently, it is not just the population of snails but their size that influences the number of parasites present.

During the cold season, if the temperature is less than 10˚C, there is no development of eggs or larval stages of the snail (Soulsby, 1982). Therefore, no fluke development takes place during winter in most countries, although it resumes as the temperatures rise in the following spring. The importance of this depends on the mortality rate of snails during winter, which can vary from region to region and from year to year (Radostits *et al*., 2007). A study by Boray (1964) found that during the winter months when the temperature was around 0˚C, snails were usually found in deeper water where the temperature was higher, and they moved to shallow water or the edges of water in summer.

In temperate countries such as the United Kingdom, the ideal conditions for fasciolosis occur from May to October, during which period there is a pronounced increase in the number of metacercariae present on pasture. This increase can be divided into two periods: the summer infection and winter infection of snails in which metacercariae appear on the pasture from August to October and from May to June, respectively (Taylor *et al*., 2007). In most European countries, the summer infection of snails is the more important phase and an increase in the numbers of metacercariae occurs annually, with the highest increase occurring in years with heavy summer rainfall. The winter infection is of less significance, but might occasionally give rise to large numbers of metacercariae in late spring or early summer from an overwintering infection of snails, particularly when the preceding months have been unduly wet (Ollerenshaw, 1970; Taylor *et al*., 2007).

In warmer areas of the world such as in the southern USA or Australia, the sequence of events has a different seasonality, but the epidemiological principles remain the same (Taylor *et al*., 2007) and overwintering may play a very important role in the survival of the parasite and snail (Shaka and Nansen, 1979). The snail activity in southern USA is highest during the cooler months of autumn, with peak numbers of metacercariae in winter. In eastern Australia, the snail continues to produce eggs throughout the year, but it is at its lowest level during winter (Taylor *et al*., 2007). Under the Australian conditions, overwintering takes place mainly in the form of infected snails, which frequently gives rise to heavy infection of sheep as early as mid-spring (Boray, 1969; Shaka and Nansen, 1979).

Other factors which have been found to have an important influence in the survival and distribution of snails include stream gradient and water current, water turbidity, light or degree of shade, type of soil, oxygen tension, salinity, aquatic vegetation, food and pollution (Kendall and Parfitt, 1953; Boray, 1964; Mzembe and Chaudhry, 1979; Ndifon and Ukoli, 1989; Ofoezie, 1999; Hourdin *et al*., 2006; Phiri *et al*., 2007b). River banks with steep slopes and a faster flow of water have been found to remove mud and vegetation which results in an environment that is unsuitable for snails (Boray, 1964; Mzembe and Chaudhry, 1979). In addition, turbid or polluted water and water with increased salinity are also unsuitable for snails, whilst muddy, alluvial soils, a pH range of 5.0 to 8.0 and the presence of suitable food, mainly unicellular algae, enhance the survival of snails (Boray, 1964; Ndifon and Ukoli, 1989; Phiri *et al*., 2007b). Studies by Boray (1964) and Ndifon and Ukoli (1989), found that the presence of aquatic vegetation was desirable for habitats of freshwater snails. However, while Boray (1964) found no definite relationship between the presence of particular plants and the distribution of snails, Ndifon and Ukoli (1989) indicated a relationship with certain types of vegetation.

2.3 Pathology of infection

2.3.1 Pathogenesis

Liver flukes are significant pathogens in domestic animals, particularly cattle and sheep (Jones *et al.*, 2006). The pathogenesis of fasciolosis is attributable in part to the invasive stages in the liver and to the blood-feeding adults in the bile ducts (Mehlhom and Armstrong, 2001). The pathological manifestation is dependent upon the number of metacercariae ingested, the phase of parasitic development in the liver and the species involved (Mehlhom and Armstrong, 2001; Taylor *et al*., 2007). There are two phases, with the first being caused by migrating larvae through the hepatic parenchyma whilst the second phase is induced by adult flukes in the bile ducts (Soulsby, 1982; Jones *et al.*, 2006; Taylor *et al*., 2007). The first phase is associated with hepatic damage and haemorrhage whereas the second phase results from the haematophagic activity of the adult flukes and damage to the biliary mucosa by their cuticular spines (Taylor *et al*., 2007). However, the effects of both stages may be present simultaneously (Jones *et al*., 2006).

The pathogenic effects of *F. hepatica* and *F. gigantica*, are considered to be essentially the same and thus result in a similar disease (Jones *et al.*, 2006; Kusiluka and Kambarage, 2006). However, it is assumed that *F. gigantica* is more pathogenic due to its ability to cause more damage because of its larger size and longer period of time in the liver (Molina, 2005) although information on the pathogenesis of *F. gigantica* is relatively limited to confirm this. The only difference reported between the pathogenesis for the two *Fasciola* spp. is that in cattle only the chronic form of the disease occurs with *F. gigantica* but not with *F. hepatica*, whereas in sheep both the acute and chronic forms occur with both species (Soulsby, 1982).

Following initial penetration of the liver parenchyma, migrating juvenile flukes cause haemorrhagic tracts of blood, fibrin, cellular debris and necrosis in the parenchyma (Hutchinson and Love, 2003; Jones *et al.*, 2006; McGavin and Zachary, 2007) and destruction of hepatic cells (Jones *et al*., 2006) resulting in blood loss (Hutchinson and Love, 2007). Sometimes, if animals are exposed to large numbers of metacercariae, the burrowing flukes lead to extensive liver damage and haemorrhage, causing an acute syndrome characterized by severe anaemia, eosinophilia and an associated peritonitis (Hutchinson and Love, 2003; Jones *et al.*, 2006). This severe anaemia may lead to liver failure with death within 8 to 10 weeks. When parasites reach the bile ducts, they ingest large quantities of blood, which can result in severe anaemia (Hutchinson and Love, 2007), and the presence of the flukes in the biliary passages provokes considerable tissue reaction, leading to chronic inflammation (cholangiohepatitis) as well as enlargement of the bile ducts (Jones *et al.*, 2006; Hutchinson and Love, 2007; McGavin and Zachary, 2007).

The disease may follow an acute or chronic course, which occur 2 to 6 weeks and 4 to 5 months, respectively, after ingestion of large and moderate numbers of metacercariae (Mehlhom and Armstrong, 2001; Taylor *et al*., 2007). The acute syndrome is less common than the chronic type, and is seen more frequently in sheep than cattle (Hutchinson and Love, 2003; Jones *et al*., 2006). The acute syndrome is essentially a traumatic hepatitis produced by the migration of large numbers of adolescent flukes (Mehlhom and Armstrong, 2001). This form of fasciolosis is due to extensive destruction of the liver parenchyma and the severe haemorrhage which occurs from migrating young flukes. The major effects seen with chronic fasciolosis include anaemia and hypoalbuminaemia (Taylor *et al*., 2007). It occurs towards the end of the acute phase, approximately 6 weeks after infection, with serious losses occurring 7 to 8 weeks after infection (Mehlhom and Armstrong, 2001).

A variety of untoward sequelae can often accompany the migration of immature flukes, including acute peritonitis, hepatic abscesses, death of the host as a consequence of acute, widespread hepatic necrosis produced by the massive infiltration of immature flukes, and the proliferation of spores of *Clostridium* species, particularly *C. haemolyticum* or *C. novyi* in necrotic tissue, which causes the subsequent development of bacillary haemoglobinuria or infectious necrotic hepatitis, respectively (McGavin and Zachary, 2007).

2.3.2 Clinical Signs

The clinical outcome of infection depends largely on the infectious dose, which is closely related to the density of metacercariae on the pasture (Radostits *et al.*, 2007). The disease in sheep may be acute, subacute or chronic, while in cattle the chronic form is far more important, although acute and subacute disease may occasionally occur under conditions of heavy challenge, especially in young calves (Taylor *et al*., 2007). A high intake of metacercariae over a relatively short time produces an acute disease, whereas an intake of lower numbers over a longer period leads to chronic disease. Subacute fasciolosis is a result of intake of moderate numbers of metacercariae over a longer period (Hutchinson and Love, 2007; Radostits *et al.*, 2007).

The clinical signs of fasciolosis vary depending on the severity of infection and the stage of the disease. The acute infection (invasive phase) is characterized by sudden death, weakness, pale mucous membranes, dyspnoea, icterus, blood-stained froth from the nostrils, bloody discharge from the anus, abdominal pain and ascites. Young calves can show similar signs, but only on rare occasions (Soulsby, 1982; Kusiluka and Kambarage, 2006; Hutchinson and Love, 2007). In the subacute form, affected animals show anorexia, lethargy, weight loss, pale mucous membranes, jaundice, loss of body condition and occasionally death (Anonymous, 2006; Hutchinson and Love, 2007).

Chronic fasciolosis, which is mainly associated with mature flukes, is seen in sheep, goats and cattle, and may be asymptomatic in mild infections. It is predominantly a persistent wasting disease characterized by a progressive loss of body condition, pale mucous membranes, emaciation, reluctance to move, submandibular oedema, ascites (hypoalbuminaemia), and possibly death (Yadav *et al.*, 1999; Anonymous, 2006; Kusiluka and Kambarage, 2006; Hutchinson and Love, 2007). Although cattle show some resistance, calves are more susceptible and display clinical signs similar to those in sheep (Anonymous, 2006). In cattle digestive disturbances with constipation, attended by diarrhoea in extreme stages, is often a feature of infection (McKay, 2007).

2.3.3 Clinical Pathology

The pathological manifestations in fasciolosis are dependent upon the number of metacercariae ingested (Mehlhom and Armstrong, 2001), however, the common haematological findings in all types of fasciolosis include anaemia, hypoalbuminaemia and eosinophilia (Anonymous, 2006; Radostits *et al.*, 2007). A profound anemia is observed in sheep but this is less marked in cattle (Soulsby, 1982). It has been estimated that each fluke results in the loss of 0.2 to 0.5 ml of blood per day (Holmes *et al*., 1968; Taylor *et al*., 2007; Lotfollahzadeh *et al*., 2008). Therefore a moderate infestation of (100 to 200) flukes in cattle can lead to blood loss of approximately half a litre a week, hence in some cases animals infected with the disease can become severely anaemic.

The exact aetiology of the anemia is controversial, however, several factors are believed to be involved, including the accidental damage to hepatic vessels and subsequent haemorrhages during the migratory phase (Mehlhom and Armstrong, 2001; Anonymous, 2006), passage of red blood cells into the gastrointestinal tract (Lotfollahzadeh *et al.*, 2008), and haemorrhage into the bile ducts and consequently loss of red blood cells due to the blood sucking effects of adult flukes in the bile ducts (Soulsby, 1982; Behm and Sangster, 1999; Mehlhom and Armstrong, 2001; Jones *et al*., 2006). Earlier studies have demonstrated that intra-biliary haemorrhage and the consequent loss of erythrocytes into the intestine occurred eight to nine weeks post-infection and thereafter increased in severity, resulting in progressive loss of iron into the gastrointestinal tract (Soulsby, 1982). However, the ultimate degree of anaemia is not related to the severity of biliary haemorrhage, but rather to the animal's erythropoietic capacity, which is influenced by the levels of dietary protein and iron (Anonymous, 2006).

In a study of cattle naturally infected with *F. gigantica*, the total erythrocyte counts, haemoglobin level, packed cell volume (PCV) and mean corpuscular haemoglobin concentration (MCHC) were significantly lower when compared with values observed in apparently healthy cattle (Taimur *et al*., 1993) Similar findings have been reported by Copeman and Copland (2008) and Lotfollahzadeh *et al*. (2008). Anaemia was also determined to be a prominent feature in riverine buffaloes that were experimentally challenged with *F. gigantica* (Yadav *et al*., 1999). In another study, *Fasciola*-infected steers exhibited lower values for haematocrit, erythrocytes, haemoglobin and iron when compared to non-parasitised animals (Coppo *et al*., 2010). The infected animals had leucocytosis and eosinophilia, which were similar findings to those found in naturally infected cattle, in Ethiopia (Mathewos *et al.*, 2001).

The destruction of the hepatic tissue during the migration of larval flukes and the presence of adult flukes in the bile ducts results in changes to the levels of serum proteins. These proteins either increase or decrease during infection, causing hypoalbuminaemia or hyperglobulinaemia, which are the most common features in liver fluke infections (Behm and Sangster, 1999), and are due to plasma leakage through bile ducts (Jones *et al*., 2006). Generally, the albumin level is reduced compared with globulins. During the period of fluke migration there is a progressive but usually mild hypoalbuminaemia with a more pronounced hyperglobulinaemia of varying severity. In contrast, when the adult flukes are present in the bile ducts, there is a further reduction in the albumin level as well as a progressive reduction in the concentration of globulins (Mehlhom and Armstrong, 2001).

The hepatic enzymes including aspartate aminotransferase (AST), glutamate dehydrogenase (GLDH), gamma glutamyltransferase (GGT), and lactate dehydrogenase (LDH), are elevated during infection with *Fasciola*. In clinical acute or chronic bovine fasciolosis, serum GGT is usually 2 to 3 times higher than normal (Braun *et al.*, 1983). In a study involving cattle, it was discovered that the plasma levels of GLDH and GGT were greatly increased (Anderson *et al*., 1981). Similar experimental studies in sheep (Ferre *et al*., 1994) water buffalo (Yang *et al.*, 1998), and goats (Martínez-Moreno *et al*., 1999) showed significant increases in the plasma activity of AST, GLDH, GGT and LDH. The increases in plasma AST and GLDH were thought to be associated with the inflammatory state of the liver and to tissue destruction provoked by the migration of immature flukes through the liver parenchyma, whereas elevated GGT was related to the penetration of the bile ducts by the migrating flukes (Ferre *et al*., 1994).

Increased levels of GGT and GLDH have been reported in an abattoir study of cattle naturally infected with *F. gigantica* (Molina *et al*., 2006) in cattle and buffalo calves (Wiedosari *et al*., 2006) and in sheep challenged with both *F. hepatica* and *F. gigantica* (Phiri *et al.*, 2007c). Further evidence of increased serum activities of the enzymes AST, GGT and alkaline phosphatase (ALP), was found in infected cattle when compared with uninfected animals in recent studies (Lotfollahzadeh *et al*., 2008; Coppo *et al*., 2010).

Therefore the changes in the serum activities of hepatic enzymes are sensitive indices of liver damage in sheep, cattle and buffalo (Ferre *et al.*, 1994; Yang *et al*., 1998; Molina *et al*., 2006; Phiri *et al.*, 2007c), and can be used as indicators of the level of infection, severity of damage and associated physiological changes caused by fasciolosis (Molina *et al.*, 2006).

2.3.4 Gross and histopathology

On inspection, the liver may have an irregular outline, and be pale and firm (Anonymous, 2006). The biliary epithelium may have evidence of papillary and glandular hyperplasia in some places and erosion in others (Jones *et al*., 2006). Most of these manifestations are related to the migration of juvenile flukes in the hepatic parenchyma and the presence of adult flukes in the bile ducts. The migratory tracts caused by larval migration fill with neutrophils, eosinophils and lymphocytes (Rahko, 1969; Jones *et al*., 2006), and are enlarged due to the growth of young flukes (Radostits *et al*., 2007), and therefore are grossly visible and dark red in colour in acute infections (McGavin and Zachary, 2007). In older lesions there is evidence of ubiquitous macrophages, epithelioid cells and multinucleated giant cells, particularly around dead larvae (Rahko, 1969; Jones *et al.*, 2006) and the tracts appear paler than the surrounding parenchyma (McGavin and Zachary, 2007).

In acute cases of the disease, the liver is enlarged, friable, haemorrhagic and honeycombed with the tracts of migrating flukes. The surface, particularly over the ventral lobe, is frequently covered with a fibrinous exudate (Taylor *et al*., 2007). Additionally, the liver may be badly damaged and swollen, with the presence of many small capsular perforations. However, the immature flukes are too small to be readily discernible and the damaged tissue is more friable than normal (Radostits *et al*., 2007). The peritoneal cavity may contain a large quantity of blood-stained serum due to subcapsular haemorrhages (Radostits *et al*., 2007; Taylor *et al*., 2007).

The gall bladder may be enlarged, and adhesion of the liver to the diaphragm or other internal organs may occur (Kusiluka and Kambarage, 2006). In the subacute form, the liver is enlarged with numerous extensive necrotic and haemorrhagic tracts visible on the surface and in the substance of the organ (Rushton and Murray, 1977; Taylor *et al.*, 2007). In chronic fasciolosis, the affected liver shows an irregular outline, and may be pale and firm, with the ventral lobe being most commonly affected and reduced in size. The liver is characterized by hepatic fibrosis and hyperplastic cholangitis (Rushton and Murray, 1977; Taylor *et al.*, 2007)

Mature flukes are usually present in the grossly enlarged and thickened bile ducts, particularly in the ventral lobe of the liver (Yadav *et al*., 1999; Radostits *et al.*, 2007) and cause necrosis and severe erosion or ulceration of the mucosa leading to peribiliary inflammation and severe hyperplasia of the epithelial layer (Anonymous, 2006; Taylor *et al.*, 2007). The walls of the ducts show significant thickening from fibrous proliferation, with partial or complete occlusion of the bile ducts (Rahko, 1969; Yadav *et al*., 1999; Jones *et al.*, 2006). Chronic cholangitis and bile duct obstruction lead to ectasia and stenosis of the ducts, and periductular fibrosis that is attended by cholestasis (McGavin and Zachary, 2007). This periductular fibrosis thickens the walls so that the ducts become prominent (McGavin and Zachary, 2007) and they may protrude above the surface of the liver, and cysts may be present due to blockage of ducts with flukes and desquamated epithelial cells (Kusiluka and Kambarage, 2006; Radostits *et al*., 2007).

The liver pathology in chronic and severe infection is typified by evidence of hepatic fibrosis and hyperplasia, resulting from cholangitis (Yadav *et al.*, 1999; Kusiluka and Kambarage, 2006). Several different types of fibrosis may be present and may induce post-necrotic scarring, ischaemic fibrosis and peribiliary fibrosis (Rushton and Murray, 1977; Taylor *et al.*, 2007). The damaged hepatic parenchyma becomes indurated and flukes may be seen in the obliterated bile ducts with small granulomata often being observed around eggs or fluke remnants that become lodged in the bile ducts (Rushton and Murray, 1977; Jones *et al*., 2006; Kusiluka and Kambarage, 2006). Extensive fibrosis and calcification of the bile duct walls is a common finding in cattle but is not a feature in small ruminants (Rahko, 1969; Behm and Sangster, 1999; Jones *et al*., 2006; Kusiluka and Kambarage, 2006; Radostits *et al*., 2007; Taylor *et al*., 2007). The hepatic parenchyma is extensively fibrosed and the hepatic lymph nodes are slightly enlarged and their cut surface is dark or greenish-brown in colour (Rahko, 1969; Radostits *et al*., 2007).

2.3.5 General effects of fasciolosis in ruminants

Animals infected with *F. gigantica* are generally in poor body condition, with overall lowered productivity. The lowered productivity in cattle and sheep is reflected by depressed appetite, decreased voluntary feed intake, reduced feed conversion efficiency, poor weight gains and in sheep, decreased wool production (Boray, 1969; Berry and Dargie, 1976; Ferre *et al*., 1994; Mehlhom and Armstrong, 2001; Mitchell, 2002; Taylor *et al.*, 2007). Some of the negative impacts associated with infection include decreased work capacity, reduction in reproductive performance and milk production, and increased susceptibility to other infections (Molina, 2005).

Reduction in work performance can be a major concern in farming communities where cattle and buffalo are an important source of draught power. As much as 27 to 35% more time is required by infected buffaloes and cattle to work a field, with a further 15% additional working time required by anaemic animals (Molina, 2005). Significant effects on performance in beef cattle have been reported in naturally infected animals (Torgerson and Claxton, 1999). In that study, animals treated for fasciolosis were used for twice as many days for preparing land for planting as the untreated ones.

Cattle and buffaloes with fasciolosis lose body condition or have reduced weight gain, resulting in poor carcass or meat quality compared with healthy livestock (Yadav *et al*., 1999; Molina *et al*., 2005b; Mungube *et al*., 2006). Infection with *F. gigantica* has greater negative impact on the carcass weight of buffaloes than cattle (Yadav *et al*., 1999; Molina *et al*., 2005b). The difference between the species was considered to be due to the limited available time for grazing by buffaloes, as they were used for draught power in contrast to cattle. Extensive infection interferes with liver function, and thereby results in weight loss or failure to gain weight normally (Jones *et al*., 2006) due to impairment of the body's ability to convert feed into body mass. The parasites can have a significant effect on production due to an impairment of appetite and to their effect on post-absorptive metabolism of protein, carbohydrate and minerals (Taylor *et al.*, 2007). Infected animals suffer from anaemia, generalized weakness and poor weight gains (El-Khadrawy *et al.*, 2008). In most cases, animals with poor body condition are often those associated with chronic infections (McGavin and Zachary, 2007). Consequently, administering suitable anthelmintics has been found to significantly increase body condition score and total weight gain (Loyacano *et al*., 2002).

The decrease in food intake, that accompanies *Fasciola*-infected animals, is actually related to liver injury since the depression in appetite usually coincides with the period of increase in liver enzymes (Boray, 1969; Ferre *et al*., 1994). Work by Yadav *et al.*, (1999) demonstrated inappetence in riverine buffaloes experimentally infected with metacercariae of *F. gigantic*a. This loss of appetite resulted in poor weight gain that became apparent from seven weeks post-infection. Appetite depression has been found to be most pronounced between 8 and 14 weeks post-infection (Ferre *et al.*, 1994).

In a study involving the experimental challenge of Friesian heifers with *F. hepatica*, it was demonstrated that the mean age at puberty of fasciolosis-infected heifers was 39 days later than that of non-infected animals (López-Díaz *et al*., 1998). A survey in Indonesia showed that challenged Ongole cows had longer inter-calving intervals of approximately 32 months compared to19 months in healthy cows that had been treated for fasciolosis (Copeman and Copland, 2008). Similarly, crossbred beef heifers treated for fluke infections have been shown to have a significantly increased body condition score after the breeding season and an increased total weight gain than non-treated animals, although there was no significant differences in the pregnancy rates

(Loyacano *et al.*, 2002). These authors reported that alterations to the hepatic metabolic processes, which stimulate heifers to reach puberty, might have been affected in infected animals with significant burdens having the potential to reduce the fertility of heifers. These data support the findings of El-Khadrawy *et al*. (2008) who reported a negative relationship between ovarian activity and fasciolosis in buffalo cows, with the prevalence of fasciolosis being higher in animals with ovarian inactivity than normal cycling animals. Mehlhom and Armstrong (2001) also found that fasciolosis in sheep had adverse effects on conception and establishment of embryos.

One of the most important effects on milking cows is a reduction in milk yield and quality, particularly of the solids-non-fat component (Mitchell, 2002; Taylor *et al.*, 2007). It is well documented that effects on milk production may be quite substantial, with production from infected animals dropping by as much as 14%, although 8% is recoverable through treatment (Ogunrinade and Ogunrinade, 1980; Torgerson and Claxton, 1999; Mehlhom and Armstrong, 2001). One study has shown that there is an increase in milk production following treatment with a flukicide in cattle infected with *Fasciola* when compared with control and non-infected untreated animals (Kumar *et al*., 2006). Similarly, a recent study also found that post-treatment average daily milk production increased by 0.67 and 0.87 litres per animal in buffaloes and cattle, respectively (Khan *et al*., 2010). However, other researchers have reported that infection with *F. gigantica* does not adversely affect milk production (Needham, 1977). These authors found that the weaning weights of calves from cows infected with *F. gigantica* did not differ significantly when compared with those of calves from cows that were treated every 8 to 12 weeks. This highlights the difficulty in estimating losses in milk production caused by fasciolosis (Molina, 2005).

There is evidence to indicate that concurrent infections with nematodes, especially the abomasal nematode *Teladorsagia (Ostertagia) ostertagi*, may complicate the clinical picture (Mitchell, 2002), and this has made fasciolosis a serious constraint on the productivity of domestic ruminants throughout Africa and Asia, and thus a significant impediment to global food production (Dargie, 1987). Fasciolosis also predisposes animals to concurrent infections, primarily *Clostridium* and *Salmonella* species, and possibly haemoparasites (Ogunrinade and Ogunrinade, 1980). Infected animals, particularly dairy cows, may have increased susceptibility to *Salmonella dublin* and metabolic disorders around the time of calving (Mitchell, 2002).

Liver damage resulting from fasciolosis is often a triggering factor for infectious necrotic hepatitis or Black's disease, an acute toxaemic and fatal disease of the liver caused by *Clostridium novyi* type B (Mehlhom and Armstrong, 2001; Hutchinson and Love, 2007; McGavin and Zachary, 2007). Black's disease is usually associated with liver damage caused by migrating young flukes since the damage creates anaerobic conditions, which allow the germination and proliferation of spores of the soil-borne toxigenic bacterium (Mitchell, 2002; Hutchinson and Love, 2007; McKay, 2007). *Clostridium novyi* is common in the environment hence black disease is found wherever populations of liver fluke and cattle or sheep overlap. Bacillary haemoglobinuria, an acute, highly fatal toxaemia of cattle and sheep caused by

Clostridium haemolyticum, has also been reported in association with liver fluke infection in cattle (Blood and Studdert, 1990; McKay, 2007).

Fasciola species have a predilection for the liver, however, occasional ectopic locations in other organs such as the lungs, kidneys, diaphragm, intestines and subcutaneous tissue can occur (Boray, 1969), and when this happens, there is development of aberrant infections associated with those organs. The aberrant migration of flukes is more common in bovines than ovines, hence encapsulated parasites are often seen in other organs, mainly the lungs (Anonymous, 2006; McKay, 2007). A study on buffaloes naturally infected with *F. hepatica* observed that these animals could develop glomerulonephritis (Tietz Marques *et al.*, 2004) due to fasciolosis. The authors pointed out that the glomerulopathy resulted from the deposition of circulating immune complexes in response to the presence of the parasite.

2.4 Epidemiology of fasciolosis

2.4.1 Prevalence, distribution and risk factors

The epidemiology and economic losses of fasciolosis have been investigated extensively in most regions of the world, including Europe (Sánchez-Andrade *et al*., 2002; McKay, 2007), the Americas (Amato *et al*., 1986; Bouvry and Rau, 1986; Knapp *et al*., 1992; Claxton *et al*., 1997; Rangel-Ruiz *et al*., 1999; Coelho and Lima, 2003; Marques and Scroferneker, 2003; Cruz-Mendoza *et al.*, 2004; Kleiman *et al*., 2007), Asia (Morel and Mahato, 1987; Suhardono *et al*., 1997; Tum *et al*., 2004; Molina *et al*., 2005a; Sothoeun *et al*., 2006; Suhardono *et al*., 2006a; Suon *et al*., 2006; Nguyen *et al*., 2010); Australia (Dixon, 1963; Baldock and Arthur, 1985; Molloy and Anderson, 2006), and Africa (Ogunrinade and Ogunrinade, 1980; Mzembe and Chaudhry, 1981; Tembely *et al*., 1988; Traore, 1988; Wamae *et al*., 1990; Malone *et al*., 1998; Yilma and Malone, 1998; Kithuka *et al*., 2002; Pfukenyi and Mukaratirwa, 2004; Keyyu *et al*., 2005b; Phiri *et al*., 2005a; Phiri *et al*., 2005b; Ekwunife and Eneanya, 2006; Mungube *et al*., 2006; Pfukenyi *et al*., 2006; Adedokun *et al*., 2008; Berhe *et al*., 2009; Mwabonimana *et al*., 2009; Abunna *et al*., 2010). Although many studies on the epidemiology of various helminthosis in developed countries have been published, there are limited data on the epidemiological aspects of helminth infections, particularly fasciolosis, in developing countries (Maqbool *et al.*, 2002).

Fasciola hepatica has a cosmopolitan distribution while *F. gigantica* is only widely distributed in cattle in tropical regions (Soulsby, 1982). However, the geographical distribution of these two species overlaps in many African and Asian countries, although the ecological requirements of the flukes and their snail hosts are quite distinct (Soliman, 2008). *Fasciola hepatica* appears to be the main cause of fasciolosis due to its very wide distribution whereas *F. gigantica* is of secondary importance because it is restricted to the old world (Mas-Coma *et al*., 2007). In the African continent, most authors have mainly reported the existence and prevalence of *F. gigantica* (Ogunrinade and Ogunrinade, 1980; Pfukenyi and Mukaratirwa, 2004; Keyyu *et al*., 2005b; Phiri *et al*., 2005a; Phiri *et al*., 2005b; Mungube *et al*., 2006; Pfukenyi *et al*., 2006; Adedokun *et al*., 2008; Mwabonimana *et al*., 2009; Nonga *et al*., 2009; Mellau *et al*., 2010). However, studies in Egypt and Ethiopia, have reported co-existence of the two species in domestic animals (Malone *et al.*, 1998; Soliman, 2008; Berhe *et al.*, 2009; Abunna *et al.*, 2010), but with varying localities of occurrence, with

F. hepatica infections being prevalent in areas above 1800 m and *F. gigantica* occurring in areas at or below 1200 m above sea level (Yilma and Malone, 1998).

The distribution of fasciolosis is largely dependent on the presence of a suitable aquatic lymnaeid snail that serves as an intermediate host (FAO, 1994). The snail is mainly found on aquatic plants, which are critical in their development (Coelho and Lima, 2003). However, the occurrence of fasciolosis in an area is influenced by multiple factors including host, parasite and environmental effects. It is important to fully understand the association between these three groups of factors so that the disease can be controlled (Maqbool *et al*., 2002).

Infection is most prevalent and a serious problem in areas with sheep and cattle production (Coelho and Lima, 2003; Soliman, 2008), with prevalence estimates as high as 100% in some countries (Soliman, 2008). In endemic areas, the prevalence of infection is often very high, even if the majority of the infected animals show only modest burdens (Torgerson and Claxton, 1999). However, the prevalence appears to vary widely from one country to another and even within the same country/continent. These differences are probably due to the agro-ecological and climatic variations between localities, although differences in the management systems adopted may influence the disease's distribution (Abunna *et al*., 2010).

A number of global investigations have shown that the prevalence of bovine fasciolosis varies considerably between countries, ranging from 0 to 97% (Table 2.1). In Africa the findings of epidemiological studies, with the exception of a recent Egyptian study by

Hussein and Khalifa (2010), have reported lower prevalences than previous reports. The lowered prevalence is believed to be due to improved systems of reporting and routine meat inspection in most slaughter houses (Kithuka *et al*., 2002) and also increased farmers' awareness about the disease resulting in regular treatment of cattle with anthelmintics (Pfukenyi and Mukaratirwa, 2004).

Studies from southern Africa have indicated that infection with *F. gigantica* is more prevalent during the wet season than in the dry season and is also more prevalent in high rainfall areas than in relatively drier areas (Mzembe and Chaudhry, 1981; Phiri *et al*., 2005a; Pfukenyi *et al*., 2006). One study in Zambia found that the highest prevalence was in the post-rainy season, however, cattle sampled in the rainy and post-rainy periods showed the highest parasite abundance (Phiri *et al*., 2005b). The peak liver condemnation period due to chronic fasciolosis has also been observed during the rainy season in Zimbabwe, Malawi and Sierra Leone (Mzembe and Chaudhry, 1981; Asanji and Williams, 1984; Pfukenyi and Mukaratirwa, 2004).

The distribution of the disease is strongly correlated with the distribution of the snail intermediate host, which has been found to be more prevalent in the higher rainfall districts than in drier districts of Zimbabwe (Pfukenyi and Mukaratirwa, 2004). However, flooding and rapid movement of water during the rainy season can result in a disturbance to the snail habitats leading to a decline in the snail population, which may subsequently affect the prevalence of the disease (Mzembe and Chaudhry, 1979). *Lymnaea natalensis* is an aquatic snail (Mzembe and Chaudhry, 1979) and therefore, the likelihood of cattle becoming infected can be expected to be higher in the high rainfall areas, that are characterized by wet or swampy grazing pastures, than in the arid or semiarid areas, with dry pastures and only focal distribution of suitable snail habitats (Pfukenyi *et al.*, 2006).

The prevalence of bovine fasciolosis from east Africa is similar to that in the southern part of the continent, with the greatest risk of the disease being reported to occur in areas of extended annual rainfall associated with high soil moisture and surplus water, with the risk diminishing in areas with a shorter wet season and/or lower temperatures (Yilma and Malone, 1998). In Tanzania it has been observed that the highest prevalence and widespread distribution of flukes occurred at the height of the rainy season (Keyyu *et al.*, 2006). These authors demonstrated a seasonal pattern of *F. gigantica* infection, with the proportion of animals excreting eggs increasing gradually from early in the dry season to reach a peak towards the end of the dry/early rainfall season. In Kenya, the highest prevalence of the disease was reported in wetter parts where flood plains exist and where large water masses accumulated (Kithuka *et al*., 2002). Higher condemnation of livers have been reported at slaughter houses during wetter years/periods (Mungube *et al*., 2006), however, in that study no distinct seasonal pattern was observed.

A study in Nigeria, in west Africa, reported a higher prevalence of fasciolosis during the rainy season than in the dry season (Adedokun *et al*., 2008). In Algeria, the prevalence of infection with *F. hepatica* in cattle was higher in the district of Jijel compared with Constantine, and these findings were related to the favourable climatic conditions (annual rainfall 750-900 mm) in Jijel (Mekroud *et al.*, 2004). In Mali, *F. gigantica* was found to be a major cause of liver condemnation at slaughterhouses, and a high prevalence of infection was found in cattle grazing pastures near an in-land delta of the Niger river (Tembely *et al.*, 1988). High rainfall areas and the rainy season favoured the development and survival of both the intermediate host snail and the developmental stages of the parasite (Torgerson and Claxton, 1999), hence arid areas are generally unsuitable for the occurrence of fasciolosis (Malone *et al*., 1984; Malone *et al*., 1998). In contrast to other countries, in Mali (Tembely *et al*., 1988), and Malawi (Mzembe and Chaudhry, 1981), the prevalence was reported to be higher during the dry season when animals congregated around the delta of the river or returned to grazing the sides of rivers, as pastures and water sources became scarce.

A study of fasciolosis in Queensland, Australia, found a prevalence of 1.4% in beef and 8.4% in dairy cattle (Molloy and Anderson, 2006). A higher prevalence of over 30% was recorded in New Zealand (Charleston and McKenna, 2002).

In Asia, the dependence of the disease on local physical and climatic conditions for the survival of the intermediate host means that the prevalence can vary significantly between locations (Copeman and Copland, 2008). In a few comprehensive, nationwide surveys conducted, the prevalence varied from 0 to 100% over a comparatively short distance, and thus any national studies may either under or overestimate the prevalence depending on the areas sampled (Copeman and Copland, 2008). A prevalence of approximately 20% in large ruminants in China has been reported, with the disease being more important in rice-producing areas (Copeman and Copland, 2008). A similar prevalence has been reported in India (Roy and Tandon, 1992), where a higher prevalence is attributed to frequent visits by ruminants to water bodies infested with snails and thereby increasing the risk of infection (Yadav *et al.*, 2007; Garg *et al*., 2009).

Other investigations in Asia have found that the prevalence of *F. gigantica* infection in cattle and buffaloes in Thailand was on average 12%, but was much higher in areas surrounding dams or large ponds, where *L. auricularia rubiginosa* was found (Srihakim and Pholpark, 1991). Epidemiological studies from the Philippines (Molina *et al*., 2005b), Cambodia (Tum *et al*., 2004); Vietnam (Holland *et al*., 2000), Indonesia and Laos (Copeman and Copland, 2008) reported comparable prevalences because of the adoption of similar farming practices.

Reports from the Middle East indicated varying results in cattle and buffaloes between countries in the region, from a low average of 17.2% in Iran (Eslami *et al*., 2009; Ahmadi and Meshkehkar, 2010; Khanjari *et al*., 2010) to a high of 65.2% in Turkey (Yildrim *et al*., 2007). The prevalence in Turkey was reported to rank among the highest, both in the Middle East and in the world. Studies from Pakistan reported a prevalence of approximately 26% (Maqbool *et al.*, 2002; Iqbal *et al.*, 2007; Khan *et al.*, 2009; Khan *et al*., 2010)

The recorded prevalence of fasciolosis in cattle from Europe indicated a mean prevalence of 25%. However, as with reports from other continents, the prevalence varied greatly from one country to another, from as low as 5% in Italy to as high as 45%

in Ireland (Torgerson and Claxton, 1999). A prevalence of 30% has been reported in cattle in Spain (Gonzalez-Lanza *et al*., 1989).

The recorded prevalence data of fasciolosis from the Americas has also shown large variations between countries. Surveys conducted in beef cattle in fluke endemic states of the USA found a mean prevalence of 19%, with the nationwide surveys indicating a relatively lower prevalence in feedlot cattle, (Knapp *et al.*, 1992; Torgerson and Claxton, 1999; Bliss, Unpublished). Data from a Canadian study reported a higher prevalence of *F*. *hepatica* infection (up to 68%) (Bouvry and Rau, 1986) than in the USA, while investigations from Latin America reported a prevalence ranging from 1 to 78% in cattle and buffaloes (Rangel-Ruiz *et al.*, 1999; Torgerson and Claxton, 1999; Clarkson and Claxton, Unpublished). The high prevalence reported from Mexico, Peru and Chile have been attributed to the favourable prevailing climatic conditions or the traditional management systems adopted in those countries (FAO, 1994).

Table 2.1 Global prevalence of bovine fasciolosis

Continent/Region	Country	Prevalence (%)	Species	Reference
Africa	Algeria	17	*F. hepatica*	Mekroud *et al* (2004)
	Cameroon	45	*F. gigantica*	Spithill *et a*l (1999)
	Chad	62	*F. gigantica*	Spithill *et al* (1999)
	Egypt	29	*F. hepatica* *F. gigantica*	Hussein & Khalifa (2010)
	Ethiopia	14-24	*F. hepatica* *F. gigantica*	Berhe *et al* (2009); Abunna *et al* (2010)
	Kenya	7-26	*F. gigantica*	Kithuka *et al* (2002); Mungube *et al* (2006); Mwabonimana *et al* (2009)
	Malawi	19-39	*F. gigantica*	Mzembe & Chaudhry (1981)
	Mali	15	*F. gigantica*	Traore (1988)
	Nigeria	11-52	*F. gigantica*	Adedokun *et al* (2008); Ekwunife & Eneanya (2006)
	Sierra Leone	25	*F. gigantica*	Asanji & Williams (1984)
	Sudan	33	*F. gigantica*	Atta El Mannan *et al* (2001)
	Tanzania	9-31	*F. gigantica*	Keyyu *et al* (2006); Swai & Ulicky (2009); Mellau *et al* (2010)
	Uganda	up to 97	*F. gigantica*	Spithill *et al* (1999)
	Zambia	31-61	*F. gigantica*	Phiri *et al* (2005a); (2005b)
	Zimbabwe	15-37	*F. gigantica*	Pfukenyi & Mukaratirwa (2004); Pfukenyi *et al* (2006)
Asia	Cambodia	12	*F. gigantica*	Tum *et al* (2004)
	China	12-27	*F. gigantica*	Copeman & Copland (2008)
	India	11-15	*F. gigantica*	Yadav *et al* (2007); Garg *et al* (2009)
	Indonesia	25-48	*F. gigantica*	Copeman & Copland (2008)
	Nepal	34-70	*F. gigantica*	Morel & Mahato (1987)
	Laos	0-81	*F. gigantica*	Copeman & Copland (2008)
	Pakistan	25-27	*F. gigantica*	Khan *et al* (2009); (2010)
	Philippines	45	*F. gigantica*	Molina *et al* (2005b)
	Thailand	0-85	*F. gigantica*	Srihakim & Pholpark (1991)
	Vietnam	22	*F. gigantica*	Holland *et al* (2000)
Caribbean	Jamaica	22	*F. hepatica*	Torgerson & Claxton (1999)
Oceania	Australia	5	*F. hepatica*	Molloy & Anderson (2006)
	New Zealand	30	*F. hepatica*	Charleston & McKenna (2002)
Middle East	Iran	2-32	*F. gigantica*	Eslami *et al* (2009); Khanjari *et a*l (2010); Ahmadi & Meshkehkar (2010)
	Turkey	65	*F. gigantica*	Yildrim *et al* (2007)
Americas	United States	6-68	*F. hepatica*	Knapp *et al* (1992); Torgerson & Claxton (1999)
	Canada	up to 68	*F. hepatica*	Bouvry & Rau (1986)
	Brazil	1-20	*F. hepatica*	Torgerson & Claxton (1999)
	Chile	Up to 94	*F. hepatica*	Torgerson & Claxton (1999)
	Mexico	Up to 75	*F. hepatica*	Rangel-Ruiz *et al* (1999)
	Peru	29-78	*F. hepatica*	Torgerson & Claxton (1999)
Europe	Belgium	13	*F. hepatica*	Torgerson & Claxton (1999)
	Germany	11	*F. hepatica*	Torgerson & Claxton (1999)
	France	17	*F. hepatica*	Mage *et al* (2002)
	Italy	5	*F. hepatica*	Torgerson & Claxton (1999)
	Ireland	45	*F. hepatica*	Torgerson & Claxton (1999)
	Poland	7	*F. hepatica*	Torgerson & Claxton (1999)
	Spain	30	*F. hepatica*	Gonzalez-Lanza *et al* (1989)
	Switzerland	9	*F. hepatica*	Ducommun & Pfister (1991)
	United Kingdom	10	*F. hepatica*	Torgerson & Claxton (1999)

Most authors have reported that the disease is generally associated with warm moist conditions and is restricted to wet areas (Amato *et al*., 1986; Bouvry and Rau, 1986; Boyce and Courtney, 1990; Luzón-Peña *et al*., 1994; Roberts and Suhardono, 1996; Claxton *et al*., 1997; Coelho and Lima, 2003; Cruz-Mendoza *et al*., 2004; Keyyu *et al*., 2005b; Phiri *et al*., 2005a; Phiri *et al*., 2005b; Mungube *et al*., 2006; Pfukenyi *et al*., 2006; Khan *et al*., 2009; Abunna *et al*., 2010). These findings are not surprising since the rainy season generally presents a more favourable climate for the life cycle of *F. gigantica*, than the dry season when the cercariae and the intermediate hosts have low survival rates (Adedokun *et al*., 2008). However, reports on the duration animals are exposed to infection with *F. gigantica* vary between habitats and the rate of infection is not constant throughout the year but concentrated over a few months (Pfukenyi *et al*., 2006).

In spite of the seasonal pattern of *Fasciola* and its intermediate host, some studies have indicated that fasciolosis appears to occur throughout the year (Amato *et al*., 1986; Roy and Tandon, 1992; Rangel-Ruiz *et al*., 1999; Yilma and Mesfin, 2000; Keyyu *et al.*, 2005b; Phiri *et al*., 2005b; Pfukenyi *et al*., 2006; Adedokun *et al*., 2008). Yilma and Mesfin (2000), recorded a high prevalence of bovine fasciolosis in Ethiopia during a wet season, which continued into the dry season. This concurred with studies by Roy and Tandon (1992), who reported that *Fasciola* infection occurred throughout the year. Although there was a gradual decrease, a relatively high prevalence was maintained throughout the long dry period of the year.

The seasonal pattern of *Fasciola* infection appears to overlap the snail biology in some areas, which is an indication of a high proportion of infected snails from the end of the rainy season into the dry season (Keyyu *et al*., 2005b). The pattern of infection in any area is a reflection of the timing and duration of ecological circumstances favourable for the population of snails and for the survival of metacercariae (Pfukenyi *et al*., 2006). Metacercariae of *F. hepatica* are present on pasture throughout the year, with the numbers decreasing during high temperatures but increasing again as temperatures reduce (Amato *et al*., 1986). Some metacercariae are able to withstand the higher temperatures of summer, provided there is sufficient rainfall to compensate for the negative effects of these high temperatures (Amato *et al*., 1986).

Epidemiological studies have shown that risk factors including age, gender, breed and the management of livestock, have a significant influence on the prevalence of fasciolosis. Age is a significant determinant of infection in cattle with *F. gigantica* (Spithill *et al*., 1999) and a study in Zambia found a higher prevalence in adult cattle than young cattle (Phiri *et al*., 2005a). Similar findings have been reported by studies in Zimbabwe (Pfukenyi *et al.*, 2006), Tanzania (Keyyu *et al*., 2005b; Keyyu *et al*., 2006), Vietnam (Holland *et al*., 2000) and Turkey (Yildrim *et al*., 2007). Studies on the susceptibility of cattle to infection with *F. gigantic*a found higher prevalences in females than males (Phiri *et al*., 2005a; Yildrim *et al*., 2007). In contrast, a study in Zimbabwe by Pfukenyi *et al*. (2006) reported opposite results, with adult bulls, pregnant and lactating cows showing a significantly higher prevalence than oxen and dry cows, and no significant differences between males and females in younger animals. Reports on the variation between breeds to infection with *F. gigantica* are limited, and such variation has been reported as a confounding factor for comparison on clinical effects of infection (Spithill *et al.*, 1999). However, one study in Indonesia found differences between different breeds of calves, therefore breed should be taken as a potential determinant of infection with *F. gigantica* (Spithill *et al.*, 1999).

Cattle management systems may influence the prevalence of infection, with infection more prevalent in livestock raised under traditional management systems than those managed under modern improved systems (Maqbool *et al*., 2002; Keyyu *et al*., 2005b; Khan *et al*., 2009; Abunna *et al*., 2010). The common practice of using animal manure as a fertilizer, such as in irrigated rice fields, in most tropical countries promotes contamination of snail habitats and subsequent infection of snails with *F. gigantica* (Spithill *et al.*, 1999). Furthermore, the grazing management of livestock may permit dung from infected stock to enter the habitat of the snails and at the same time allows livestock to drink water or graze vegetation fringing such contaminated sites (Spithill *et al.*, 1999; Suon *et al*., 2006). Infection with *F. gigantica* was identified as a serious health problem in Mali among migratory cattle which grazed the wet inland delta compared with sedentary animals (Tembely *et al*., 1988). Similarly, cattle originating from river-bank villages in Cambodia, with access to herbage and water in irrigation canals and dams on the river bank, had a higher prevalence than those from other areas (Suon *et al*., 2006).

A study undertaken on dairy farms in Tanzania found that the prevalence of the disease was associated with the grazing habits adopted, and was highest in livestock raised under the traditional grazing system, compared with moderate to low in fenced or zero grazed dairy farms (Keyyu *et al*., 2005b). Similarly, studies in Pakistan conducted on dairy farms with different management systems reported a higher prevalence in farms having grazing practices than in stall fed stock (Khan *et al.*, 2009). A study in Turkey also found a higher prevalence in traditional farms than in small scale dairy farms. This difference was attributed to contaminated pastures and insufficient treatment and control associated with these traditional farming systems (Yildrim *et al.*, 2007).

2.4.2 Economic Importance

Fasciolosis is a widespread ruminant health problem resulting in significant economic losses to the livestock industry (Abunna *et al*., 2010). It is regarded as one of the most important parasitic diseases, particularly for farmers from poorer countries where treatment is often too expensive to apply and other control measures are difficult or impossible to implement (Borgsteede, 2002). The economic losses are due to mortality, morbidity, reduced growth rate, condemnation of livers, increased susceptibility to secondary infections and the cost of implementing control or treatment protocols (Malone *et al*., 1998). Subclinical infections, which often go unnoticed, can result in marked economic losses (Torgerson and Claxton, 1999). The disease has a significant economic impact on buffaloes in Asia due to condemnation of livers, decreased milk and meat production, loss of weight and poor carcass quality (Maqbool *et al*., 2002). Even if the majority of the animals show only modest burdens, the economic effects of the parasite on a global scale has been estimated to be billions of dollars (Torgerson and Claxton, 1999).

Accurate assessment of the economic loss from infection with *F. gigantica* is hampered by several factors including incomplete information of the extent to which meat, milk and fibre production, as well as mortality, reproduction, draught output, feed conversion efficiency and appetite are adversely affected by infection; the variation in importance of each of these productive indices from place to place; variation between animal breeds in their resilience and resistance to infection; and the extent to which production loss is influenced by the level of infection, level of nutrition, age, gender and concurrent infection with other parasites or infectious agents (Ogunrinade and Ogunrinade, 1980; Spithill *et al*., 1999). There are also risks associated with extrapolation of information derived from studies with *F. hepatica* to infections with *F. gigantica* due to the significant differences in the host-parasite relationships between the two species (Spithill *et al*., 1999).

Estimation of the economic loss due to fasciolosis at the regional and national level is hindered by a lack of accurate estimation of the prevalence of disease (Adedokun *et al*., 2008) as most reports are based on prevalence estimates derived from examination of animals from slaughterhouses or from faecal egg examination studies. Estimates of the loss have predominantly been undertaken in developing countries, and have primarily looked at the value of livers condemned at slaughter as unfit for human consumption (Ogunrinade and Ogunrinade, 1980; Mzembe and Chaudhry, 1981; Baldock and Arthur, 1985; Morel and Mahato, 1987; Kithuka *et al*., 2002; Ashrafi *et al*., 2004; Pfukenyi and Mukaratirwa, 2004; Phiri *et al*., 2005a; Ahmedullah *et al*., 2007; Yildrim *et al.*, 2007; Molina *et al.*, 2008; Berhe *et al*., 2009; Mwabonimana *et al*., 2009; Swai and Ulicky, 2009; Abunna *et al*., 2010; Ahmadi and Meshkehkar, 2010;

Mellau *et al*., 2010) and in some cases on the value of meat lost through lower carcass weights of infected animals (Harrison *et al.*, 1996). This is likely to underestimate the real cost of infection.

The fact that only healthy animals are slaughtered could mean that the true prevalence might be considerably higher than that reported from abattoir studies. This is further compounded by the fact that livers are condemned based on the presence of gross pathological lesions, which is likely to have low sensitivity and there is likely to be poor record keeping in some situations (Mellau *et al*., 2010). Adoption of serological tests in developing nations may provide a more accurate estimate of the prevalence of infection (Spithill *et al.*, 1999). In the face of such incomplete and diverse information, it is worthwhile to question the value of making broad estimates of loss. Nevertheless, there is no doubt that control programmes need to be justified and supported by accurate economic data as there is increasing competition for limited public and private investment. However, even broad estimates would help to direct research towards new approaches to control the disease and to identify projects most likely to be beneficial (Copeman and Copland, 2008). Therefore, in spite of the difficulties in estimating the global economic losses, it is clear that fasciolosis is widespread and endemic in many countries, and causes significant losses to agricultural producers and smallholders (Spithill *et al*., 1999).

Estimates of the annual economic losses due to fasciolosis have been made by several authors (Ogunrinade and Ogunrinade, 1980; Schillhorn Van Veen *et al.*, 1980; Morel and Mahato, 1987; Spithill *et al*., 1999; Torgerson and Claxton, 1999; Kithuka *et al.*, 2002; Mungube *et al.*, 2006; Sothoeun *et al.*, 2006; Copeman and Copland, 2008; Berhe *et al.*, 2009; Mwabonimana *et al.*, 2009; Swai and Ulicky, 2009; Abunna *et al.*, 2010). Economic losses of several millions of (US) dollars per year have been reported due to fasciolosis in terms of mortality, liver condemnation, and low calf weight at birth (Yilma and Malone, 1998). The economic and social impact is huge, with estimates of over 600 million animals infected resulting in damage up to US$2 billion worldwide (Borgsteede, 2002), with half of these animals being cattle (Copeman and Copland, 2008).

The economic loss from fasciolosis in cattle from the African continent has, to date, not been well studied. Records from a few regions, including west Africa, indicate losses ranging from US$10 million to more than US$40 million(Spithill *et al*., 1999; Harrison, Unpublished). Reports from Kenya indicate that the meat industry loses at least £7 million (about US$11 million) through a combination of poor productivity, death of livestock, condemnation of infected livers and reduction in carcass quality (Harrison, Unpublished). Although the prevalence of fasciolosis in other eastern African countries is similar to that of Kenya, the economic losses reported have been much lower (Kithuka *et al*., 2002; Mungube *et al*., 2006; Berhe *et al*., 2009; Mwabonimana *et al.*, 2009; Swai and Ulicky, 2009; Abunna *et al*., 2010).

The economic losses from fasciolosis in cattle and buffaloes in Asian countries vary. In Indonesia, the impact has been estimated through a number of studies. In one study, looking at the losses from reduced meat production, lost draught power and reduced fertility in infected cattle and buffaloes, the annual losses were estimated at US$107 million (Spithill *et al.*, 1999; Copeman and Copland, 2008), which represents an annual loss per animal of US$42 (Spithill *et al*., 1999).

The economic loss from fasciolosis in cattle and buffaloes in Thailand has been assessed at not less than 100 million Baht (~US$3 million) (Srihakim and Pholpark, 1991; Copeman and Copland, 2008). In Nepal, an annual economic loss of US$20 million was reported by Morel and Mahato (1987), however, recent studies have indicated a higher loss of up to US$37 million due to poor animal health, low reproduction, high mortality rates and decreased buffalo milk and meat production (Copeman and Copland, 2008; Hughes and Harrison, Unpublished).

In 1997, the size of the cattle and buffalo herd in Asia was estimated at 589 million (Spithill *et al*., 1999; Copeman and Copland, 2008) These authors stated that using a conservative prevalence of 10% and losses per infected animal of US$42, that the economic losses in cattle and buffaloes alone exceeded US$2.4 billion in Asia, each year. Using similar calculations for the African cattle herd of 201 million animals, an annual loss of US$0.84 billion was predicted (Spithill *et al.*, 1999; Copeman and Copland, 2008). The estimated worldwide total annual loss attributed to fasciolosis is substantial by any valuation, and has been estimated to be more than US$3 billion per year, which is much greater than the earlier estimate of US$2 billion by Borgesteede (2002).

Fasciolosis in Australia has been estimated to cost the livestock industry approximately AU$80 million per year (Molloy and Anderson, 2006). According to a 1999 study, graziers spent approximately AU$10 million a year on fluke drenches alone and a further AU$50-80 million were lost annually through reduced production of cattle and sheep (Hutchinson and Love, 2007). This figure is comparable to an estimated average annual loss, from bovine fasciolosis, of €52 million (US$72million) in Switzerland (Schweizer *et al*., 2005). The effect of liver fluke disease has been estimated to cost the British cattle industry approximately £23 million (~US$36.5 million) per year (Bennett and Ijpelaar, 2003), while the Department of Agriculture and Food in Ireland estimated the cost of liver fluke to farmers to be around €25 million (~US$34 million) annually (McKay, 2007). In the USA, losses to the beef industry in Florida from liver fluke have been estimated at US$10 million per year (Irsik *et al.*, 2007). Although no specific estimates, in monetary terms, have been made in South America, fasciolosis in Peru is believed to cause massive losses of production and productivity in the vitally important dairy industry (Harrison, Unpublished). The annual economic losses due to fasciolosis from other Latin American countries, in particular Brazil where the disease appears to be endemic, could be much higher than currently envisaged.

2.4.3 Zoonotic Importance

Fasciolosis is an infectious parasitic disease infecting not only domestic ruminants, but also humans (Claxton *et al.*, 1997; Mas-Coma *et al*., 2007). However, the human disease tends to be sporadically reported (Hillyer, 1999) and has been neglected for decades (Mas-Coma *et al*., 2009). The epidemiological picture of human fasciolosis has changed in recent years, and the disease has now become an important emerging public health problem of increasing concern (Lotfy, 2002).

Human fasciolosis, like the disease in animals, occurs worldwide; however, although animal fasciolosis is distributed in countries with high cattle and sheep populations, the human disease appears to occur in developing countries. The disease is an important plant or food-borne trematode zoonoses, and humans usually acquire infection through ingestion of aquatic plants that contain infected metacercariae (Mas-Coma *et al*., 2007; Taylor *et al*., 2007), eating vegetables contaminated with metacercariae (Soliman, 2008) or even drinking untreated water containing metacercariae. Humans may also become infected through the consumption of raw livers from infected sheep, goats or cattle (Taira *et al.*, 1997; Soliman, 2008). Therefore, the prevalence is highest in areas where dietary practices include the consumption of raw aquatic vegetables or dishes made from raw liver. Fasciolosis occurs mainly in children living in rural settings but also can occur in people living in urban areas, with anaemia the most frequent finding in affected individuals (Soliman, 2008).

Human infection with fasciolosis has been sporadic, although during the past 30 years clinical cases and outbreaks have been reported. Since then the number of infected humans has increased significantly and several geographical areas have reported endemics of varying intensity (Soliman, 2008). The disease is an emerging parasitic infection that impacts significantly on both veterinary and human health worldwide (Rojas *et al*., 2009), and has become an endemic public health problem in several areas of the world, including Bolivia, Ecuador, Peru, Egypt, central Vietnam and northern Iran (Soliman, 2008).

Although fasciolosis is a well known disease of livestock (Mas-Coma *et al*., 2007; Mas-Coma *et al*., 2009), the low reports of the disease in humans is one reason why fasciolosis in humans has been neglected (Mas-Coma *et al*., 2007; Rojas *et al.*, 2009). Until 20 years ago, fasciolosis was considered a secondary disease, however, the World Health Organization (WHO) suspected this was changing due to reports from a range of countries indicating the presence of fasciolid infection (Mas-Coma *et al.*, 2007). Fasciolosis infection was, in the past, limited to specific and typical geographical areas such as populations within well defined watershed boundaries, however, recent environmental changes and changes in human behavior are defining new geographical limits and increasing the population at risk (Soliman, 2008). Endemic foci are not limited only to areas of extensive livestock farming but can be found in other places owing to the parasite's ability to colonize new intermediate hosts and adapt to new environments (Rojas *et al.*, 2009).

Even though the prevalence is highest in areas where sheep and cattle occur, a high prevalence in humans is not necessarily found in areas where fasciolosis is a major veterinary problem, since in some areas transmission can be maintained by infected people excreting eggs, especially where the habit of defaecating outdoors is still widespread (Mas-Coma *et al*., 1999; Soliman, 2008). Recent urbanisation, population migration and development of dams and irrigation systems have increased the population at risk, leading to a significant increase in the incidence in humans over the past 30 years (Soliman, 2008).

Fasciolosis is now widespread throughout the world, with human cases being increasingly reported from Europe, the Americas, Oceania, Asia and Africa (Soliman, 2008). The disease is increasingly recognized as a significant human problem, with an estimated 2.4 million people infected, and a further 180 million people at risk of infection (Soliman, 2008; Mwabonimana *et al*., 2009). These infection rates are high enough to make fasciolosis a serious public health concern, and therefore the parasite should be considered as a zoonosis of major global and regional importance (Soliman, 2008).

2.5 OBJECTIVES

The overall objectives of the research reported in this thesis were to determine the epidemiology (occurrence, distribution, transmission and economic importance) of fasciolosis in Botswana and to determine the geographical distribution and abundance of the parasite's intermediate host snail, *Lymnaea* species.

CHAPTER THREE

3 A retrospective study of the prevalence of bovine fasciolosis based on data from the main abattoirs in Botswana

3.1 Introduction

Fasciolosis is caused by the two most important fasciolid flukes, *Fasciola hepatica* and *F. gigantica*. These trematodes affect numerous mammalian species, mainly ruminants, in most countries of the world (Gajewska *et al*., 2005) and are significant pathogens of domestic animals, in particular cattle and sheep (Jones *et al*., 2006). As *Fasciola* spp. are haematophagous, their infection usually results in anaemia (Phiri *et al*., 2007c) and can cause very high mortalities, especially in small ruminants and calves (Mungube *et al.*, 2006).

Lymnaeid snails, the intermediate hosts of *Fasciola* species, play a crucial role in the epidemiology of fasciolosis (Coelho and Lima, 2003). As a result, fasciolosis is prevalent in areas where climatic conditions, such as marshland pastures, are favourable for the survival and proliferation of the intermediate host snail (Pfukenyi and Mukaratirwa, 2004; McGavin and Zachary, 2007).

In most African countries the prevalence of fasciolosis in ruminants has been determined mainly through slaughter house surveys (Phiri *et al*., 2005a; Mungube *et al*., 2006; Mwabonimana *et al*., 2009). Therefore, information gathered on animals slaughtered at an abattoir can be a convenient and relatively inexpensive source of information (Roberts and Suhardono, 1996) with condemnation rates proving a useful guide to the prevalence of the subacute, mild or chronic forms of the diseases in the regions served by the various abattoirs (Pfukenyi and Mukaratirwa, 2004).

In Botswana, traditional (cattle post) grazing establishments and commercial ranches (large fenced farms) send their animals for slaughter to various abattoirs throughout the country. The cattle are usually brought to these abattoirs on-foot or by road or rail transport. Meat inspection in the abattoirs is carried out independent of the owners by certified meat inspectors, hence the owners do not have any influence on the result of the inspection, and records are kept at the abattoirs by the government.

The prevalence of a number of diseases, notably bovine cysticercosis, has been reported from data collected at abattoirs in the country. However, no recorded studies have been carried out to determine the prevalence of fasciolosis in cattle using abattoir records. The objective of the present study was, therefore, to determine the prevalence of *F. gigantica* infections in slaughtered cattle based on records from the two main export abattoirs in Botswana during the period from 2001 to 2010.

3.2 Materials and Methods

3.2.1 Sampling method

A retrospective abattoir study was conducted that involved the retrieval and analysis of existing meat inspection records from two major exporting abattoirs in Botswana.

These data covered a period of ten-years, from January 2001 to December 2010. The data allowed estimation of the baseline prevalence of fasciolosis in cattle in Botswana.

Data were collected from two Botswana Meat Commission (BMC) export abattoirs located at Lobatse and Francistown (Figure 3.1). These are the largest abattoirs in Botswana with wide catchment areas, and are located in the southern and northern parts of the country, respectively, based primarily on livestock concentration and slaughter capacities (Table 3.1). The BMC abattoirs are parastatal organizations jointly owned by the Government of Botswana and private companies. The selected abattoirs slaughter animals from all regions of the country, and consequently cover animals from a range of climatic conditions, and service both the communal and commercial beef sectors.

The actual meat inspection process is performed by certified meat inspectors in accordance with the standards of the Livestock and Meat Industries (LMI) Act (2007) of the Republic of Botswana, under the supervision of the Chief Veterinary Officer (CVO) in the Department of Veterinary Services, Ministry of Agriculture.

3.2.2 Data collection and computation

These data were obtained on visits to the two abattoirs. Records of monthly and annual returns from the abattoirs were scrutinized with regard to the number of cattle slaughtered and the corresponding number of livers condemned as a result of infection with *F. gigantica* for the period from January 2001 to December 2010. The prevalence of fasciolosis was calculated as the number of cattle infected with *Fasciola* *gigantica* expressed as a percentage of the total number of cattle slaughtered, and was calculated annually for each abattoir. The overall prevalence for the ten year period (2001-2010), for each abattoir, was also determined, along with their 95% confidence intervals (CI). The Exact binomial method was used to work out the 95% CI.

3.2.3 Data analysis

Data obtained for the prevalence of bovine fasciolosis were entered, validated and calculated in a Microsoft Excel 2007 for Windows spreadsheet and later transferred into IBM Statistics Programme for Social Sciences (IBM SPSS) version 21.0 for Windows (IBM Corporation, Software Group, Somers, New York, USA) for analysis.

Data were tested for normality by running normality function tests (Kolmogorov-Smirnov and Shapiro-Wilk tests). The data showed abnormal distribution, which was an indication that a non-parametric test should be used instead of a parametric test. The Mann-Whitney test, which is an equivalent of the independent *t*-test, was used in this abattoir survey. The mean and standard error of the mean, as well as their 95% CI were also calculated.

Table 3.1 Annual rainfall and temperature for the catchment areas of the two export abattoirs

Abattoir	Catchment areas	Range in annual rainfall (mm)	Range in temperature (˚C)
	Gaborone	400 – 850	18 – 28
	Kgatleng	400 – 850	18 – 30
	Kweneng	350 – 600	20 – 30
Lobatse	Ngwaketse	350 – 600	18 – 30
	Borolong	350 - 600	18 - 30
	Kgalagadi	150 – 400	22 – 40
	Gantsi	150 – 400	22 – 40
	North-east	650 – 1200	22 – 35
	Boteti	400 – 650	22 – 35
Francistown	Serowe	400 – 650	20 – 32
	Tswapong	400 – 550	20 – 32
	Bobirwa	400 – 550	20 – 32

Source: Department of Meteorological Services (DMS), Botswana, 2003

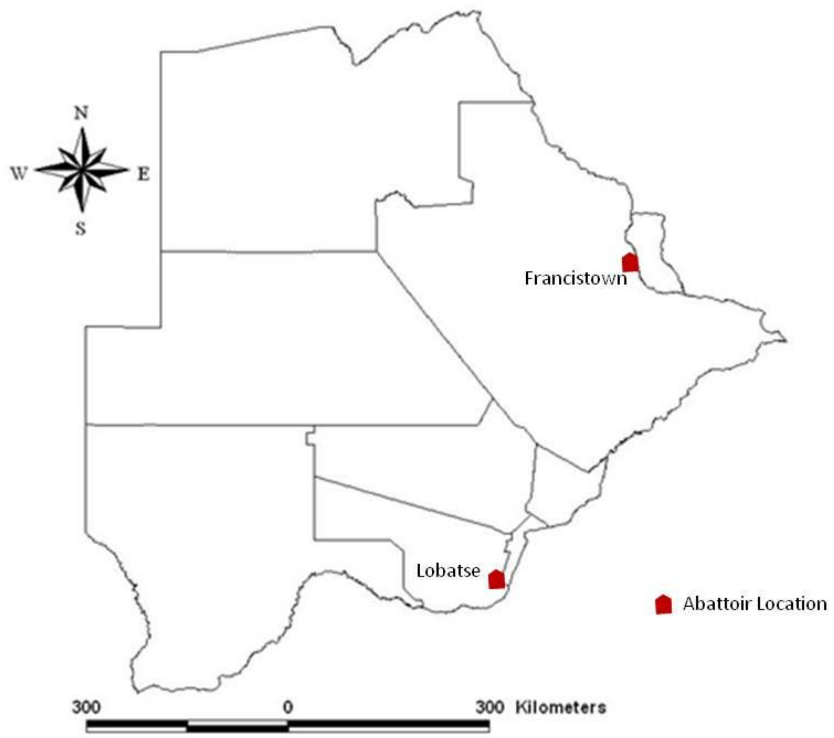


Figure 3.1 Map showing location of the two main export abattoirs, Lobatse (South) and Francistown (North) in Botswana

3.3 Results

The results of the total number of condemned livers and cattle slaughtered in each of the two abattoirs over the ten-year period are displayed in Table 3.2.

According to abattoir records, during the ten-year period, a total of 1,394,721 cattle were slaughtered at the two export abattoirs, and only 1,250 livers were condemned as a result of *F. gigantica* infection. Therefore, the overall prevalence of fasciolosis in cattle for the period from 2001 to 2010 was 0.09% (95% CI: 0.085, 0.095 %) with a range of 0% to 1.35 % in individual years.

At the Lobatse abattoir, the prevalence ranged from 0 to 0.01%, with no cases detected in 2003, 2004, 2005, and 2007 to 2010. In contrast, the Francistown abattoir recorded higher values, with no cases only in 2003 and 2004, and a much higher prevalence of 0.89% in 2006 and 1.35% in 2007 (Figure 3.2). There were no significant differences in prevalence between the years at the two abattoirs (p (0.08 and 0.13) > 0.05) of Lobatse and Francistown, respectively.

The prevalence of fasciolosis was significantly higher at the Francistown abattoir (0.265%; 95% CI: 0.25, 0.28%) than at the Lobatse abattoir (0.002%; 95% CI: 0.001, 0.003%) [p (0.004) < 0.05] (Table 3.2 and Figure 3.3).

Table 3.2 The number of cattle slaughtered and liver condemnations due to *F. gigantica* infections at two main export abattoirs in Botswana

Year Abattoir	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	Total for 10 years
Lobatse											
No cattle slaughtered	92,466	84,739	100,195	75,698	74,107	95,902	114,018	76,602	91,761	124,449	929,937
No livers condemned	7	4	0	0	0	7	0	0	0	0	18
Percentage infected	0.008	0.005	0.000	0.000	0.000	0.007	0.000	0.000	0.000	0.000	0.002
95% CI	0.00-0.02	0.00-0.01	0.00-0.00	0.00-0.00	0.00-0.00	0.00-0.02	0.00-0.00	0.00-0.00	0.00-0.00	0.00-0.00	0.001-0.003
Francistown											
No cattle slaughtered	71,200	28,825	53,301	54,022	44,093	41,452	57,211	36,690	43,525	34,465	464,784
No livers condemned	9	13	0	0	17	369	773	35	13	3	1,232
Percentage infected	0.013	0.045	0.000	0.000	0.039	0.890	1.351	0.095	0.030	0.009	0.265
95% CI	0.01-0.02	0.02-0.08	0.00-0.00	0.00-0.00	0.02-0.06	0.80-0.99	1.26-1.45	0.07-0.13	0.02-0.05	0.00-0.03	0.25-0.28
Annual Totals											
Total cattle slaughtered	163,666	113,564	153,496	129,720	118,200	137,354	171,229	113,292	135,286	158,914	1,394,721
Total livers condemned	16	17	0	0	17	376	773	35	13	3	1,250
Percentage infected	0.010	0.015	0.000	0.000	0.014	0.274	0.451	0.031	0.010	0.002	0.090
95% CI	0.01-0.02	0.01-0.02	0.00-0.00	0.00-0.00	0.01-0.02	0.25-0.30	0.42-0.48	0.02-0.04	0.01-0.02	0.00-0.01	0.085-0.095

The Lobatse abattoir, which had a lower prevalence, gets its supply of cattle from the southern half of the country, mainly from the two major cattle producing areas of Kgalagadi and Gantsi districts in the south-west and western parts of the country, respectively, but some animals do come from the Southern, South-east, Kgatleng and Kweneng districts. In contrast, the Francistown abattoir (higher prevalence) is supplied by the Central and North-east districts. The southern and western parts of the country receive less rainfall when compared to the north-eastern and central areas of the country with higher annual rainfall (Table 3.1), and where more and larger rivers and dams also exist. The monthly and seasonal difference in prevalence of the disease was not evident.

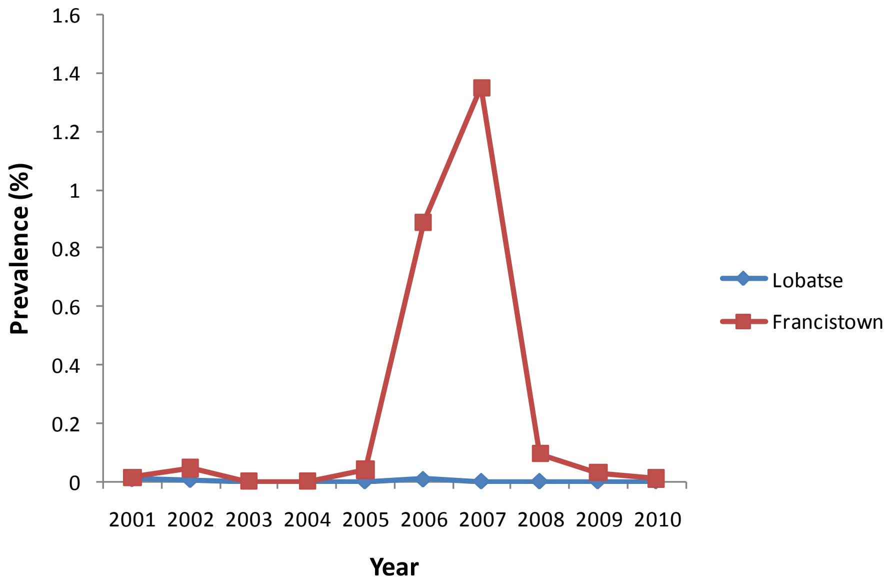


Figure 3.2 Ten-year annual trend of the prevalence of fasciolosis in cattle at two main export abattoirs in southern (Lobatse) and northern (Francistown) Botswana (2001 to 2010)

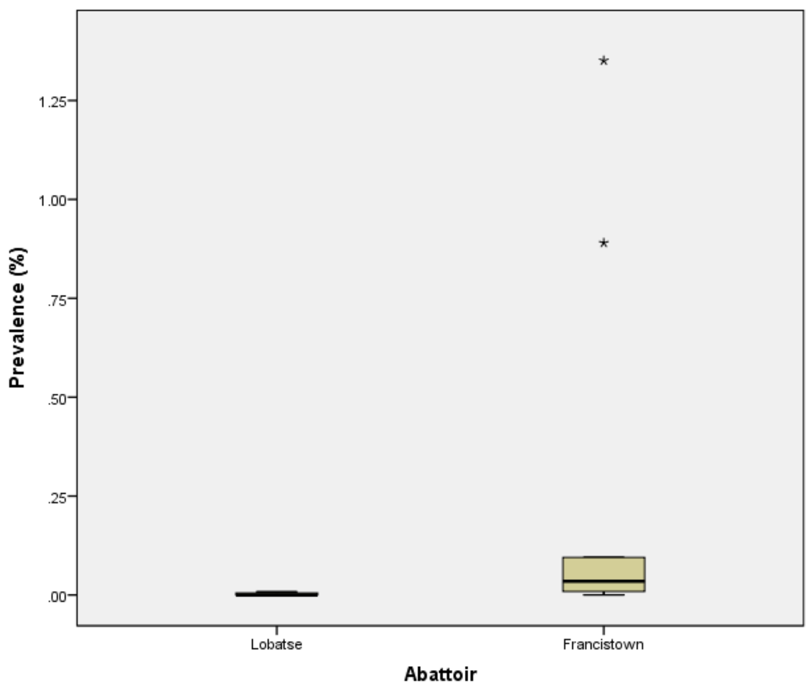


Figure 3.3 Mean prevalence of fasciolosis in cattle at the two export abattoirs in the southern and northern regions of Botswana

3.4 Discussion

The findings from this study have demonstrated that although fasciolosis was present in cattle slaughtered at the two main export abattoirs in Botswana, the prevalence was extremely low, in particular at Lobatse abattoir. This is the first systematic examination of the prevalence of bovine fasciolosis in the country, and it provides evidence for the need for more extensive epidemiological investigations to be undertaken in different regions of Botswana, to ascertain the actual prevalence in live animals according to their area of origin.

The mean overall prevalence of fasciolosis of less than 0.1%, detected in cattle slaughtered at the two main export abattoirs of Botswana, was much lower than that reported from other countries in sub-Saharan Africa. Similar abattoir studies in Zimbabwe (Pfukenyi and Mukaratirwa, 2004), Kenya (Kithuka *et al*., 2002; Mungube *et al.*, 2006) and Tanzania (Nonga *et al*., 2009; Mellau *et al*., 2010) have reported a significantly higher prevalence of 37.1%, 8%, 26%, 16.5% and 8.6%, respectively. The low prevalence in Botswana would indicate that fasciolosis is a negligible cause of liver condemnation when compared to other African countries, such as Tanzania, where the disease is the leading cause of liver condemnations in cattle (Mellau *et al*., 2010). These findings suggest that liver fluke infection in slaughtered cattle in Botswana is not of clinical or economic importance.

The abnormal surge in condemnations of *Fasciola*-infected livers in 2006 (269) and 2007 (773) at the Francistown abattoir, even though the latter year was a drought year in Botswana, could be attributed, in part, to the good rains in 2005 and the above average rainfall received by the country in 2006, which led to higher infections due to increased contact between cattle and contaminated pastures. The most possible explanation, though, could be the result of the actual drought that prevailed in 2007, which could have led to an increase in the supply or sale of cattle to the Francistown abattoir as a management measure by farmers, to mitigate the high costs usually associated with the purchase of supplementary feeds for the cattle during dry seasons or drought years. However, abattoir studies can be biased with uneven presentation of different age groups and under-representation of animals with clinical disease (Robertson and Blackmore, 1985). Consequently, extrapolating findings from meatworks for the general population needs to be undertaken with caution and appreciation of the biases of the population presented to abattoirs.

The difference in prevalence of fasciolosis between studies might also be attributable to the ecological and climatic variations existing in the various locations sampled, as well as animal husbandry practices which differ between countries. In particular, adoption of different parasite control measures between countries and awareness of the disease by farmers may vary between locations and countries. In Botswana, the commonly used (over-the-counter) veterinary drugs, such as dips, anthelmintics and some antibiotics, are sold to farmers at subsidized prices from the Livestock Advisory Centres (LACs) located throughout the country. Farmers in Botswana regularly use anthelmintics. One commonly used anthelmintic is albendazole (valbazen) which is a broad-spectrum drug with flukicidal activity against adult trematodes, as well as having the ability to kill nematodes and cestodes. However, triclabendazole, a highly effective flukicide, which is effective against both adult and juvenile flukes and the drug recommended in most countries, is not available in Botswana. However, given the low prevalence detected in this study, supply of triclabendazole, which is more expensive than other flukicides, is probably not warranted in Botswana.

The low prevalence might also be attributed to the provision of better veterinary services to the farming community by private veterinary practitioners in Botswana. The number of private practitioners has increased in various parts of the country in recent years (Aganga, pers comm.), and these provide advice to farmers on livestock management, which could result in a reduction the prevalence of fasciolosis. In addition, owing to the large size of the bovine liver, it is also possible that the prevalence of fasciolosis was underestimated in the present study since some livers with partial infection could have been passed as fit for human consumption after trimming of the affected parts. Another possibility might be that farmers chose to send their healthiest animals to the main abattoirs and the less healthy or poor conditioned cattle to the local council abattoirs and meat inspection slabs situated around the country. At these latter facilities, carcasses would undergo less rigorous scrutiny during meat inspection than at the export abattoirs.

In this study, animals processed at the Francistown abattoir, in the northern region, had a higher prevalence of fasciolosis than those processed at Lobatse, in the southern part of the country. These results are not surprising since the Francistown abattoir receives cattle from higher rainfall areas and where larger dams and rivers are located, in the North-east and Central districts. The North-east district in Botswana shares the border with Matebeleland province in Zimbabwe where a prevalence of 36.1%, based on an abattoir survey, has been reported (Pfukenyi and Mukaratirwa, 2004). The north-east region receives an annual rainfall of more than 1000 mm, which provides suitable conditions for the survival of the intermediate host snail, *L. natalensis*.

The lowest prevalence of fasciolosis was recorded at Lobatse abattoir, in the southern part of the country, and this could be attributed to the fact that the abattoir gets a large supply of cattle from Kgalagadi and Gantsi districts, where relatively dry conditions exist, which are unfavourable for the survival of the intermediate host snail. The mean annual rainfall for Lobatse is 550 mm, and it is comparatively lower in most areas of the southern part of the country, which supply cattle to the abattoir, and even much lower, with an average of 250 to 300 mm, in Gantsi and Kgalagadi districts, in the west.

The observation from this study is, to some extent, in agreement with studies from other parts of Africa where a higher prevalence of fasciolosis in cattle was reported following periods of high rainfall than during drought periods and from areas with higher rainfall than areas with lower rainfall amounts (Kithuka *et al*., 2002; Pfukenyi and Mukaratirwa, 2004; Mungube *et al*., 2006). The cattle sent to the Francistown abattoir could have come predominantly from fasciolosis endemic areas, such as the eastern margin of the country. Most livestock farmers in the northern and central part of the country use dams and rivers as water sources for their animals, and therefore take their cattle to drink directly from such sources on a regular basis, which could increase the risk of infection with *F. gigantica*.

The origin of cattle examined at a particular abattoir would be expected to have a strong influence on the prevalence of the disease (Phiri *et al*., 2005a), as was observed in this study. Fasciolosis is endemic in areas with a mean annual rainfall of over 1000 mm where *L. natalensis* is widely distributed (Pfukenyi and Mukaratirwa, 2004). Therefore, the cool and humid climate in the Central and North-east districts of Botswana could probably provide the optimal conditions for the survival of the intermediate host snail and hence the liver fluke.

Lymnaea natalensis, a freshwater snail, is the most common and widely distributed snail in tropical and subtropical Africa, including Botswana (Brown and Kristensen, 1989; Seddon *et al*., 2010) and can tolerate a wide range of conditions including changes affecting regional wetlands (Seddon *et al.*, 2010). The snail is found in a great variety of habitats including natural permanent water bodies, man-made dams, reservoirs, ponds and even cattle drinking troughs (Pfukenyi and Mukaratirwa, 2004; Seddon *et al*., 2010). These water bodies increase the risk of acquisition of infection (Ogunrinade and Ogunrinade, 1980). Consequently, the relatively higher prevalence of the disease in cattle slaughtered at the Francistown abattoir was probably due to the presence of larger and permanent rivers, as well as numerous streams in the North-east and Central district catchment areas, as opposed to the ephemeral water system that is prevalent in most of the southern part catchment areas, where the Lobatse abattoir is located. A similar study in Zambia by Phiri *et al* (2005a) found a higher prevalence of the disease in areas prone to flooding. Such areas are found in the North-west district of Botswana, where fasciolosis is believed to be endemic, however, it was not included in the present study due to logistical reasons associated with sampling this remote region.

The present study, nevertheless, did not observe any distinct seasonal pattern in liver condemnation rates and therefore the prevalence of fasciolosis. In contrast, elsewhere in Africa, seasonal differences have been observed, with a high prevalence of the disease reported during the rainy season and post-rainy periods (Mzembe and Chaudhry, 1981; Asanji and Williams, 1984; Pfukenyi and Mukaratirwa, 2004; Nonga *et al*., 2009). A study in Zimbabwe found that the snail population builds during the beginning of the dry season, and then drops during the cold dry months of winter, but again increases during the rainy season, with a concomitant peak in liver condemnations at the abattoirs (Pfukenyi and Mukaratirwa, 2004), and this is the likely scenario in the Central and North-east districts, and eastern parts of Botswana.

The findings of the present abattoir study provided preliminary baseline data on the prevalence of bovine fasciolosis in Botswana. These results have indicated that fasciolosis does not appear to be a major cause of liver condemnation in abattoirs in Botswana, and correspondingly, only low annual financial losses are likely as a consequence of condemnation of *F. gigantica* infected livers during the ten year period reviewed in this study. However, there is a need for a cross-sectional survey of fasciolosis in cattle of all ages in the country to determine the situation in live cattle in Botswana, and to have a better understanding of the epidemiology of this important parasitic disease of ruminants. Such information is essential for the design and implementation of appropriate control measures. In the following chapter, the results of a cross-sectional study conducted in Botswana are reported.

CHAPTER FOUR

4 Epidemiological survey of *Fasciola gigantica* infections in communal and commercial cattle farms in Botswana

4.1 Introduction

Fasciolosis has been recognized as one of the most important parasitic diseases in tropical countries limiting the productivity of ruminants, in particular cattle (Keyyu *et al*., 2005b). The prevalence of the disease in cattle has been reviewed by a number of authors across the world, and has been found to vary depending on a number of environmental and management factors. The disease occurs in areas where environmental conditions, typically low swampy areas, exist for the survival and proliferation of the intermediate host (McGavin and Zachary, 2007). Therefore, the disease is likely to be endemic and the prevalence high in areas with marshland pastures, regions of high moisture and temperatures, and areas regularly irrigated and poorly draining, which suit the survival of the snail intermediate host (Pfukenyi *et al*., 2006).

Infection with *F. gigantica* is regarded as one of the most important helminth infections of ruminants in Asia and Africa (Harrison *et al.*, 1996; Roberts and Suhardono, 1996; Wamae *et al.*, 1998) due to its wide distribution and spectrum of definitive hosts (Rondelaud *et al.*, 2001). It is considered a major source of production losses in domestic ruminants (Mage *et al.*, 2002), and even subclinical infections result in reduced feed efficiency, weight gains, milk production, reproductive performance, carcass quality and work output in draught animals, and condemnation of livers at slaughter (Pfukenyi *et al.*, 2006). Infections with *Fasciola* also predispose animals to other infections such as infectious necrotic hepatitis and salmonellosis (Ogunrinade and Ogunrinade, 1980).

Surveys on livestock diseases can give a useful guide to the prevalence or incidence of mild or chronic diseases, such as fasciolosis (Pfukenyi and Mukaratirwa, 2004). An effectively implemented survey can provide data which can be used to rank the importance of diseases to determine if a control programme is required (Kithuka *et al*., 2002). Repeated surveys can identify changes in the disease prevalence with time and identify potential factors to account for these changes (Kithuka *et al*., 2002).

The epidemiology and economic losses of fasciolosis are well known in most countries (Keyyu *et al*., 2005b). The prevalence of *F. gigantica* has been well documented in a number of tropical countries in Africa (Mzembe and Chaudhry, 1981; Tembely *et al*., 1988; Kithuka *et al*., 2002; Keyyu *et al*., 2005b; Phiri *et al*., 2005a; Phiri *et al*., 2005b; Pfukenyi *et al*., 2006). However, there are only a few reports on the prevalence and possible impact of fasciolosis in cattle in Botswana. The existing data on the disease are based on a few laboratory reports in some areas of the country, and cover only a few months of the year. The objective of the present study was to determine the prevalence and distribution of *F. gigantica* in cattle in Botswana.

4.2 Material and methods

4.2.1 Livestock farm surveys

4.2.1.1 Study location

The study was undertaken in six of the nine districts of Botswana (Southern, Southeast, Kweneng, Kgatleng, Central and Northeast districts - Figure 4.1). These districts are located between two villages at the extreme ends of the country, Ramatlabama in the south, bordering South Africa, and Ramokgwebana in the north-east, bordering Zimbabwe. The study area was located between latitude 20˚ 36' 38" and 25˚ 39' 55"S and longitude 25˚ 34' 23" and 27˚ 36' 50"E. The districts were included in the study owing to their topographical features and the prevailing weather conditions. These areas are characterized by hills, valleys, rivers, streams, drainage depressions, dams (natural and man-made), lakes and swampy or marshland areas. These rivers, streams, dams, lakes and marshlands serve as watering places/points for livestock.

The rainy season in Botswana extends from October to April, and the dry season from May to September. The mean annual rainfall is 650 mm (range 400 – 1200 mm) in the north-east, falling through the central and south-east areas to 500 mm (range 200 – 850 mm), to a minimum of 250 mm (range 100 – 400 mm) in the south-west. The rainfall is both erratic and unevenly distributed, and varies in total from year to year, making the country prone to periodic droughts (Figure 4.2 a and b) (Department of Meteorological Services, 2003).

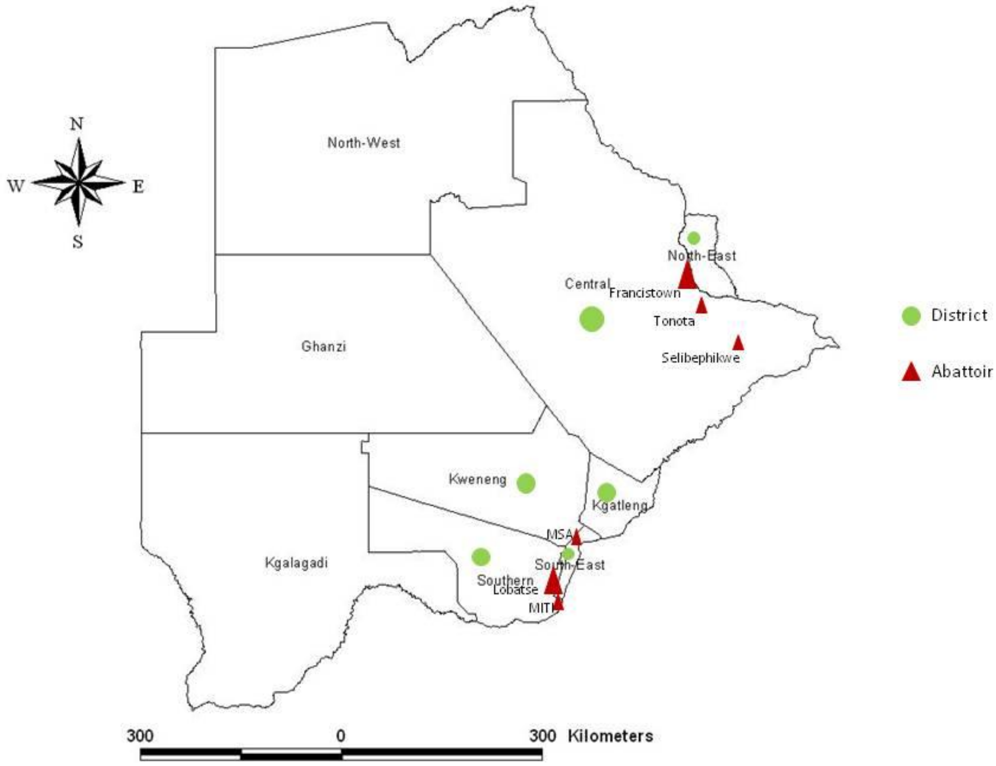
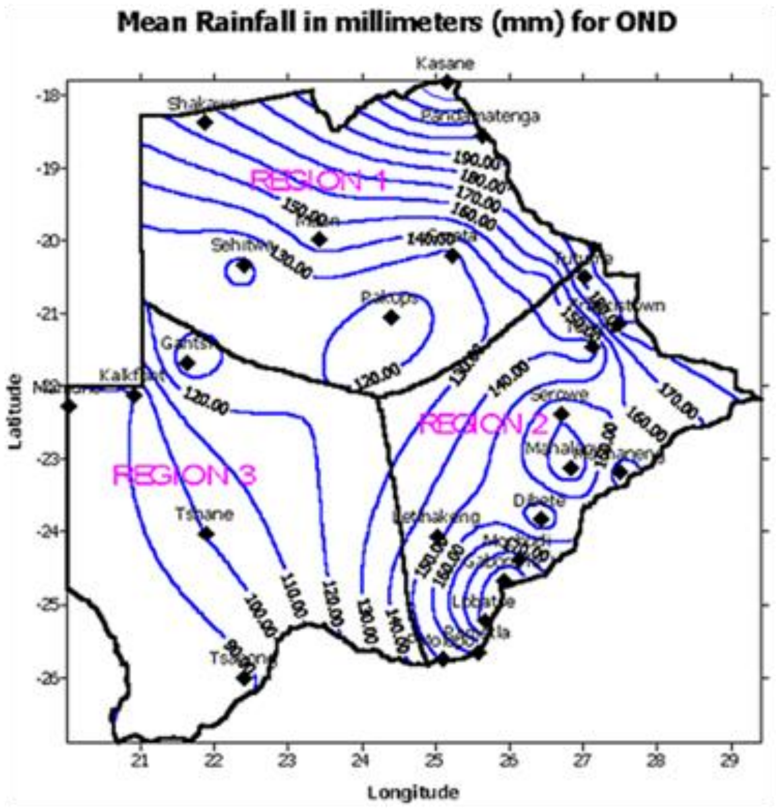


Figure 4.1 Map showing districts and abattoirs included in the study

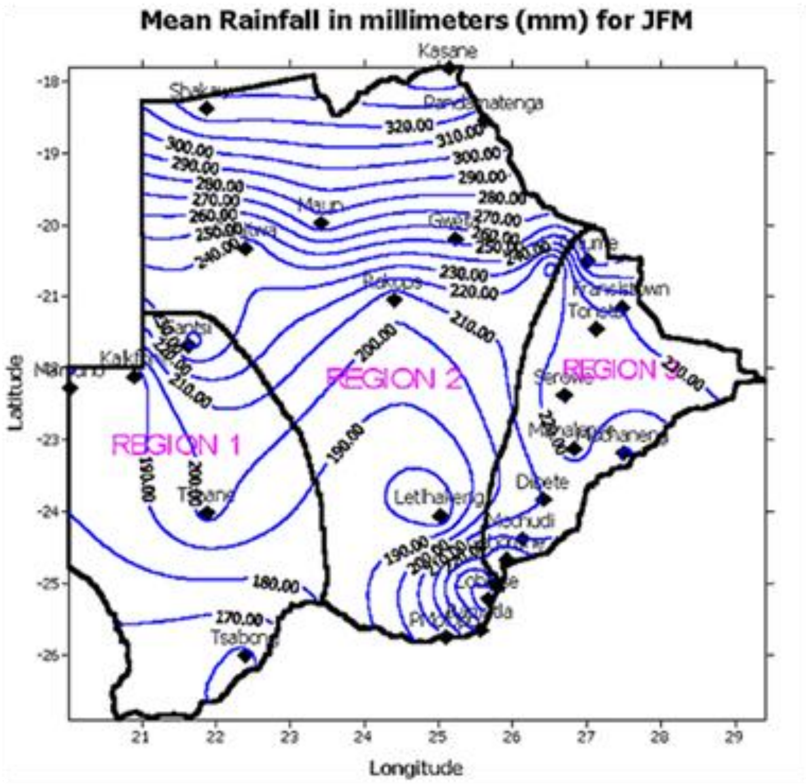
Dip tanks and crushes, used as handling facilities for large animals, and boreholes, used as water sources, were chosen as study sites. Cattle are regularly taken to the crushes for husbandry practices such as dipping, deworming, branding and loading/off-loading for transportation as well as for individual or national livestock census, mass vaccination campaigns or bolus insertion (for livestock identification) throughout the year, and to the boreholes for drinking water especially during the dry season. A few crushes/dip tanks or boreholes were randomly selected from each of the six districts.

4.2.1.2 Study animals

The breed of the sampled animals was recorded and included Brahman, Simmental, Charolais, Limousin, Hereford, Tuli, the native Tswana, Nguni and their crosses. Cattle were categorized into three age groups: calves (≤2 months old); weaners (12 to 24 months old); and adults (≥24 months old) and were further subdivided into male and female animals.



a)



b)

Figure 4.2 ***a* and *b*.** Isohyets showing long-term mean rainfall (mm) for October, November and December; and January, February and March, respectively.

4.2.1.3 Cattle management systems

Cattle were managed under the traditional or communal system and the commercial beef cattle sector. In the traditional or '*cattle post*' system, farmers keep small herds of between five and 50 animals per herd. Animals were grazed on unfenced tribally administered communal rangeland and watered at central watering points such as hand dug wells, dams, boreholes and streams/rivers. Cattle grazed natural pastures during both the wet and dry seasons, and supplementary feeding was rarely undertaken. Crop residues, available after harvesting grain, were grazed in *situ* rather than harvested as feed for livestock during the dry season, when natural pastures deteriorated both in quantity and quality. Livestock were allowed to wander freely over the grazing land during the day but were kraaled or housed close to the households at night. Some farmers practised crop production in small fields in addition to livestock production. The traditional cattle herds were given little or no effective disease control, apart from the annual national vaccinations conducted by the government. The common breeds kept were the Brahman, Simmental and Tswana and their crosses.

The commercial farmers raised their cattle on leasehold or freehold fenced large grazing lands or ranches. They had far more cattle (hundreds to thousands) than the traditional farmers. The sector adopted an improved management system, with supplementary feeding of livestock whenever necessary. Although cattle still used the extensive rangelands, most farmers are wealthy and could afford to buy either locally or imported feed resources, either roughage or concentrates. Animals were let out onto pasture to graze and could roam for several days before returning to drink. Cattle in the commercial sector were also kraaled regularly, but not handled frequently as in the traditional sector, unless some husbandry practices had to be carried out. In some farms, intensive livestock production systems, such as feedlotting were practised. Disease control was much better in the commercial sector than in the traditional sector. The majority of the cattle kept on commercial farms were exotic beef cattle breeds, Tswana and Nguni and their crosses.

4.2.1.4 Selection of farms and sampling of animals

The study was an observational cross-sectional study conducted in six districts across the country. A stratified random sampling method (proportional to size) was used to select animals from districts, villages, farms, management systems, age, gender and breed groups. Farms in each district or village were categorized as either traditional or commercial. Animals on each farm were categorized as calves (≤12 months), weaners (12 to 24 months) or adults (≥24 months). The survey covered the period from June 2011 to May 2013. The total number of animals sampled was 8,646, which was a large sample size, based on an estimated prevalence of 10% (Thrusfield, 2003).

4.2.1.5 Coprological studies

Faeces were collected per rectum from cattle in a crush using a gloved hand (Appendix 1). The faeces were placed into 40 g universal bottles, labelled, placed in a cooler box and transported to the Veterinary Parasitology Laboratory of the Department of Animal Science at the Botswana College of Agriculture in Gaborone. The presence of *F. gigantica* eggs was determined quantitatively by the standard sedimentation technique (Foreyt, 2001; Zajac and Conboy, 2006). The eggs of *F. gigantica* were distinguished from those of *Paramphistomum* species on the basis of their colour. *Fasciola* eggs are yellow-brown (Figure 4.6) whereas those of *Paramphistomum* are colourless (Taira *et al.*, 2003; Zajac and Conboy, 2006).

4.2.2 Abattoir based survey

4.2.2.1 Sampling method

A two-year prospective study was undertaken from June 2011 to May 2013 to determine the relative occurrence of *F. gigantica* infection in the livers of cattle presented to six abattoirs across the country. This prospectively acquired data validated the historical data from the retrospective study described in Chapter 3.

4.2.2.2 Hepatic inspection, Fasciola recovery, identification and count

During the study period, regular visits were made to the selected two export and four local council (municipal) abattoirs to collect samples of *Fasciola*-infected livers from cattle. During the visits, condemned and non-condemned livers, and their associated gall bladders, were thoroughly inspected visually for the presence of liver flukes. Evidence of *Fasciola* infection was based on liver enlargement, with raised or depressed areas and a firm consistency on palpation. In order to determine the presence of *F. gigantica*, the livers and bile ducts were incised longitudinally using a sharp knife or scalpel blade. The liver was incised into sections or slices of 1 cm thickness (Figure 4.7), and squeezed to force out any flukes from the bile ducts. The gall bladder was also opened, drained and inspected for any flukes as described by Bindernagel (1972).

The liver flukes were removed from the livers by the use of blunt forceps (Figure 4.8) and counted for each liver before being preserved in universal bottles containing 70% alcohol. The fluke samples or sometimes livers were subsequently transported, for further identification, to the Veterinary Parasitology Laboratory at the Botswana College of Agriculture. Details with reference to geographical origin, age, gender and breed of the animal were recorded during meat inspection for each animal from which the samples were collected. In the laboratory, identification of the species of liver flukes collected was confirmed by morphological features and measurements as described by Soulsby (1982) and Taylor *et al* (2007). Liver flukes which were ≤ 35 mm in length, leaf-shaped with broad shoulders and pointed caudal ends were classified as *F. hepatica*. Those flukes which were ≥ 35 mm in length, elongated with sloping shoulders and had rounded caudal ends were classified as *F. gigantica* (Figure 4.9)

4.2.3 Statistical analysis

The prevalence of *F. gigantica* was calculated along with their 95% confidence intervals for each sampling site and group of animals (Thrusfield, 2003; Dohoo *et al*., 2009).

Data obtained for the prevalence of bovine fasciolosis were entered and validated in a Microsoft Excel 2007 (for Windows) spreadsheet and later transferred into the IBM Statistics Programme for Social Sciences (IBM SPSS) version 21.0 for Windows (IBM Corporation, Software Group, Somers, New York, USA) for analysis.

The proportion of animals with *Fasciola* eggs in the faeces was compared between geographic location (district of origin), age, gender and breed using the Pearson's *Chi*-square (χ^2) test for independence and odds ratios (OR) and their 95% CI were calculated to identify risk factors for infection. The effect of age, gender and breed on transformed faecal egg counts [Log_{10} (egg count + 1)] were analysed with an Analysis of Variance (ANOVA). The intensity of infection based on the number of eggs per gram (epg) was classified into three levels namely:

Low intensity: Egg count ≤ 10 epg

Moderate intensity: Egg count between 10 and 25 epg

High intensity: Egg count ≥ 25 epg

The prevalence was also determined in the abattoir survey, and was calculated as described in the previous chapter (Section 3.2.2). In all analyses, the statistical level was considered significant if $p < 0.05$.

4.3 Results

4.3.1 Prevalence based on coprological examination

The overall prevalence of *F. gigantica* infections in cattle is summarized in Table 4.1. A total of 8,646 bovine (4,618 adults, 2,843 weaners and 1,185 calves) faecal samples were examined during the 24 month study, and only 64 (0.74%; 95% CI = 0.57 to 0.94%) animals were positive for *Fasciola* eggs.

4.3.1.1 Influence of geographic location or district of origin

Of the 8,646 cattle sampled, 7,052 originated from communal farms and 1,594 from commercial farms. A total of 2,733 were from the Central district (1,407 from communal and 1,326 from commercial farms), 1,256 from Kgatleng, 1,115 from Kweneng, 478 from the North-east, 1,463 from the South-east (which were all communal farms) and 1,601from Southern district (1,333 communal and 268 from commercial farms). The recorded prevalence from the coprological examination in each district was as follows: Central, 2.34% (95% CI: 1.81, 2.98%); Kgatleng, 0% (0.00, 0.29%); Kweneng, 0% (0.00, 0.33%); North-east, 0% (0.00, 0.77%); South-east, 0% (0.00, 0.25%) and Southern, 0% (0.00, 0.23%).

According to the Pearson's *Chi* square test for independence, there was great variation between the prevalence of bovine fasciolosis and the geographical areas of origin. The difference was significant (χ^2 (5) = 139.50, $p < 0.001$), with no infections in five of the study districts.

Table 4.1 Prevalence of *F. gigantica* infection in cattle according to village of origin within study district based on coprological examination

Geographical Location	Number Examined	Number Positive	Prevalence (%) (95% CI)
Central district			
Machaneng	709	64	9.03 (7.02, 11.38)
Mahalapye	751	0	0 (0.00, 0.49)
Mmadinare	215	0	0 (0.00, 1.70)
Sefhophe	97	0	0 (0.00, 3.73)
Selibe-phikwe	141	0	0 (0.00, 2.58)
Shakwe	203	0	0 (0.00, 1.80)
Shoshong	617	0	0 (0.00, 0.60)
Total	2,733	64	2.34 (1.81, 2.98)
Kgatleng district			
Bokaa	143	0	0 (0.00, 2.55)
Malolwane	301	0	0 (0.00, 122)
Mochudi	716	0	0 (0.00, 0.51)
Modipane	96	0	0 (0.00, 3.77)
Kweneng district			
Kopong	311	0	0 (0.00, 1.18)
Kumakwane	67	0	0 (0.00, 5.36)
Lentsweletau	206	0	0 (0.00, 1.77)
Molepolole	531	0	0 (0.00, 0.69)
Northeast district			
Masunga	126	0	0 (0.00, 2.89)
Tati	352	0	0 (0.00, 1.04)
Southeast district			
Otse	379	0	0 (0.00, 0.97)
Ramotswa	1,084	0	0 (0.00, 0.34)
Southern district			
Goodhope	295	0	0 (0.00, 1.24)
Mabule	130	0	0 (0.00, 2.80)
Mmathethe	214	0	0 (0.00, 3.73)
Metlojane	582	0	0 (0.00, 0.63)
Ramatlabama	91	0	0 (0.00, 3.97)
Ranaka	289	0	0 (0.00, 1.27)
Total	8,646	64	0.74 (0.57, 0.94)

The disease was found in only one (Central district) of the six districts sampled, and was restricted to only one of the 25 villages sampled (Machaneng). All the 64 *Fasciola*-positive animals originated from commercial farms in this village. These results should, however, be interpreted with caution since, from the results, the Cramer's statistic (0.13) was small, which is indicative of a weak strength of association between the geographical location and the prevalence of *F. gigantica* infections. *Fasciola gigantica* infections were localized within the Tuli Block ranches in the eastern part of Machaneng. A total of 709 cattle were sampled for coprological examination in the area and only 64 or 9.03% (95% CI: 7.02, 11.38%) had a positive faecal sample.

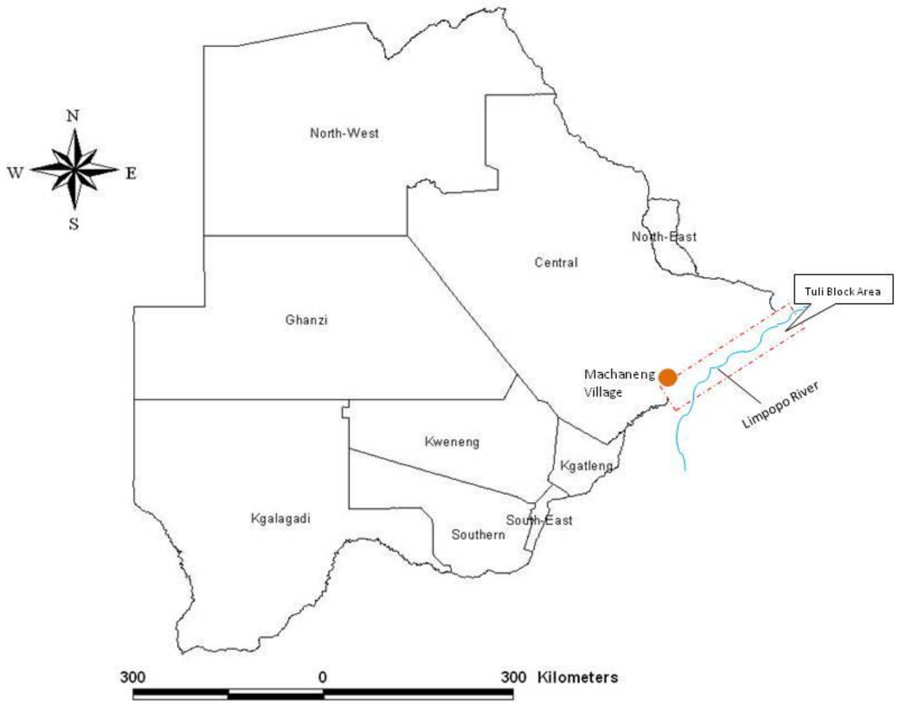


Figure 4.3 Map indicating the area where positive faecal samples were detected in this coprological study

4.3.1.2 Influence of age

The prevalence in the Tuli Block Table 4.2 was significantly higher in adults (12.85%; 95% CI: 9.72, 16.54%) than in weaners (6.49%; 95% CI: 3.40, 11.06%) and calves (0.79%; 95% CI: 0.02 to 4.31%), (χ^2 (2) = 19.01, $p < 0.001$). By calculating odds ratios, it was demonstrated that infection with *F. gigantica* was more common in adult cattle (OR = 18.57; 95% CI: 2.54, 135.81%) and weaners (OR = 8.74; 95% CI: 1.12, 68.09) than calves.

4.3.1.3 *Influence of gender*

Female cattle (7.76%; 95% CI: 5.90, 9.98%) had a significantly higher prevalence than males (1.27%; 95% CI: 0.58, 2.40%), (χ^2 (1) = 9.73, $p = 0.002 < 0.05$). Females were three times more likely to be infected than males (OR = 3.01; 95% CI: 1.46, 6.21). When gender were separated into individual age categories, only adult females showed a significantly higher prevalence than males (χ^2 (1) = 4.28, $p = 0.04 < 0.05$) whilst weaners and calves did not show significant gender differences, (χ^2 (1) = 1.27, $p = 0.26 > 0.05$) and (χ^2 (1) = 0.77, $p = 0.38 > 0.05$), respectively.

Table 4.2 Age and gender-specific prevalence in Tuli Block farms in Machaneng village

Age class	Gender	No. examined		No. positive		Prevalence (%) (95% CI)	
Adult	Female	397	305	51	45	12.85(9.72, 16.54)[a]	14.75
	Male		92		6		6.52[a]
Weaner	Female	185	127	12	10	6.49(3.40, 11.06)[b]	7.87
	Male		58		2		3.44[b]
Calves	Female	127	55	1	0	0.79(0.02, 4.31)[c]	0
	Male		72		1		1.38[c]
Total	Female	709	487	64	55	9.03(7.02, 11.38)	11.29
	Male		222		9		4.05

[a,b,c] Values with different superscript in a column are significantly different ($p < 0.001$)

A comparison of the prevalence in different cattle breeds in the Tuli Block commercial farms in Machaneng village (Table 4.3) revealed that the pure Brahman (8.33%; 95% CI: 5.56, 11.89%) and Brahman crosses (12.80%; 95% CI: 9.18, 17.21%) were positive for *F. gigantica* eggs. In contrast, no cattle of the Nguni breed showed were infected (0%; 95% CI: 0.00, 3.77%), and this prevalence was significantly lower than the pure Brahman and Brahman crosses (χ^2 (2) = 14.73, p = 0.001 < 0.05).

Table 4.3 Breed-specific prevalence in Tuli Block farms in Machaneng village

	Fasciola faecal result		
Breed	No. examined	No. positive	Prevalence (%) (95% CI)
Brahman	324	27	8.33 (5.56, 11.89)[a]
Brahman cross	289	37	12.80 (9.18, 17.21)[a]
Nguni	96	0	0 (0.00, 3.77)[b]
Total	709	64	9.03 (7.02, 11.38)

[a,b] Values with different superscript in a column are significantly different (p = 0.001)

The overall mean monthly and annual precipitation was well below 100 mm (ranged from 0 to 92 mm), with most months recording below 50 mm of rainfall for the 24 months of study. The mean temperature ranged between 12 and 26 ˚C, with very little variation between the two years (Figure 4.2).

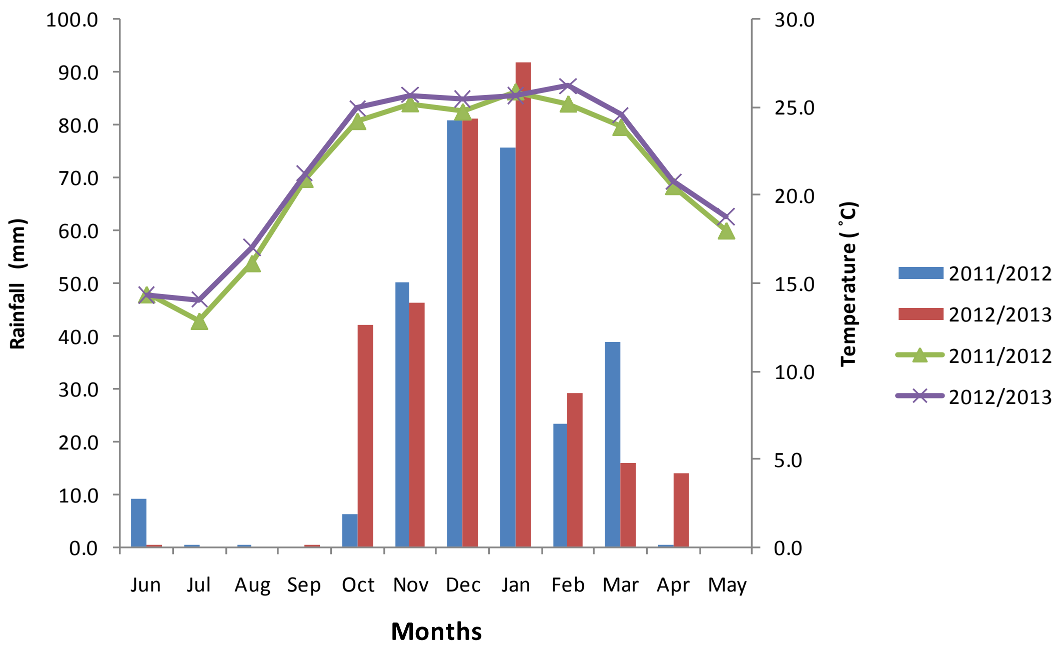


Figure 4.4 Mean monthly rainfall (columns) and temperature (lines) in the six study districts of Botswana for the period from June 2011 to May 2013 (Source, DMS, 2003)

The intensity of infection based on the number of *egg per gram* (epg) of faeces, regardless of age, gender or breed, showed that almost all cattle examined (97.18%; 95% CI: 95.68, 98.27%) had either no or low infection level. Only a few (2.40%; 95% CI: 1.40, 3.81%) had a moderate infection and a negligible number (0.40%; 95% CI: 0.09, 1.23%) had severe infections. The mean egg count was 0.98 ± 0.14 epg. Of the 64 *Fasciola* spp. egg-positive animals, the majority (44 - 68.75%) had a low count, ≤10 epg, 17 (26.56%) had a moderate infection (>10 ≤ 25 epg) and only three (4.69%) had evidence of high infections (>25 epg) (Table 4.4).

Table 4.4 Intensity of infection with *F. gigantica* in different categories of cattle from the Tuli Block farms in Machaneng village

	Number infected		
Animal category	Low (≤10 epg)	Moderate (>10 ≤25 epg	High (>25 epg)
Adult	35	14	2
Weaner	8	3	1
Calves	1	0	0
Total	44	17	3

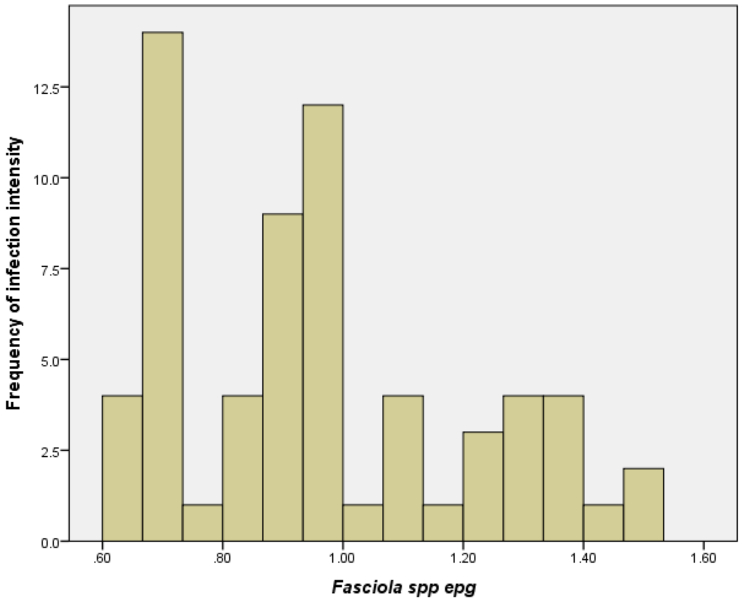


Figure 4.5 Infection intensity (epg of faeces) of *F. gigantica* at Tuli Block farms in Machaneng

Figure 4.6 *Fasciola gigantica* egg (arrow) detected during coprological examination

4.3.2 Prevalence as determined by the abattoir survey

A total of 268,957 cattle were slaughtered and their livers inspected during the 24 month period. Of these, only 78 (0.03%; 95% CI: 0.02, 0.04%) livers were condemned due to fasciolosis, including 28 (0.02%; 95% CI: 0.01, 0.02%) at the Lobatse abattoir, 11 (0.09%; 95% CI: 0.05, 0.17%) at Multi Species Abattoir (M.S.A.) in Gaborone and 39 (0.06%; 95% CI: 0.04, 0.08%) at the Francistown abattoir (Table 4.5). Approximately half of the condemned livers (n = 41; 52.6%; 95% CI: 40.93, 63.99%) had light intensity, 26 (33.3%; 95% CI: 23.06, 44.92%) had moderate intensity and 11 (14.1%; 95% CI: 7.26, 23.83%) exhibited severe intensity of fluke infection (Table 4.6). The mean overall prevalence of 0.03% recorded from the inspection of livers was significantly lower than that found from the coprological examination (χ^2 (1) = 16.13, $p < 0.001$).

Infection with liver fluke was evenly distributed between abattoirs in the south (Lobatse, Meat Inspection Training Institute (M.I.T.I.) also in Lobatse and M.S.A in Gaborone) and those in the north (Francistown, Tonota and Selibe-phikwe), with a similar mean prevalence of 0.04% and 0.02%, respectively. No infections were detected at the M.I.T.I. in Lobatse, Selibe-phikwe and Tonota abattoirs. Examination of flukes recovered from slaughtered cattle confirmed the presence of both juvenile and adult flukes.

Table 4.5 Prevalence of *F. gigantica* infection in the livers of cattle processed at different locations between June 2011 and May 2013

Abattoir Location	No. examined	No. positive	Prevalence (%) (95% CI)
Lobatse	173,350	28	0.02 (0.01, 0.02)
M.I.T.I. (Lobatse)	8,741	0	0.00 (0.00, 0.04)
M.S.A. (Gaborone)	11,673	11	0.09 (0.09, 0.17)
Selibe-phikwe	5,362	0	0.00 (0.00, 0.07)
Tonota	6,427	0	0.00 (0.00, 0.06)
Francistown	63,404	39	0.06 (0.04, 0.08)
Total	268,957	78	0.03 (0.02, 0.04)

Table 4.6 Fluke intensity of infection of affected livers (See Figures 4.7 to 4.9)

Infection intensity	No. livers affected	Prevalence (%) (95% CI)
Light intensity	41	52.56 (40.93, 63.99)
Moderate intensity	26	33.33 (23.06, 44.92)
Severe intensity	11	14.10 (7.26, 23.83)
Total	78	100

Figure 4.7 Bovine liver incised into 1 cm slices during inspection

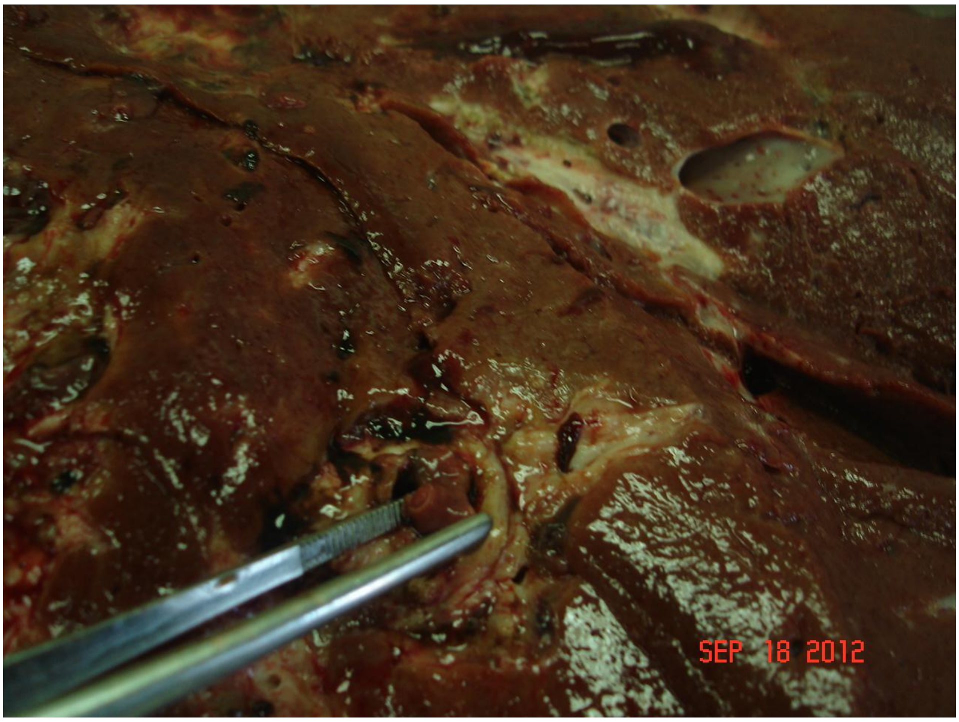


Figure 4.8 Adult liver flukes being removed from the bile ducts

Figure 4.9 Adult *F. gigantica* recovered from a bovine liver during inspection

4.4 Discussion

The findings of 0.74% prevalence from the present study have shown that although liver fluke is present in beef cattle in Botswana, infection is not widespread throughout the country. This study is the first report of infection with *Fasciola* in the study areas. Prior to this study, it was believed that infection was widely distributed in Botswana albeit based on a few unsubstantiated records (Department of Veterinary Services, 2008). These results have clearly indicated that the geographical distribution of *Fasciola* infection is narrower than previously envisaged, being prevalent in only one of the six districts included in this study, and localized within the Tuli Block area in Machaneng village, in the eastern margin of the country. Larger permanent water bodies exist in the Central district than in the other districts, where it is relatively drier. The prevalence of 2.34% in the Central district (and 9.03% in the Tuli Block area) was significantly lower than that reported from the neighbouring countries of Malawi, Zambia and Zimbabwe (range 15 to 37%) (Mzembe and Chaudhry, 1981; Phiri *et al*., 2005b; Pfukenyi *et al*., 2006).

The occurrence of fasciolosis in cattle in different areas around the world is influenced by a range of factors (Maqbool *et al*., 2002) including the hosts, the parasites, environmental and climatic conditions, snail population and the choice of diagnostic technique used to detect infection (Yildrim *et al*., 2007). The prevalence of *F. gigantica* has been found to differ between continents, countries and within a country. This variation has been attributed to differences in agro-ecological and climatic variations between localities (Yilma and Mesfin, 2000; Copeman and Copland, 2008; Abunna *et* *al.*, 2010), however, in some situations it may be due to the management system practised (Yilma and Mesfin, 2000; Keyyu *et al*., 2006; Yildrim *et al*., 2007; Abunna *et al*., 2010).

In the present study, the mean annual rainfall recorded was less than 100 mm during the rainy season in all the areas studied, and was far below the normal annual average of 500 to 650 mm (Department of Meteorological Services, 2003). This low precipitation could have negatively affected the prevalence of infection with *Fasciola* in cattle. This pattern of rainfall persisted during the 24 months of study. In contrast, the mean annual temperature ranged from 12 to 26 ˚C, and was suitable for the proliferation of the intermediate host snail as well as the development of the intra-molluscan stages of *F. gigantica* (Soulsby, 1982; Taylor *et al*., 2007). However, temperature alone is not sufficient to support the development of *Fasciola* infection in ruminants, there is, additionally, the requirement for adequate moisture.

The Tuli Block area is a strip of land at Botswana's eastern margin where the practice of raising large beef cattle herds is common. The ranches in this area are located on the fringes of the Limpopo River, which is a large perennial river (the second largest river in Africa after the Zambezi River) that drains into the Indian Ocean. The river holds a large amount of water during the year and thus serves as a good source of drinking water for cattle in the ranches throughout the year. Therefore, this study area presents prominent epidemiological significance due to its geographical location. The water from the river is reticulated to the farms through metal pipes, however, cattle from some paddocks, in particular the native breeds, such as Nguni and Tswana, are usually driven to the river and drink directly from the river. This practice would increase the likelihood of infection if *Fasciola* was present in snails in this region.

The prevalence of fasciolosis observed in this study in the Tuli Block ecological zone of eastern Botswana is consistent with reports from other African countries with similar ecological conditions. In Kenya, Malawi, Tanzania, Zambia and Zimbabwe, the pattern of distribution of fasciolosis follow zones of high rainfall, high livestock density and wetland areas infested with the intermediate host snail (Bitakaramire and Bwangamoi, 1969; Mzembe and Chaudhry, 1981; Kithuka *et al*., 2002; Keyyu *et al*., 2005b; Phiri *et al*., 2005a; Phiri *et al*., 2005b; Pfukenyi *et al*., 2006). A study in Zimbabwe found that fasciolosis was more prevalent in the high rainfall areas of the highveld than in the drier areas of the lowveld (Pfukenyi *et al.*, 2006) since the high rainfall areas favour the development and survival of both the intermediate host snail and the intra-molluscan stages of the parasite (Malone *et al*., 1984; Malone *et al.*, 1998; Yilma and Malone, 1998; Yadav *et al.*, 2007) and thus increase the likelihood of infection in livestock. This is further supported by recent findings in Britain (Pritchard *et al*., 2005), where the emergence of fasciolosis in cattle in East Anglia was linked with higher summer rainfall, which favoured the development of the intermediate host, *L. truncatula* and the free-living stages of *F. hepatica*.

A high prevalence of fasciolosis has been reported in wetter areas of Kenya where flood plains exist, allowing accumulations of large water masses which favour the survival of *L. natalensis* (Bitakaramire and Bwangamoi, 1969). Although these large water bodies provide a good source of water for drinking by animals, there is increased risk of infection (Ogunrinade and Ogunrinade, 1980), and the high population of livestock in such areas helps to maintain the disease (Kithuka *et al.*, 2002). The same findings were reported in another study in Tanzania where the grazing of cattle in marshland areas, valleys and flood plains exposed livestock to contaminated pastures (Keyyu *et al*., 2005b). These reports are similar to the findings of a study in Zambia, which showed that the occurrence of wetlands in grazing areas of cattle and the high livestock density increase the risk of infection with *F. gigantica* (Phiri *et al*., 2005a). In Europe, the recent emergence of fasciolosis in cattle in Britain, was attributed to the increased influx of sheep from endemic fluke areas - for seasonal grazing and the wetter grazing conditions (Pritchard *et al.*, 2005).

The prevalence of the disease in West Africa varies widely according to the availability and distribution of the intermediate host snail (Schillhorn Van Veen *et al.*, 1980). Similar findings have been reported in Algeria where a higher prevalence was recorded in a district where favourable climatic conditions prevailed (Mekroud *et al.*, 2004). An investigation in Mali found a higher prevalence of *Fasciola* infection in cattle grazing the wet inland delta of the Niger river (Tembely *et al.*, 1988). A similar trend was observed in Nigeria where a high prevalence of fasciolosis was attributed to a high density of cattle in the area during the time of increased water drinking (Schillhorn Van Veen *et al*., 1980). In Ethiopia, bovine fasciolosis has been reported in most regions, although the prevalence differs with locality and is dependent upon climatic and ecological conditions such as altitude, rainfall and temperature (Yilma and Mesfin, 2000; Abunna *et al*., 2010). In other parts of the world, a higher prevalence has been recorded in wet countries with mild temperatures (Dorchies, 2006).

The North-west district in Botswana, where the wetlands of the country, the Okavango delta and swamps, exist, and where fasciolosis is believed to be endemic and consequently the prevalence presumably high, was not included in the present study due to a limitation of resources. The marshy pastures there, which serve as part of the grazing and drinking sources for livestock in the area, are potential habitats for the snail intermediate hosts and as a result increase the risk of infection with *F. gigantica*. In other countries, fasciolosis is endemic in lowland areas with extensive seasonally flooded pasture land surrounded by undulating hills where environmental conditions are favourable for the intermediate hosts (Rangel-Ruiz *et al*., 1999; Faria *et al.*, 2005).

The findings reported in this study are amongst the lowest documented in Africa, in terms of both prevalence and infection intensity. However, the low prevalence detected at the Tuli Block farms might be attributable to the sedimentation technique used to determine the proportion of infected animals because of the low sensitivity and thus characteristically poor detection of fluke eggs by this method. Therefore, the prevalence of flukes in livestock from the sampled farms and consequently the country might actually be underestimated in this study. Use of serological tests, such as the ELISA, which are more sensitive for the diagnosis of *F. gigantica* infection in cattle, should provide more accurate estimates of the true prevalence, especially in developing countries where the molecular diagnoses of *F. gigantica* infections are in their infancy (Hillyer, 1999; Spithill *et al*., 1999; Awad *et al*., 2009).

Most recent studies from around the globe have reported a much higher prevalence and infection rates than that found in the present study. As a result, fasciolosis is currently regarded as an emerging or re-emerging disease in many countries (Esteban *et al*., 2003; Phiri *et al*., 2005a). In contrast, as reported in Chapter 3 of this thesis, Botswana does not appear to have a serious problem with the disease. Therefore bovine fasciolosis can be regarded as a parasitic disease of low prevalence especially in the widespread semi-arid regions of the country.

The prevalence of infection with *F*. *gigantica* has been reported to increase with age (Gonzalez-Lanza *et al*., 1989; Spithill *et al*., 1999). Similarly in the current study, although the overall prevalence of *F. gigantica* was low, older cattle had a significantly higher prevalence than younger livestock. Similar results have been reported in other countries (Baldock and Arthur, 1985; Gonzalez-Lanza *et al*., 1989; Holland *et al*., 2000; Waruiru *et al*., 2000; Keyyu *et al*., 2005b; Phiri *et al*., 2005a; Pfukenyi *et al*., 2006). The higher infection in older cattle is believed to be associated with increased opportunity for infection from pasture with increasing age (Schillhorn Van Veen *et al*., 1980; Baldock and Arthur, 1985; Waruiru *et al*., 2000; Keyyu *et al*., 2005b; Pfukenyi *et al*., 2006).

This study found that female cattle had a statistically higher prevalence than males. Similar findings have also been reported in other parts of Africa, including Sierra-Leone (Asanji and Williams, 1984), Zambia (Phiri *et al*., 2005a) and Egypt (Kuchai *et al*., 2011). These results were also consistent with findings from other parts of the world including Turkey (Yildrim *et al*., 2007) and Switzerland (Ducommun and Pfister, 1991). The higher prevalence in females could be explained by the fact that female to male ratio is usually high since most, if not all, female animals are attributed to the practice of retaining for breeding and in some cases milk production purposes, as reported by Phiri *et al*.(2005a). Stress associated with pregnancy and parturition may increase the risk of infection in females (Spithill *et al*., 1999). However, age could be a confounding variable because when gender is compared within each age category, only female adults indicated a significantly higher prevalence than male adult cattle. The weaner and calf categories did not have significant differences in prevalence, implying that infection with liver fluke was almost similar regardless of gender.

In this study, a significantly higher prevalence was observed in Brahman cross cattle (12.20%) and pure Brahman (8.33%) compared with the indigenous Nguni cattle (0%). These findings are in agreement with a similar recent study carried out in South Africa where lower *Fasciola* egg counts were reported in Nguni than in Bonsmara and Angus breeds (Ndlovu *et al*., 2009). In contrast, other authors (Tembely *et al*., 1988; Kato e*t al*., 2005; Keyyu *et al*., 2006) have reported a higher prevalence in traditional breeds compared to *Bos taurus* breeds. However, Dorchies *et al*. (2006) reported that the Charolais breed was more commonly infected than Limousins or cross bred cattle. In contrast, some authors have found no difference in prevalence in different cattle breeds (Sánchez-Andrade *et al*., 2002; Yildrim *et al*., 2007; Yeneneh *et al*., 2012).

The absence of infection in Nguni cattle could be due to an innate resistance, as opposed to acquired immunity that occurs over time, since both Brahman and Nguni breeds were exposed to the same conditions on the farms. Genetics may in fact play a major role in determining differences in resistance and resilience to *F. gigantica* infection in cattle (Molina, 2005). The Nguni cattle were, in fact, watered directly from the river whereas other breeds had water reticulated to them through water pipes. Consequently, it could be expected that the Nguni cattle would have a higher prevalence than other breeds. Generally, acquired resistance is known to be only partially protective, with cattle remaining susceptible to re-infection every year (Spithill *et al*., 1999).

In the present study, evidence of *Fasciola* infection was only present in cattle reared under the commercial farming system. However, in the current study, the most probable source of infection, the Limpopo River in the Tuli Block area, was only used by the commercial farms and was not accessible to communally grazed cattle. In contrast to the findings of this study, research from other tropical countries has generally shown that a higher prevalence of infection is associated with traditional or communal grazing of cattle with modern systems of faming tending to have a lower prevalence (FAO, 1994; Maqbool *et al*., 2002; Keyyu *et al*., 2005a; Yildrim *et al*., 2007; Khan *et al*., 2009; Abunna *et al*., 2010; Tsegaye *et al*., 2012). The high prevalence in communal grazing areas is ascribed to firstly allowing cattle to drink water or graze contaminated pastures adjacent to river banks, dams and irrigation canals (Spithill *et al*., 1999; Suon *et al*., 2006; Munguía-Xóchihua *et al*., 2007) and secondly is likely to be linked with insufficient adoption of anthelmintic treatment and control measures for flukes (Phiri *et al*., 2005a; Yildrim *et al*., 2007). In a Japanese study, the high prevalence of fasciolosis was linked to the management whereby native cattle were grazed on potentially contaminated rice straw whereas other breeds remained kraaled and thereby reduced their risk (Kato *et al*., 2005).

The overall prevalence due to liver condemnation from the abattoir study was 0.03%. A significantly higher prevalence has been reported from similar surveys carried out in Malawi (38.67%), Zambia (53.90%), Ethiopia (24.32 and 90.65%) and Tanzania (14.05%) (Mzembe and Chaudhry, 1981; Yilma and Mesfin, 2000; Phiri *et al*., 2005a; Berhe *et al*., 2009; Swai and Ulicky, 2009). The result from the current abattoir survey revealed that the only fluke species involved was *F. gigantica*. These findings are therefore suggestive of an association with the existence of a favourable biotic environment for *L. natalensis*, the recognized snail intermediate host of *F. gigantica* in southern Africa. The prevalence of fasciolosis was relatively similar between abattoirs in the south and those in the north of the country. The findings of both juvenile and adult flukes in slaughtered cattle at abattoirs from August/September implied that the first metacercarial (infective) stages could have been ingested around May/June with transmission occurring thereafter. Studies from elsewhere have indicated that the pre-patent period for *F. gigantica* varies between 9 and 12 weeks in susceptible young animals, but may be longer in older or previously exposed animals (Soulsby, 1982; Radostits *et al*., 2007; Taylor *et al.*, 2007).

The prevalence of fasciolosis recorded from the coprological examination of live cattle in this study was higher (0.74%) than that recorded from the inspection of livers at abattoirs (0.03%). These results are in contrast to those reported from studies in Zambia (Phiri *et al*., 2005a), Zimbabwe (Pfukenyi *et al*., 2006) and Egypt (Kuchai *et al*., 2011). The difference could also be associated with the low sensitivity of liver examination at the abattoirs which is based only on the presence of gross pathological lesions (Mellau *et al.*, 2010). Furthermore, only healthy cattle are slaughtered, which would also result in underestimating the prevalence in the total population (Yilma and Mesfin, 2000; Mellau *et al*., 2010). Another possible limitation is the speed at which data has to be recorded in the abattoirs, which is directly related to the rate of slaughter and processing of the particular plant (Robertson and Blackmore, 1985). Some studies have reported that approximately one-third of infected livers are not detected in abattoir surveys (Rapsch *et al*., 2006).

In the current study, infected livers were detected in cattle processed at abattoirs in the southern part of the country. In contrast, no animals from this region were positive on coprological examination, implying that the positive animals at the abattoirs may have originated from the northern part of the country. The transportation of animals around the country, for example from FMD free zones in the north to the south, or from the Tuli Block ranches where infection appears to be prevalent, for slaughter at abattoirs in the south of the country, would result in the detection of *Fasciola*-infected livers in abattoirs located in the southern part of the country. Similar findings have been observed in Montana, USA, where the practice of shipping infected cattle around the state or from areas where the prevalence of liver flukes was highest to areas where suitable snail vectors were present provided good opportunities for the spread of the parasite (Knapp *et al.*, 1992). However, in this study, it was sometimes difficult to precisely trace the geographical origins of the cattle slaughtered and hence associate infection with a particular location. This highlights the need for a monitoring system to track animal movements which could be beneficial for diseases such as fasciolosis, and also assist with livestock trace back of diseases such as FMD.

Several epidemiological studies have highlighted that the prevalence of fasciolosis has a strong seasonal variation, with a higher prevalence in the wet season (when climatic conditions are more favourable) than the dry season. However, the present survey was not able to establish any distinct seasonal pattern associated with the prevalence of *F. gigantica*. The limitation associated with this could be attributed to the fact that the monthly visitation of farms and abattoirs that was planned at the beginning of the study became impractical due to logistical issues. The findings from studies in other countries of Africa have indicated a higher prevalence of fasciolosis during the rainy or post-rainy periods than in the dry season (Phiri *et al*., 2005b; Keyyu *et al*., 2006; Adedokun *et al*., 2008; Kuchai *et al*., 2011). In contrast, observations from Pakistan and Brazil by Khan *et al*., (2009) and Faria *et al*., (2005), respectively, found a higher prevalence during the dry season. However, others have reported the presence of bovine fasciolosis throughout the year (Amato *et al*., 1986; Morel and Mahato, 1987; Roy and Tandon, 1992; Rangel-Ruiz *et al*., 1999; Holland *et al*., 2000; Yilma and Mesfin, 2000; Maqbool *et al*., 2002; Keyyu *et al.*, 2005b; Phiri *et al*., 2005b; Mungube *et al*., 2006; Pfukenyi *et al*., 2006; Adedokun *et al*., 2008; Kuchai *et al*., 2011).

The mean intensity of infection or the epg was extremely low in this study. This result could be associated with acquired resistance due to frequent contact of the trematode with the animals, thereby enhancing the development of immunity which provides some protection (Bouvry and Rau, 1986; Munguía-Xóchihua *et al.*, 2007). Acquired resistance has been reported in cattle, goats and sheep (Spithill *et al*., 1999) but with considerable variation (Hurtrez-Boussès *et al*., 2001). These findings suggest that ruminants are capable of mounting immune responses that can kill *Fasciola* (Spithill *et* *al*., 1999) with cattle showing higher ability to develop acquired immunological resistance than small ruminants (Hillyer *et al*., 1996; Hurtrez-Boussès *et al*., 2001). Although the results from the current study have indicated a low prevalence of infection with *F. gigantica* infection in cattle, with the disease being localized only to the Tuli Block ranches in Machaneng village of the Central district, the disease could still be present in other districts of Botswana which were not sampled in the current study. Similarly, there may have been insufficient samples collected from some districts in this study to detect infection.

Therefore, detailed epidemiological surveillance studies, involving more cattle and areas, are required to confirm the accurate and definite prevalence and distribution of the disease. Additionally, monthly visits would have to be carried out to determine if any seasonal variation exists. Such data would assist to design appropriate control programmes for the farmers, since in order to accomplish an effective flukicide treatment, a properly timed strategic programme has to be implemented. Also, the design of future approaches for the control of bovine fasciolosis in Botswana would require a good understanding of the epidemiology of this trematode disease. In order to ascertain whether bovine fasciolosis is economically important in Botswana, a gross pathological examination of the livers to determine the extent of damage and a comparison of fluke numbers in each liver was carried out and these findings are presented in the following chapter.

CHAPTER FIVE

5 Hepatic pathology, trematode burden and economic significance of fasciolosis in slaughtered cattle in Botswana

5.1 Introduction

Fasciolosis, caused by *F. gigantica*, is a major constraint to ruminant production in tropical countries, causing extensive pathological changes due to the migratory habits of the flukes through the liver parenchyma (Mungube *et al*., 2006; Phiri *et al*., 2007c). Some of the significant pathological alterations include hepatic fibrosis, thickening and calcification of the bile ducts (Phiri *et al*., 2007c) which compromises liver function (Gajewska *et al*., 2005). These changes result in condemnation of affected livers at meat inspection.

Infections with these trematodes reduce productivity resulting in serious losses in domestic ruminants (Vercruysse and Claerebout, 2001; Mekroud *et al*., 2006; Adedokun *et al*., 2008). However, the estimated cost of infection with *F. hepatica* and *F. gigantica* could vary slightly due to differences in the farming purposes. In most areas where *F. hepatica* is endemic, animals are kept primarily for profit and measured in terms of output of meat, milk and reproductive efficiency whereas in areas where *F. gigantica* is endemic, animals are kept mainly for reasons other than profit, such as giving status to owners, production of dung for fertilizer, draught power and are sold only when money is needed for special or unforeseen events. As a result, the costs and derived benefits from parasite control are likely to be calculated differently (Spithill *et al*., 1999). Although *Fasciola*-infected cattle rarely display signs of clinical disease, subclinical infections are recognized as the cause of economically important reductions in animal productivity (Kaplan, 2001).

The economic significance of fasciolosis is, therefore, mainly due to direct losses from increased condemnations of infected livers during meat inspection at abattoirs (McKown and Ridley, 1995; Kaplan, 2001; Gajewska *et al*., 2005) and indirect losses caused by lowered reproductive performance, reduced calf weaning weights, reduced growth rate and weight gains, increased susceptibility to infections, morbidity, mortality and expenses of control measures (McKown and Ridley, 1995; Malone *et al*., 1998; Kaplan, 2001; Gajewska *et al.*, 2005; Abunna *et al*., 2010). Generally, bovine fasciolosis can be considered a subclinical disease (Kaplan, 2001) However, in situations where cattle become clinically sick as a result of fluke infection, the economic ramifications are quite obvious (Kaplan, 2001).

A number of abattoir studies have been conducted on tropical fasciolosis in cattle from sub-Saharan African countries (Ogunrinade and Ogunrinade, 1980; Tembely *et al*., 1988; Kambarage *et al*., 1995; Kithuka *et al.*, 2002; Mungube *et al*., 2006; Berhe *et al*., 2009; Mwabonimana *et al*., 2009; Nonga *et al*., 2009; Swai and Ulicky, 2009; Abebe *et al*., 2010; Abunna *et al*., 2010; Mellau *et al.*, 2010) as a relatively inexpensive method of collecting information on the prevalence and economic significance of fasciolosis in various ruminant production systems (Roberts and Suhardono, 1996; Pfukenyi and

Mukaratirwa, 2004; Sothoeun *et al*., 2006). However, only a few such studies have been carried out in southern Africa (Mzembe and Chaudhry, 1981; Pfukenyi and Mukaratirwa, 2004; Phiri *et al*., 2005a) and none have been conducted in Botswana.

Estimation of the economic losses due to fasciolosis in developing countries is limited by lack of accurate information on the prevalence of the disease (Phiri *et al*., 2005a; Mwabonimana *et al*., 2009). Even though it has been shown that fasciolosis is not a major cause of liver condemnation in Botswana (Chapters 3 and 4) direct losses associated with the rejection of bovine livers at meat inspection could still affect farmers, butchers and consumers. However, currently there has been no such evaluation on the socio-economic losses resulting from condemned livers in the country. Therefore, the present study was designed to determine the gross pathological lesions induced on the hepatic tissue and estimate the direct losses associated with bovine liver condemnations at selected abattoirs in Botswana.

5.2 Material and methods

5.2.1 Data collection

The study involved a retrospective survey which entailed the retrieval and analysis of meat inspection data from two major abattoirs in Botswana, covering a ten-year period, from 2001 to 2010. Records of monthly and annual returns from the abattoirs were scrutinized with regard to the number of cattle slaughtered and the corresponding number of livers condemned as a result of infection with *F. gigantica*. The data allowed estimation of the baseline prevalence of fasciolosis in cattle in Botswana (Chapter 3). In addition to the retrospective study, in order to assess the significance of *F. gigantica* infection in cattle, a two-year prospective abattoir survey was undertaken from January 2011 to December 2012. These prospectively acquired data were used to validate the retrospective study, and were obtained by regularly visiting the two main abattoirs and four selected local council abattoirs in Botswana, to inspect cattle livers for evidence of fasciolosis (details covered in Chapter 4).

5.2.2 Liver inspection

All the infected livers and gall bladders were thoroughly examined for pathological abnormalities. Livers exhibiting gross pathological changes were cleaned with normal saline. All infected livers were weighed immediately on collection after the animal was slaughtered. A comparison between the severity of liver lesions and the intensity of fluke infection (burden) was carried out on infected livers. The infected livers were classified into three categories based on the severity of liver lesions detected as per the criteria previously described by Ogunrinade and Adegoke (1982) as follows:

Lightly affected: a quarter of the liver was affected, and only one bile duct was prominently enlarged on the visceral surface of the organ

Moderately affected: half of the liver was affected with two or more bile ducts enlarged

Severely affected: almost all the entire liver was affected which was cirrhotic and triangular in outline with the right lobe atrophied

5.2.3 Data analysis

Data obtained for the prevalence of bovine fasciolosis were entered, validated and calculated in a Microsoft Excel 2007 (for Windows) spreadsheet. The retrospective and prospective data were analysed using IBM SPSS 21.0.

The prevalence of the disease was determined as described in previous chapters. The mean infection intensity from different abattoirs was compared using the *Chi* square test for independence and the associated severity of infection was compared with the Kruskal-Wallis H test and the Games-Howell *post hoc* test to compare the three categories of infection because of unequal variances of such infections.

5.2.4 Economic assessment

The total economic loss due to fasciolosis, in slaughtered cattle, presented in this study was derived from a summation of the annual liver condemnation losses (that is, direct losses), using both the prospective and retrospective data obtained from the selected six abattoirs, during the period from 2001 to 2012 (12 years).

Several parameters were used to estimate the economic significance attributable to liver condemnations due to fasciolosis. These included the total number of animals slaughtered, the total number of livers condemned due to fluke pathological lesions per year, the average weight of the liver in a mature animal, in kilograms (kg) and the average selling price of a bovine liver (price/kg), calculated on an annual basis. The average selling price of the bovine liver was established from the abattoirs and butcheries. Information on the exchange rate for the Botswana Pula (BWP) to the Australian Dollar (AUD) was obtained from the Bank of Botswana (BOB).

The information obtained on losses accrued as a result of liver condemnations due to bovine fasciolosis was computed with Microsoft Excel 2007, in accordance with a mathematical formula set earlier by Ogunrinade and Adegoke (1982) but with the following modifications:

ALC = CSR * LP * LM

Where:

ALC denotes the annual loss from liver condemnation

CSR represents the total annual number of livers condemned at various abattoirs

LP represents the average selling price of a bovine liver

LM represents the average liver mass of a mature bovine

The average bovine liver was estimated to have a mass of 4.5 kg, and the average price per kilogram was determined to be approximately AUD5.00 (BWP35.00). At the time of writing this thesis, the Australian dollar was almost on a par with the United States of America dollar.

5.3 Results

5.3.1 Intensity of infection and pathological lesions of affected livers

A total of 2, 376 flukes were recovered from the 78 infected and condemned livers (Chapter 4), with an overall mean fluke count of 30.46 flukes from infected livers. The range of flukes per liver varied from 3 to 213.

Most infected livers had few flukes in them (≤ 20) with 41 of the 78 (52.56%) livers showing light infection intensity, followed by moderate infection intensity (> 25 ≤ 50) with 26 (33.33%) livers, and only 11 (14.1%) livers showed a severe intensity of infection (> 50 flukes). The variation in fluke count between the three levels of infection was significant ($p < 0.003$). However, the results indicated that there was no direct relationship between the fluke burden and the severity of pathological lesions observed since the mean fluke number of flukes recovered from the severely affected livers was lower than the mean fluke number recovered from the moderately affected livers (Figure 5.1). The moderately affected livers showed the highest mean rank fluke count of 73 whereas the lightly affected livers presented the lowest count at 21 (Figures 5.2 and 5.3). The severely affected livers had a mean count of 54.5 livers flukes per liver (Figure 5.4). The variation in the level of infection between the three lesion categories was statistically significant (χ^2 (2) = 62.86, $p < 0.001$), with most animals exhibiting light infections. The weight of the liver was higher in infected (> 5 to 8 kg) than in non-infected animals (3 to 5 kg).

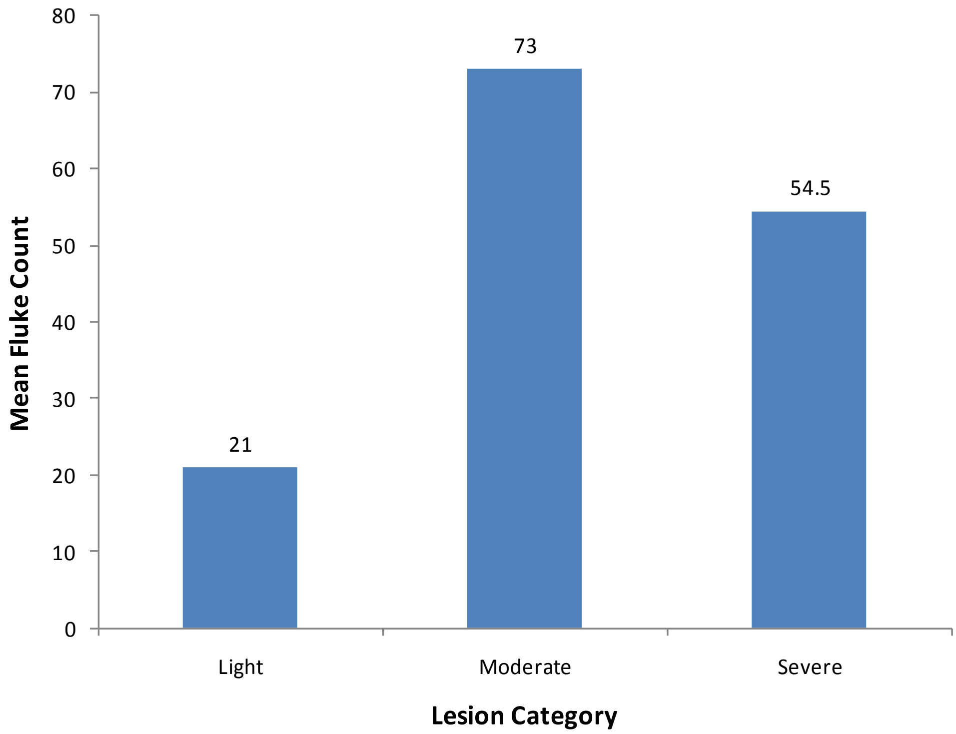


Figure 5.1 Mean fluke intensity of infection of affected livers

Table 5.1 Classification of gross pathological lesions of infected livers with their corresponding mean fluke burden

Pathological category	No of livers affected	Percentage (95% CI)	Mean fluke burden
Lightly affected	41	52.56 (40.93, 63.99)	21.00[a]
Moderately affected	11	14.10 (7.26, 23.83)	73.00[c]
Severely affected	26	33.33 (23.06, 44.92)	54.50[b]
Total	78	100	30.46

[a,b,c] Values with different superscripts in a column are significantly different ($p < 0.003$)

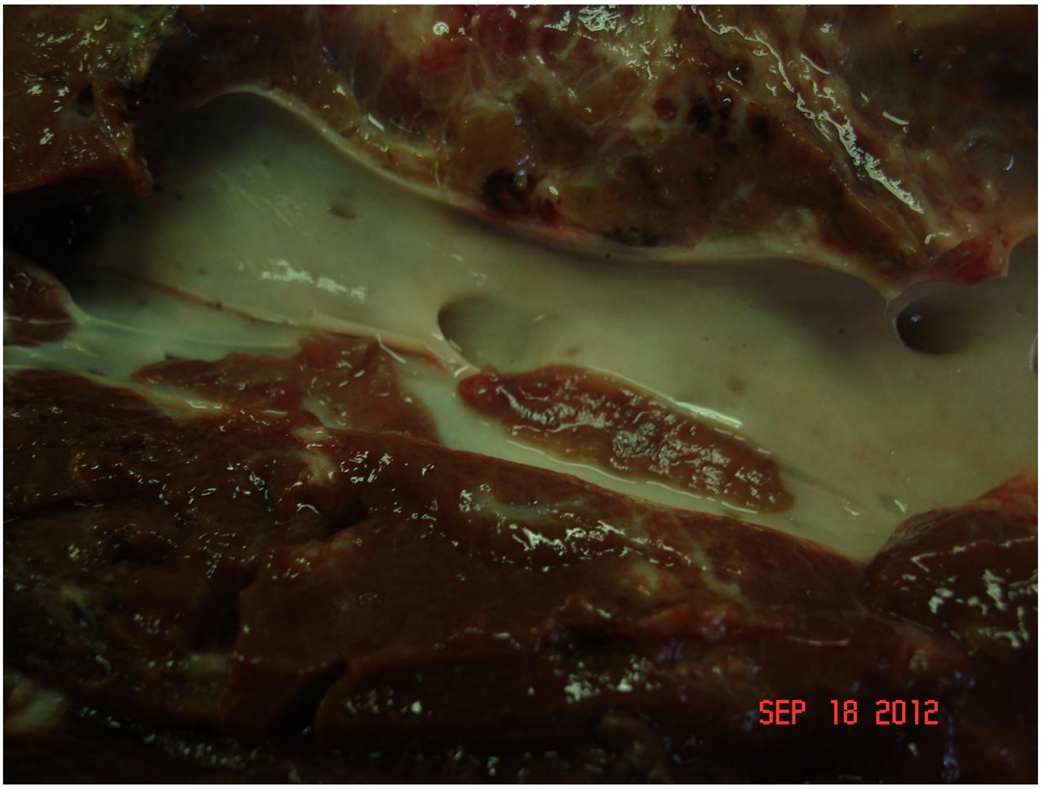


Figure 5.2 Light infection of the bovine liver. Note the presence of flukes in rather smooth lumen of the normal bile duct

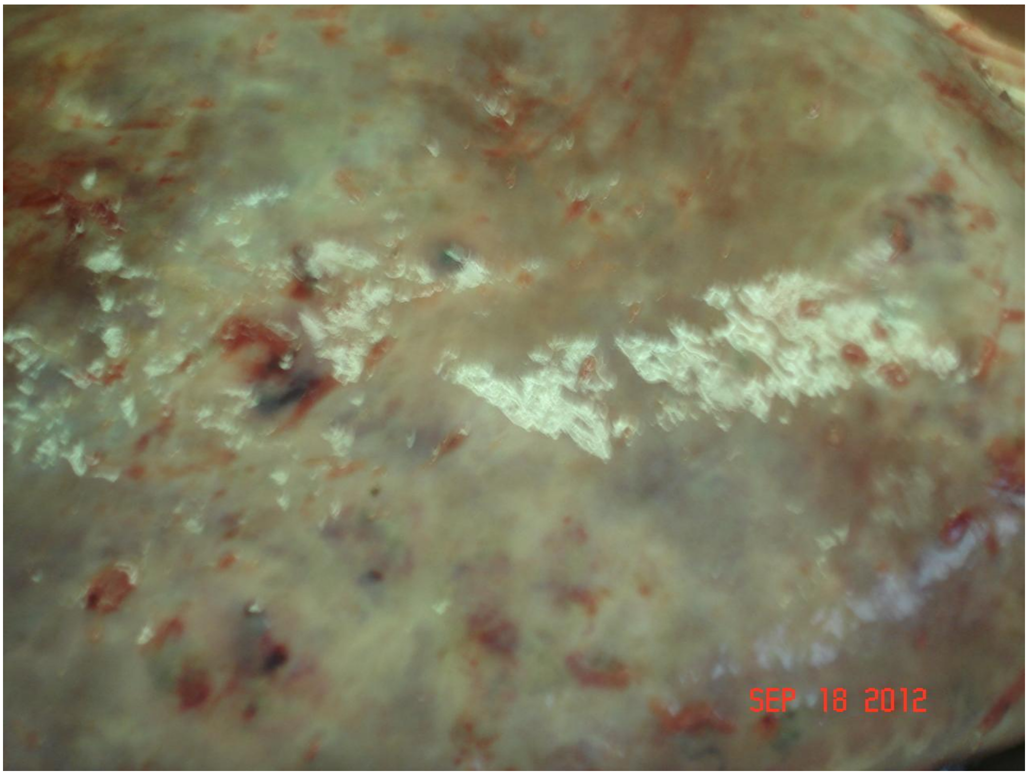


Figure 5.3 Typical moderate gross pathology (haemorrhaging) associated with fluke-infected bovine livers

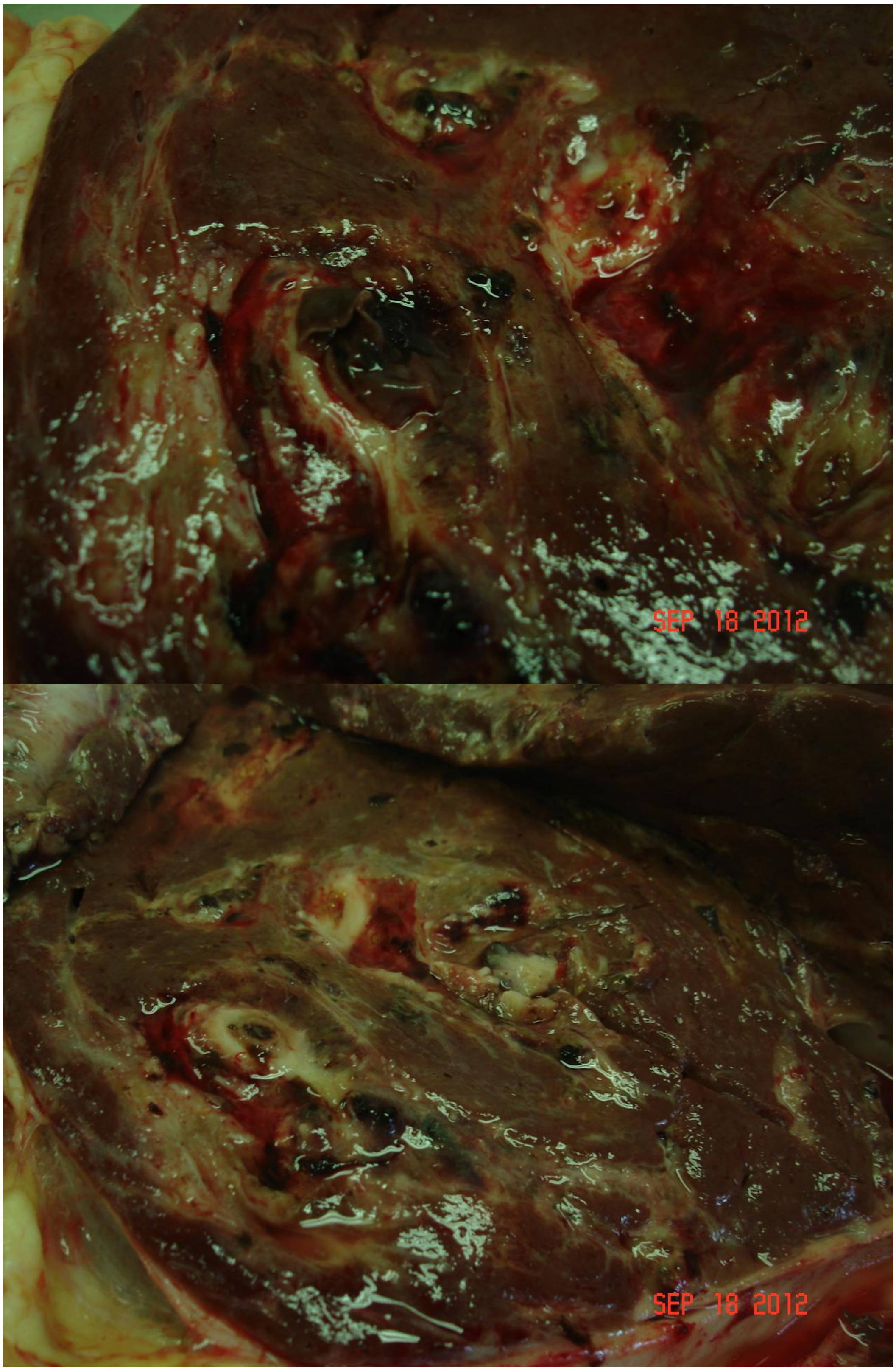


Figure 5.4 Severely infected liver. Note the severe parenchymal fibrosis and haemorrhage, and grossly thickened, dilated and calcified bile ducts due to *F. gigantica*. Ducts contain a dark tarry substance, probably deposited by flukes. Liver flukes are quite discernible from incised bile ducts.

Table 5.2 Annual number of cattle slaughtered at two main and four council abattoirs, livers condemned due to *F. gigantica* infections and estimated economic loss during the period from 2001 to 2012 in Botswana

Year	Number of cattle slaughtered	Number infected with *F. gigantica*	Total weight of condemned livers(kg)	Economic loss (AUD)
2001	163,666	16	72.0	360.00
2002	113,564	17	76.5	382.50
2003	153,496	0	0.0	0.00
2004	129,720	0	0.0	0.00
2005	118,200	17	76.5	382.50
2006	137,354	376	1,692.0	8,460.00
2007	171,229	773	3,478.5	17,392.50
2008	113,292	35	157.5	787.50
2009	135,286	13	58.5	292.50
2010	158,914	3	13.5	67.50
2011	113,153	55	247.5	1,237.50
2012	130,554	19	85.5	427.50
Total	1,638,728	1,324	5,958.0	29,790.00

As reported earlier in Chapter 4, all infected and condemned livers in this study were due to infection with *F. gigantica.*

5.3.2 Economic assessment

The summary of the direct losses resulting from liver condemnations in abattoirs across the country is given in Table 5.2. A total of 1,638,728 cattle were slaughtered at the six selected abattoirs and only 1,324 livers (0.08%), with a total mass of 5,958 kg, were condemned due to fasciolosis during the period from 2001 to 2012. This amounted to a total national economic loss of approximately AUD29,790 (BWP208,530) during the 12 years or an average of AUD2,483 (BWP18,622), annually.

The total number of cattle slaughtered varied from year to year, but the differences were not significant. Similarly, the number of infected and condemned livers, and correspondingly the annual economic loss, did not vary greatly, with the exception of the year 2007 when 773 livers were condemned compared to an annual average of 110 livers (AUD2,483), and as a result a considerable financial loss of AUD17,393 was incurred in that year. The year 2006 was also of some significance with a total loss of AUD8,460. The lowest economic loss occurred in 2003 and 2004, when no condemnations were reported, and in 2010, with only three livers condemned (AUD67).

5.4 Discussion

In this study infected livers had raised surfaces, irregular outlines and some of them were firm in consistency. Rahko (1969) and Jones *et al*. (2006) reported that livers infected with *Fasciola* spp. had uneven surfaces irregular outlines, a pale colour and felt firm, had evidence of papillary and glandular hyperplasia and there were erosions of the biliary epithelium. These lesions are associated with the migration of juvenile flukes through the hepatic parenchyma and the presence of mature flukes in the biliary tract (Jones *et al*., 2006; McGavin and Zachary, 2007; Radostits *et al*., 2007). The flukes digest the hepatic tissue, thereby causing considerable parenchymal destruction with intensive haemorrhagic lesions (Gajewska *et al.*, 2005).

The results also indicated that the severity of pathological lesions was not directly related to the number of flukes, with fluke counts in moderately affected livers being significantly higher than those recovered from severely affected livers. These observations were consistent with the findings of Yilma and Mesfin (2000) in Ethiopia, who reported that fluke counts in moderately affected livers exceeded those found in more severely affected bovine livers. The possible explanation for this is that severe fibrosis impedes the passage of young flukes and the acquired resistance associated with infection culminates in the expulsion of adult flukes from the bile ducts (Yilma and Mesfin, 2000; Mungube *et al*., 2006).

Earlier studies reported that the presence of a mean fluke count of more than 50 flukes per liver was an indication of high pathogenicity (Soulsby, 1982) and that a mean fluke count of approximately 171 flukes per liver would lead to development of clinical disease (Bliss, Unpublished). In the present study, the majority of infected livers had light infections, exhibiting few flukes (mean 21 flukes) with a mean overall infection intensity of 30.46 flukes, which implied moderate pathogenicity. These results were lower than those reported from recent studies by Yilma and Mesfin (2000) and Sothoeun *et al*., (2006) who found mean trematode burdens of 66.23 and 39.9 flukes, respectively. The lowered infection intensity in Botswana could be attributable to the conditions that are unfavourable for the survival of the intermediate hosts and thus maintenance of infection by larval stages of the flukes. Consequently, there are few metacercariae on pastures ingested by cattle.

Gross hepatic destruction, such as parenchymal fibrosis and haemorrhaging, were also noticeable in this study. Generally, the walls of the bile ducts were strikingly thickened, calcified and moderately dilated, with stenotic lumina present in a few livers. Similar findings have been reported previously in various countries (Yadav *et al*., 1999; Kaplan, 2001; Gajewska *et al*., 2005; Phiri *et al*., 2006b). The lesions were more pronounced in the severely affected livers, and were indicative of chronic cholangio-hepatitis, as a result of prolonged infection, which is a common finding in cattle (Rahko, 1969; Jones *et al*., 2006; Kusiluka and Kambarage, 2006). The cut surface of the liver showed numerous flukes, which were quite conspicuous, protruding from the bile ducts. The exudation of a dark tarry substance by the bile ducts, most probably deposited by the flukes, was quite discernible. Occasionally, there was evidence of rasping sounds on incision of the affected livers, which was a sign of mineralization of the bile ducts. Similarly, Yadav *et al*., (1999) also observed grating sounds when slicing livers of buffaloes infected with *F. gigantica*, and earlier research detected flakes of gritty material from the affected bile ducts of cattle in Finland (Rahko, 1969).

The present study has also revealed, for the first time, the presence and extent of infection of *F. gigantica* in Botswana. The current findings are also further evidence that, whereas the only species of *Fasciola* recognized in Australia, the Americas and Europe is *F. hepatica*, the distribution of *F. hepatica* and *F. gigantica* overlaps in most areas of Africa (and Asia), and that in Botswana the only species present appears to be *F. gigantica*. The livers of infected animals weighed more than those of non-infected, and this result is in agreement with the findings of Molina *et al*.,(2005b) from a similar study of cattle and buffaloes in the Philippines. These authors suggested that the increased weight of the infected liver might be attributable to the necrotic and calcified lesions due to compensatory hypertrophy of the liver parenchyma caused by infection with *F. gigantica*. This explanation was also supported in a previous study by Rahko (1969) who described extensive calcification in the bile ducts as a counter-balancing mechanism against prolonged survival of mature *F. hepatica*.

The migrating juvenile flukes inflict mechanical destruction to the liver leading to extensive toxic damage of the hepatic tissue (Rahko, 1969; Kaplan, 2001; Gajewska *et al*., 2005). Abrasions caused by spines and the prehensile action of the suckers appear to account for the majority of the damage induced in the liver (Behm and Sangster, 1999). In fact, there is evidence that macerated hepatic cells have been observed inside the oral sucker and pharynx of flukes (Gajewska *et al*., 2005). In most cases, death of the definitive host is a consequence of the haemorrhage caused by this damage and the widespread hepatic necrosis (Behm and Sangster, 1999; McGavin and Zachary, 2007).

Bovine fasciolosis is generally considered a chronic disease, with the hepatic parenchymal tissue able to resolve the damage, heal and regain normal function. Nevertheless, extensive infection by the parasite resulting in extensive hepatic damage compromises the function of the liver, which is reflected in alterations of plasma protein concentration (Gajewska *et al*., 2005; Jones *et al*., 2006). Sometimes blood loss to the intestines may be so extensive that the synthetic capacity of the liver may not be sufficient to restore the lost albumin, more especially in field infections, where incoming metacercariae induce further damage to the liver, and thereby compromising its function further (Behm and Sangster, 1999). There is also some evidence that fluke-infected hosts have significant disturbances to the liver even when only small areas of the organ are overtly damaged (Behm and Sangster, 1999).

Food animals, such as cattle, are beneficial as a source of quality protein and revenue to humans (Mellau *et al.*, 2010) but diseases of such animals, including fasciolosis, may lead to poor returns. The economic impact of bovine fasciolosis resulting from direct losses, such as liver condemnation at slaughter, is relatively easy to measure whereas the indirect losses, such as slow growth rate and lowered reproductive efficiency, can be a challenge to quantify, even though they are deemed to be far more economically important (Kaplan, 2001). The present study estimated the economic repercussions of bovine fasciolosis based only on direct losses from liver condemnations at abattoirs, and it was the first economic assessment of the disease in Botswana.

The total loss was estimated to be only AUD29,790 or an average of only AUD2,483 annually. These findings showed that only modest financial losses occurred in Botswana during the period from 2001 to 2012 as a result of condemnation of *Fasciola*-infected livers. The loss is much lower and not comparable to losses from similar studies in other countries in Africa. In Kenya, Kithuka *et al*., (2002) reported a loss of USD2.6 million while Mungube *et al*., (2006) reported a loss of USD72,272 for similar study duration (10 and 16 years, respectively) from similar numbers of cattle slaughtered. Mwabonimana *et al*.,(2009) estimated an annual loss of USD18,000 in Tanzania, but from only a very small number of cattle (4,329 animals). Estimates of annual losses from Ethiopia were also relatively higher, with losses ranging from USD2,245 to USD8,313 with cattle numbers ranging from 406 to 1,000 animals (Abebe *et al*., 2010; Abunna *et al*., 2010; Equar and Gashaw, 2012) compared to the annual average of 136,560 cattle used in this study. Therefore, it is quite evident that the economic losses in Tanzania and Ethiopia would be much higher with an equivalent number of cattle to that used in the present study.

The low financial losses realized from the present study is not surprising since the prevalence from both the field and abattoir surveys were extremely low, and the distribution was localized when compared to other African countries. These findings make bovine fasciolosis of lower importance economically than other livestock diseases in Botswana. However, the economic loss might only be of minor importance when considered on a national scale, but still could be significant on individual farms, particularly in the local Tuli Block ranches, where commercial farming is practiced. In fact, all the condemned livers at the MSA abattoir in Gaborone and the majority of livers at Francistown abattoir were from cattle originating from these commercial ranches in the Tuli Block area.

Estimates of losses as a result of fasciolosis have often been restricted to direct losses such as the value of livers condemned at slaughter as unfit for human consumption (Morel and Mahato, 1987; Spithill *et al.*, 1999; Kithuka *et al*., 2002; Mungube *et al*., 2006; Berhe *et al.*, 2009; Mwabonimana *et al.*, 2009) or on the value of meat lost through lowered carcass weights of infected animals (Harrison *et al*., 1996; Molina *et al.*, 2005b; Swai and Ulicky, 2009). However, this parasitic disease may result in significant indirect losses such as loss of body condition, slow growth rate and reduced reproductive efficiency, which affect the economy of the livestock industry (Saleha, 1991; Kaplan, 2001). Nevertheless, monitoring at abattoirs is valuable as it has high accuracy and precision resulting in low error rates (Herenda *et al*., 2000; Mellau *et al*., 2010).

The direct and indirect losses from fasciolosis can be significant. Annual losses were estimated at USD107 million in Indonesia and USD37 million in Nepal (Spithill *et al*., 1999; Copeman and Copland, 2008). In Europe, estimated average annual losses were between USD34 million and USD72 million (Bennett and Ijpelaar, 2003; Schweizer *et al*., 2005; McKay, 2007). In the USA, the average losses to the beef industry in the states of Florida and Kansas were estimated at USD10 million and 300,000, respectively (McKown and Ridley, 1995; Irsik *et al.*, 2007). These figures were much higher than those estimated in the current study. The present findings are indicative that bovine fasciolosis is a minor cause of liver condemnation with extremely low financial losses in Botswana. This is in contrast to other African countries where fasciolosis remains the most common disease condition encountered in slaughter houses and the leading cause of liver condemnation resulting in significant losses (Kithuka *et al*., 2002; Mellau *et al*., 2010; Raji *et al*., 2010).

Human infection with *F. gigantica* is believed to be underestimated in tropical countries through a lack of thorough investigations (Hammond, 1973) and the disease has recently been recognized as a re-emerging and widespread zoonosis (Esteban *et al.*, 2003; Mas-Coma *et al*., 2007). Research on the presence and significance of the disease in humans, more especially from humans residing close to wetlands, is warranted (Phiri *et al*., 2005a). Accordingly, it is essential to carry out surveillance studies in the Okavango swamps in the North-west district of Botswana, in order to monitor the disease throughout the country and in particular in areas where the prevalence is likely to be higher.

In Botswana, beef production is of primary importance and thus the beef industry is significant, however, subclinical diseases, including parasitosis, are likely to be limiting production. Such subclinical diseases, including fasciolosis, require further investigation as they are often under-diagnosed and not recognized by farmers. Implementation of an educational campaign on the role of fasciolosis and other parasitosis on livestock production is important, especially in the Tuli Block area.
The present study has highlighted the extent of *F. gigantica* infection on the livers of cattle, and the associated economic losses in the country. In spite of the low prevalence and negligible economic importance of bovine fasciolosis, it is clear that the

disease is endemic in the Tuli Block ranches in the Central district, and further investigation into the disease in that area is warranted as is formulation of better control measures. The implementation of proper control methods requires knowledge of the distribution of the snail intermediate host, which will be covered in the next chapter.

CHAPTER SIX

6 Population dynamics and biogeography of *Lymnaea natalensis* and its natural infection by *Fasciola gigantica* in Botswana

6.1 Introduction

Lymnaeid snails, the intermediate hosts of liver fluke, play a vital role in the epidemiology of fasciolosis. The snail, *L. natalensis*, is known to be the main and habitual intermediate host of *F. gigantica* (Brown and Kristensen, 1989; Dar *et al.*, 2004) the cause of fasciolosis in tropical countries, including Africa and Asia. Previous studies have shown that *L. natalensis* is the most abundant and widely distributed fresh water snail in Africa (Frandsen and Christensen, 1984; Brown and Kristensen, 1989; Moema *et al.*, 2008). It occurs mostly in permanent waters, and is present mainly in the eastern part of southern Africa (Brown and Kristensen, 1989) where the prevalence of fasciolosis is expected to be high since it is related to the availability of the snail intermediate host and the density of the definitive host animals.

The occurrence of *Lymnaeid* snails depends on the presence of suitable food and the stability of the habitat. Suitable habitats include springs, streams, rivers, natural and man-made dams, irrigation channels and swamps whereas lakes do not usually provide good habitats for snails (Boray, 1964). The presence of aquatic vegetation also provides a good habitat for the snail (Boray, 1964; Ndifon and Ukoli, 1989). Therefore transmission patterns of *F. gigantica* are dependent, to a large extent, on the availability of appropriate habitats for the proliferation of the intermediate host snail and the larval (intra-molluscan) stages of the parasite (Rapsch *et al.*, 2008).

Large wetland areas found in and around lakes, marshlands or swamps, lagoons, ponds and canals, accompanied by high temperatures, provide suitable conditions for the existence and breeding of snails of veterinary and medical importance (Phiri *et al*., 2007b). *Lymnaea natalensis* has a preference for minimal shade in its habitats (Ndifon and Ukoli, 1989). The seasonal or sometimes daily hydrodynamics of rivers often create more favourable conditions for communities of freshwater pulmonates which live on river banks, and when the rivers do not flow into dams, snail habitats become scarce and populations of snails decline (Hourdin *et al*., 2006). *Lymnaea natalensis* is likely to be less frequent or have low diversity in habitats which frequently dry up because of its preference for permanent water bodies and intolerance to desiccation (Ndifon and Ukoli, 1989). Thus the snail is likely to be abundant during the rainy season or at the end of the rainy season and scarce during the dry season or during drought years and in areas with transitory water sources.

The data presented in this paper were collected over a period of 24 months and in geographic locations across the country, covering six districts in the eastern one third of the country. As no previous studies had been conducted on the occurrence and geographical distribution patterns of *L. natalensis,* the objective of the present study was to determine the distribution of the snail intermediate host of *F. gigantica* in various cattle raising areas in Botswana.

6.2 Materials and methods

6.2.1 Study location

The study location was the same as those described in Chapter 4

6.2.2 Snail studies

In each of the study districts, both grazing sites and drinking sources were identified, which represented potential habitats of the snail intermediate hosts (Appendices 2 and 3). The sites included swampy or marshland areas, lakes, dams, rivers and streams within the grazing areas. Every attempt was made to examine as many freshwater bodies as possible throughout the areas surveyed, and each grazing site identified was searched and sampled for potential habitats of *L. natalensis* snails on a regular basis from June 2011 to May 2013, by using a method described by Boray *et al*. (1985) which involved locating and accessing all snail habitats in the area. Snails were then collected either by sieving the surface mud and aquatic vegetation with metal kitchen sieves or strainers (Boray *et al*., 1985) or from the water with a metal kitchen strainer (1 mm) or hand picked off aquatic plants with gloved hands (Coelho and Lima, 2003; Phiri *et al*., 2007b).

Each drinking source was sampled for snails using the scooping method (Coulibaly and Masden, 1990) whereby a scoop made from a kitchen sieve was supported by an iron frame and mounted on a one and half metre long handle. The collection at each site was carried out by one or two people during a period of one hour or 30 minutes of investigation, respectively. Snails were then placed in wet gauze strips as described by Coelho and Lima (2003) or transferred into jars with perforated lids containing aquatic vegetation in a small amount of water as described by Boray *et al.* (1985) and transported live to the laboratory.

The snails were counted and each snail collected was identified and measured according to the identification key described for African freshwater snails (Mandahl-Barth, 1962; Brown and Kristensen, 1989). Measurements were taken of the length and shell height of each snail collected, and the snails were categorized into two groups, namely small (4-10 mm in length and 2-5 mm shell height) and large (>10 mm in length and >5 mm shell height) (Pfukenyi *et al*., 2006). The sampling method collected only snails with a minimum length of 4 mm and a minimum shell height of 2 mm. All snails sampled at each site were dissected under a stereoscope at a magnification of 40X to detect patent infections of *F. gigantica* larval stages.

The harvesting of cercariae was by natural emergence whereby groups of snails (20 snails per 200 ml) were placed in containers with river water as described by Frandsen and Christensen (1984) or snails were placed individually in small (12.5 ml) plastic beakers with river/dam water (Coulibaly and Masden, 1990) The snails were then exposed to indirect sunlight to stimulate the natural shedding of cercariae (Moema *et al.*, 2008) or artificial electrical light for a period of approximately 2 hours after which the presence of cercariae was checked under a dissecting microscope as described by Coulibaly and Masden(1990). If negative, the snails were then crushed between slides and examined for the presence of *Fasciola* larvae (Frandsen and Christensen, 1984; Boray *et al*., 1985). Harvested cercarial stages were then identified using the identification and naming procedure of African freshwater snails as described by

Frandsen and Christensen (1984). The percentage of snails infected with *F. gigantica* cercariae was calculated for each site.

6.2.3 Aquatic vegetation

Aquatic vegetation and other grass samples from the drinking and grazing areas, which serve as snail habitats, were recorded on a regular basis. Some plants were collected and examined for the presence or absence of *F. gigantica* metacercariae. Water and soil samples were, however, not obtained nor subjected to the chemical analyses, during the 24 month study period because of the absence of metacercariae in the sampled vegetation.

6.2.4 Meteorological data

The mean monthly temperatures and mean monthly rainfall data were obtained for the weather bureau station nearest to each study site. These measurements were obtained from the recordings maintained by the DMS in Gaborone.

6.2.5 Data analysis

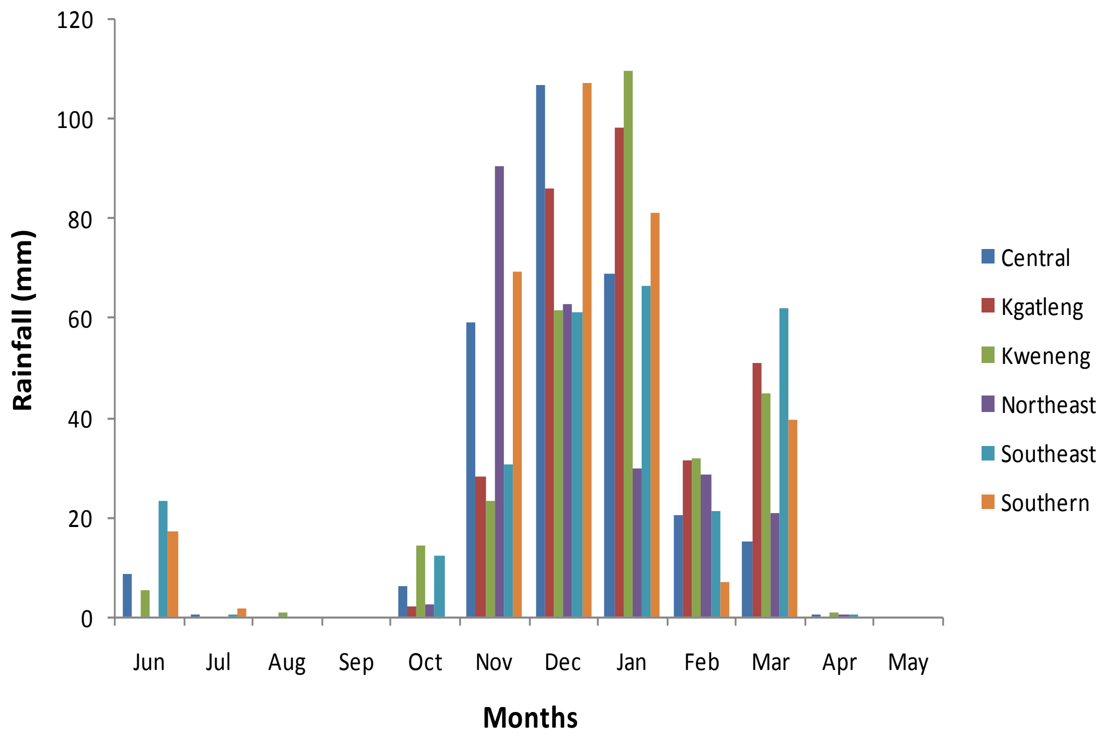
The effect of geographical location, type of habitat and season on snail counts and the relationship between snail density and climatic factors (rainfall and temperature) were not analysed because of the complete absence of snails in nearly all of the study sites. Also, the infection rates of snails with flukes could not be carried out because the snails recovered were too small.

6.3 Results

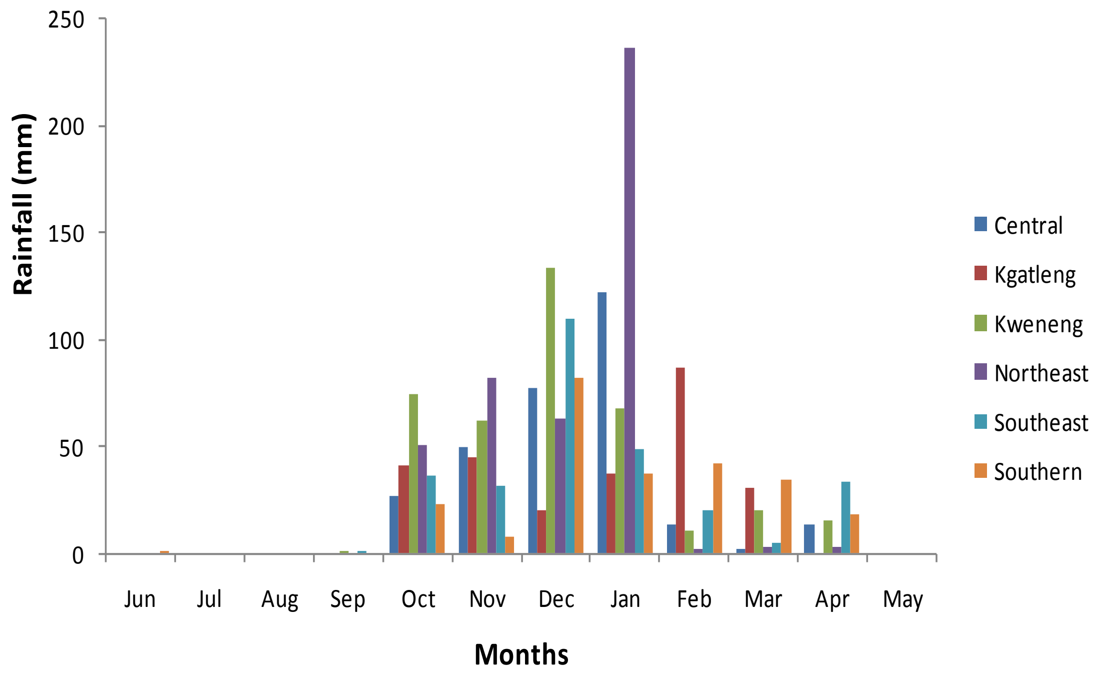
A total of 37 potential habitats were sampled on livestock farms during the study period from six districts. No lymnaeid snails were detected from any of the study habitats, including the Tuli Block area in the Central district, where fasciolosis had been previously detected (Chapter 4). However, 13 small *Lymnaea*-like snails were found along the banks of the Notwane River at Ramotswa village in the South-east district (Table 6.1).

In all study sites, cattle kraals were located near grazing areas and water sources and cattle dung was deposited in all habitats. There was evidence of human contact in most habitats, more especially in sites used for watering livestock and fishing. There were empty snail shells observed at water sources in northern Botswana, including on the banks of the Limpopo, Motloutse and Shashe rivers, in the Tuli Block area, Mmadinare and Tonota villages, respectively. Snail shells were also seen at Letsibogo dam in Mmadinare village and Shashe dam in Tonota village. The 13 snails collected from the South-east district could not be identified definitively because they were immature (Mzembe and Chaudhry, 1979; Pfukenyi *et al*., 2006).

The variation in mean precipitation and temperature were recorded during the study as indicated in Figures 6.1 (*a* and *b*) and 6.2 (*a* and *b*), respectively. The mean annual rainfall varied from a minimum of 0 mm in May to a maximum of approximately 80 mm in December, during 2011/2012, and a minimum of 0 mm in May to a maximum of 90 mm in January, during the 2012/2013 study period.

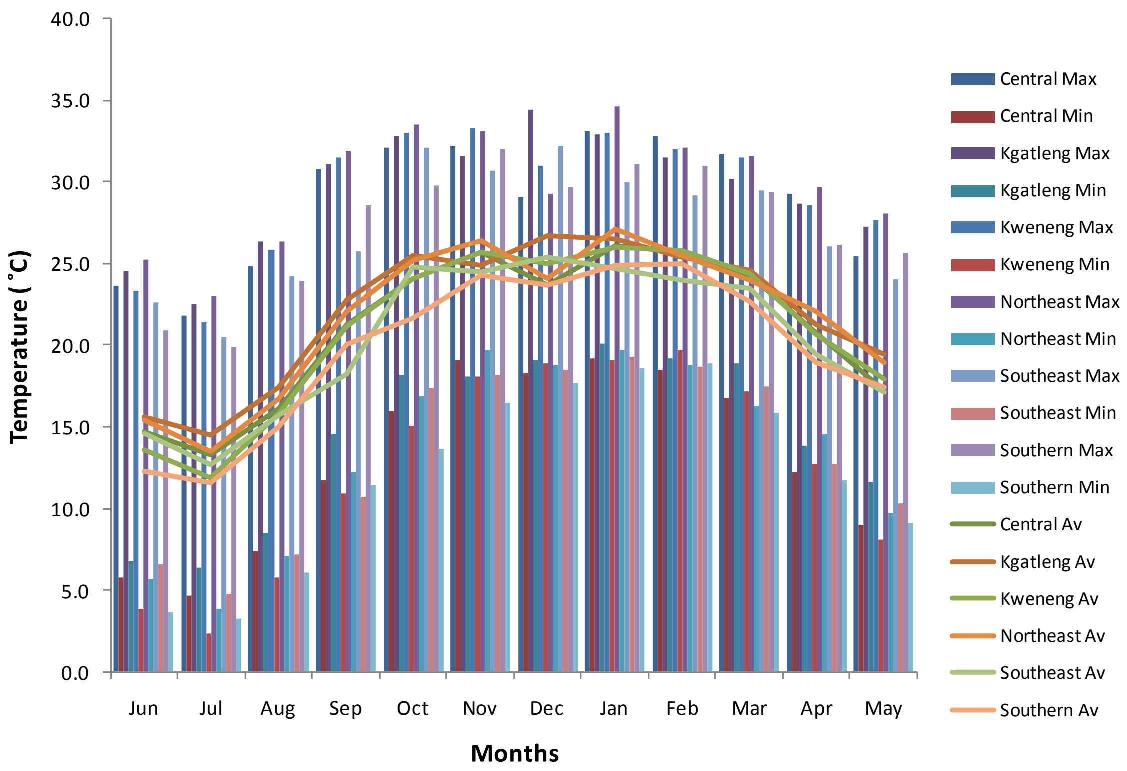


a)

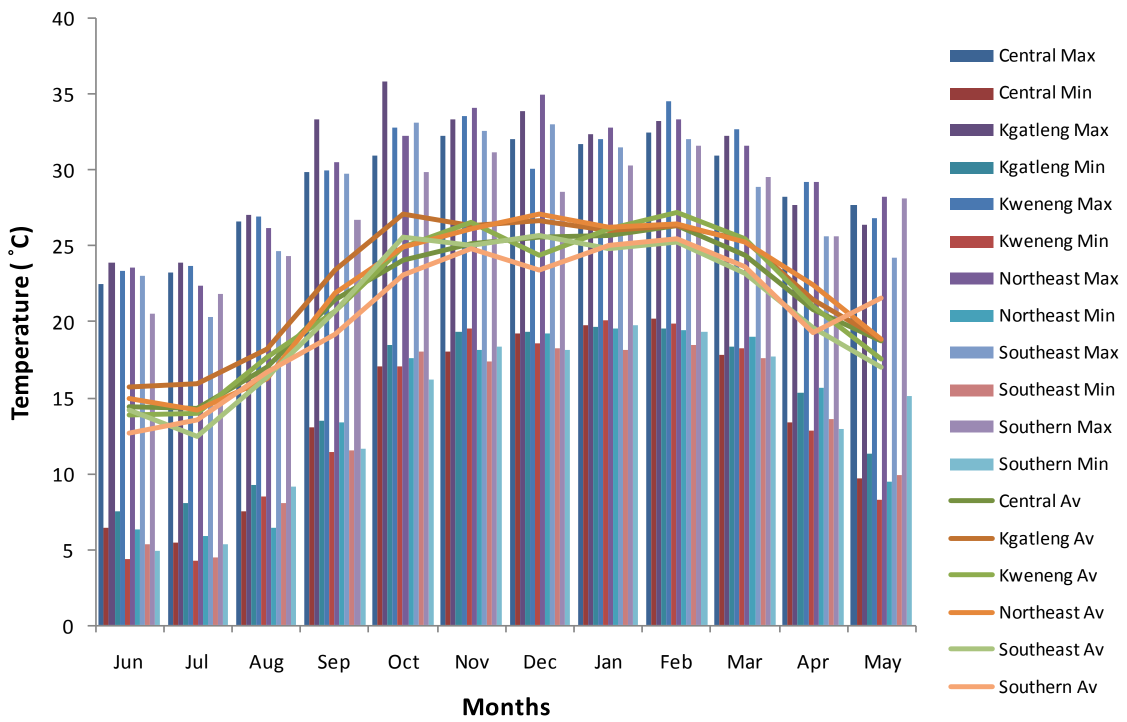


b)

Figure 6.1 *(a)* Mean monthly rainfall during the period from June 2011 to May 2012 and *(b)* mean monthly rainfall from June 2012 to May 2013 in the six surveyed districts



a)



b)

Figure 6.2 *(a)* Mean monthly temperature during the period from June 2011 to May 2012 and *(b)* mean monthly temperature from June 2012 to May 2013 in the six surveyed districts

There was a small variation in the overall rainfall during the 24 months of study, with a slightly higher amount in the 2011/2012 rainy season. The mean annual temperature recorded varied from a minimum of 11˚C in July to a maximum of approximately 26˚C in January 2011/2012, and from a minimum of 12˚C in July to a maximum of 26˚C in February 2012/2013 study period.

Most of the rivers and small dams in all the districts had little water during the rainy season in summer and were dry during the dry season in winter, with dry sand in the river beds and dried mud in the dams. The larger rivers and dams contained water throughout the year, with more during the rainy season in summer than in winter months, but not as much as they would have been expected to contain during years of good rain (personal observation). As a result of this, there were no or only patchy aquatic plants available at the edges of water or snail habitats in all six study districts, and subsequently no aquatic plants could be collected for identification as to which ones might represent suitable habitats for snail development and *Fasciola* metacercariae. from any of the water sources in all the six study districts. Ultimately, no soil or water samples could be collected for chemical analysis to determine the pH most appropriate for the survival of snails.

Table 6.1 Location of study sites, potential snail habitats and number of snails collected and infected with *Fasciola* in different natural pastures in Botswana for the period from June 2011 to May 2013

Location	Site	Habitat type	No. snails collected	No. snails infected
Central				
	Machaneng	River (1)	0	0
	Machaneng	Water tank (9)	0	0
	Mahalapye	River (2)	0	0
	Mmadinare	River (1)	0	0
	Mmadinare	Dam (2)	0	0
	Selibe-phikwe	River (1)	0	0
	Shakwe	River (1)	0	0
	Shakwe	Water tank (1)	0	0
	Shoshong	River (1)	0	0
	Shoshong	Water tank (3)	0	0
Kgatleng				
	Bokaa	Dam (1)	0	0
	Bokaa	Water tank (1)	0	0
	Malolwane	River (1)	0	0
	Mochudi	River (1)	0	0
	Mochudi	Water tank (4)	0	0
Kweneng				
	Kopong	River (1)	0	0
	Kumakwane	River (1)	0	0
	Kumakwane	Pasture (1)	0	0
	Molepolole	River (1)	0	0
North-east				
	Francistown	Stream (1)	0	0
	Tonota	River (1)	0	0
	Tonota	Dam 91)	0	0
South-east				
	Boatle	Dam (2)	0	0
	Boatle	Stream (1)	0	0
	Taung	River (1)	0	0
	Ramotswa	River (1)	0	0
Southern				
	Goodhope	Lake (2)	0	0
	Goodhope	Pasture (2)	0	0
	Goodhope	Dam (1)	0	0
	Metlojane	Lake (1)	0	0
	Metlojane	Dam (1)	0	0
	Metlojane	Water tank (6)	0	0
	Ramatlabama	River (1)	0	0
	Ramatlabama	Pasture (1)	0	0
	Ranaka	Stream (1)	0	0

***The number in parentheses indicates the number of habitats at the study sites which were sampled for snails

6.4 Discussion

The epidemiology of fasciolosis in an area is influenced by the ecology of the intermediate host, the availability of a suitable habitat in which it lives and the climatic conditions, in particular precipitation and temperature (Yilma and Mesfin, 2000; Coelho and Lima, 2003). In the present study, no *L. natalensis* were obtained from the six districts, including the Tuli Block area, in the Central district, where clinical evidence of infection in cattle had been observed based on the presence of *Fasciola* eggs in the faeces (Chapter 4). Consequently, statistical evaluation could not be performed to determine the association between the presence of suitable snails and areas positive for fasciolosis. Alves *et al*.(2011) also failed to find any snails in areas where fasciolosis was prevalent in Brazil. In contrast, in most studies where fasciolosis has been observed, investigators have consistently reported an association between the presence of relevant lymnaeid snails and infection (Mzembe and Chaudhry, 1979; Knapp *et al*., 1992; Tembely *et al*., 1995; Mage *et al*., 2002; Coelho and Lima, 2003; Pfukenyi *et al.,* 2006; Phiri *et al*., 2007b; Walker *et al*., 2008).

A variety of lymnaeid snails are capable of being infected by *F. gigantica*, however it is generally accepted that *L. natalensis* remains the predominant intermediate host in Africa. However, natural infection of the snail with the associated tropical fluke could not be ascertained in this study because of the unavailability of snails, including at the Tuli Block area. Although this investigation could not find any snails to determine the habouring and shedding of intra-molluscan stages by *L. natalensis*, the presence of *F. gigantica* infections in cattle has been revealed in the Tuli Block farms by coprological examination of the bovine definitive hosts. The prevalence of fasciolosis in the absence of the snail intermediate host could be related to the chronic nature of fasciolosis in cattle since infection by adult flukes in the bile ducts are capable of persisting for years such that they may still be present several years after the source of infection has disappeared (Behm and Sangster, 1999). The chronicity of bovine fasciolosis can, at times, make the interpretation of the observed disease trends somewhat difficult.

The scarcity of aquatic vegetation and the resultant absence of snails at the study sites precluded the identification of plants and collection of soil and water to determine their relationship with the presence or absence of snails. Other studies have reported an association of lymnaeid snails with certain types of aquatic plants, including *Potamogeton* spp in Zimbabwe (Pfukenyi *et al*., 2006) *Heteranthera* spp and *Eichornea* spp in Brazil (Coelho and Lima, 2003) and *Juncus* spp, *Glyceria* spp and *Agrostis* spp in France (Rondelaud *et al*., 2011). The absence of aquatic plants, either by removal, overgrazing or lack of adequate rainfall, may lead to a serious decline in the snail population (Coelho and Lima, 2003). The optimum soil pH for snail development has been determined to be approximately 6.5, with a range of 5.0 to 8.0 and water should not be polluted and have low salinity (Boray, 1964).

Climate can influence the infection dynamics of lymnaeid snails with *Fasciola* species, with seasonality being of importance in infection rates (Coelho and Lima, 2003) with a well balanced host-parasite relationship between the fluke and the intermediate host snail evident (Boray, 1978). Temperature and rainfall have an enormous effect on both the spatial and temporal abundance of snails as well as the rate of development of liver fluke eggs and larvae (Radostits *et al*., 2007). A dense population of snails usually develops in a suitable environment of moderate temperature and wet pastures (Soulsby, 1982), which occurs during the rainy season.

The absence of snails and the possible infection with liver fluke larvae in this study could be explained by the prolonged drought that prevailed during the entire period of the present study. It is likely that with adequate (normal) rainfall and the subsequent growth and maintenance of aquatic vegetation, then the snail intermediate host would be expected to be present. The mean annual temperature of approximately 18.5˚C, (range 11˚C to 26˚C) in the study districts was appropriate for snail development (Soulsby, 1982; Taylor *et al*., 2007). The absence of snails observed in this study was in contrast with previous studies from Africa and other parts of the world (Mzembe and Chaudhry, 1979; Boray *et al.*, 1985; Amato *et al*., 1986; Morel and Mahato, 1987; Coelho and Lima, 2003; Pfukenyi *et al*., 2006; Moema *et al.*, 2008).

The distribution of the snail intermediate hosts for *Fasciola* species is dependent upon favourable environmental factors (Dunkel *et a*l., 1996) and considering the semi-arid climate that prevails in most parts of the country, it could be expected that moisture would remain a critical factor to their survival. Liver fluke infection involves a two-host cycle: adult flukes in the bile ducts of the definitive mammalian hosts such as cattle, and the developmental stages within the snail intermediate host (Claxton *et al.*, 1997). All the stages outside the definitive host are susceptible to environmental factors, principally moisture and temperature (Claxton *et al*., 1997) and in this study it is likely that the snail population was severely affected through desiccation during the study period.

In general, the important snail intermediate hosts of *F. gigantica* differ from those of *F. hepatica* in that they are aquatic, with little evidence to suggest that they can aestivate, thus requiring the continuous existence of free water for their development (Torgerson and Claxton, 1999). This has been confirmed in West Africa, where it has been demonstrated that *L. natalensis* survived for only 15 to 90 days in the absence of moisture during periods of high temperature and a lack of surface water (Tembely *et al*., 1995). Additionally, a study in Mali found that *L. natalensis* was present in relatively low numbers and with a narrow distribution. The snail population was observed in only two geographical areas and only during a short period of time, for approximately two months (Tembely *et al*., 1995). In the current study, a lack of rain/moisture for some months in the affected area in Botswana made the prevailing conditions at the time unsuitable for the survival and proliferation of this potential intermediate host snail.

The ecological characteristics of the Tuli Block farms, including the Limpopo River, numerous small water bodies, temperatures between 20 ˚C and 25 ˚C during the rainy season and the presence of large numbers of cattle as definitive hosts, are all suggestive of suitable conditions for the transmission of *F. gigantica*. Accordingly, the detection of infection in cattle in the Tuli Block area was indicative of the previous presence of *L. natalensis*, despite the failure to detect any snails in this study. One study in Tanzania found that the determining factor in the spread and establishment of

F. gigantica was not temperature, but the pre-existing presence of *L. natalensis* (Walker *et al.*, 2008).

Earlier studies on snails in West Africa have shown an increase in the snail population during the rainy season, with peak abundance in March and April at the height of the rainy season, followed by a severe decline in June (Tembely *et al*., 1995). In contrast, in Southern Africa the snail population is usually low from December to March, increasing at the end of the rains, in April, and reaching peak abundance at the end of the dry season, in September to October (Mzembe and Chaudhry, 1979; Pfukenyi *et al*., 2006). The latter findings can be explained by the fact that the period of receding water levels increases the requirement for the majority of cattle to graze near and drink water at permanent water sources, which are snail habouring sites, and therefore cattle are more likely to be exposed to metacercariae (Pfukenyi *et al*., 2006).

During the period when the present study was conducted, there was lower than normal rainfall. This resulted in transient water bodies containing little or no water and permanent water bodies only had water to last several months, and consequently metacercariae, if available, would only survive for a short time. Such conditions are extremely difficult for the development and maintenance of snail populations, including the Tuli Block area in Machaneng where some cattle were positive for *Fasciola* eggs. Ultimately, the likelihood of recovery of *L. natalensis*, the snail intermediate host which plays a major role in the epidemiology of infection of cattle with *F. gigantica*, diminished. Since lymnaeid snails are capable of being infected with more than one trematode, the potential co-infection of *F. gigantica* and amphistomes in cattle is investigated and reported on, in the following chapter.

CHAPTER SEVEN

7 Epidemiology of natural bovine *Fasciola gigantica* infection and its association with infection of Amphistome species in cattle from Botswana

7.1 Introduction

Trematode parasitosis has been recognized as one of the most common helminth problems and an important cause of lost productivity in livestock worldwide (Vercruysse and Claerebout, 2001). Among the trematode infections reported around the world, the fasciolid and faramphistomid flukes have been recorded in several published and unpublished reports as some of the major parasitic problems of ruminant livestock. These infections are economically important helminth diseases through limiting livestock production (Vercruysse and Claerebout, 2001; Fromsa *et al.*, 2011). Fasciolosis and amphistomosis are the two most important trematode parasitoses of farm livestock (Mage *et al*., 2002), even though fasciolosis has been reported as a more important constraint to ruminant production than amphistomosis (Mungube *et al.*, 2006).

The biotopes of the various trematodes have a lot in common, particularly as the intermediate hosts occupy identical niches in the food chain. As two or more species of snails may be commonly found together in the same habitat (Pfukenyi *et al*., 2005a), mixed infections with different trematodes are common since they are able to utilize the same snail intermediate hosts and have similar life cycles (Abrous *et al*., 2000;

Szmidt-Adjide *et al*., 2000; Hordegen, 2005; Diaz *et al*., 2006; Yabe *et al.*, 2008). As a result, the prevalence of snail-borne diseases are influenced more by the abundance and productivity of the snails than by the number of infected animals (Pfukenyi *et al*., 2005a). Concurrent trematode infections may influence the epidemiology of individual fluke populations (Yabe *et al*., 2008).

As fasciolosis can have important economic consequences, the disease has been well researched (Spithill *et al*., 1999; Mage *et al*., 2002). In contrast, data on the prevalence of amphistomosis are scarce, even from European countries (Mage *et al*., 2002; Keyyu *et al*., 2005b). Amphistomosis has only been investigated in a few countries (Phiri *et al*., 2006a) and data on its prevalence and distribution is lacking (Mage *et al*., 2002). However, infection with amphistomes can result in clinical disease and mortality and economic losses in cattle (Keyyu *et al*., 2005b; Phiri *et al*., 2006a).

In contrast to *Fasciola*, where only one species, *F. gigantica*, has been reported in ruminants in southern Africa (Mzembe and Chaudhry, 1981; Pfukenyi *et al*., 2005a; Phiri *et al*., 2005a; Pfukenyi *et al*., 2006; Phiri *et al*., 2006b) ten species belonging to the genera *Paramphistomum*, *Calicophoron*, *Cotylophoron* and *Carmyerius* have been recognized (Pfukenyi *et al*., 2005a). The most important species responsible for outbreaks of acute amphistomosis in ruminants in Africa has been reported to be *Paramphistomum microbothrium* (Keyyu *et al*., 2005b; Pfukenyi *et al*., 2005a; Pfukenyi *et al*., 2005b).

Epidemiological investigations based on coprological and abattoir surveys for trematode infections have been reported worldwide. However, studies on the prevalence of amphistome infection and concurrent infections of *Fasciola* and apmhistomes in livestock are lacking in Botswana. The objective of this study was to determine the prevalence and extent of association of natural infections between *Fasciola gigantica* and amphistomes in cattle in the country.

7.2 Materials and methods

7.2.1 Study areas and parasitological examinations

The study was carried out for 24 months in the six districts as described in Chapter 4. *Fasciola* and amphistome eggs were detected and quantified by the sedimentation technique (Chapter 4). The prevalence of infection was calculated by comparing the proportion of infected cattle with the two parasites.

7.2.2 Statistical analysis

Faecal egg counts (epg) from sampled animals were transformed into logarithmic values (1 was added to all counts prior to log transformations) to normalise the variances. The frequencies were calculated using SPSS version 21.0 for Windows, as described earlier, and analysis of correlation in mixed infections was done on $\log_{10}$ transformed egg counts. The association of geographic origin, age, gender and breed were determined by the Pearson's correlation coefficient and linear regression to describe the relationship between the two trematodes.

7.3 Results

In the 8,646 cattle sampled, the prevalence of *F. gigantica* was 0.74% (95% CI: 0.57, 0.94%), as reported in Chapter 4, and amphistomes was 11.59% (95% CI: 10.92, 12.28%). Only 14 animals (0.16%; 95% CI: 0.09, 0.27%) were infected with both trematodes and 267 cattle (3.09%: 95% CI: 2.73, 3.47%) were positive for one trematode (50 for *Fasciola* and 217 for amphistomes). Faecal egg counts were variable throughout the present study for both trematodes, but ranged from 0 to 30 with a mean (± SEM) of 0.08 ± 0.01 for *F. gigantica*, and 0 to 250 with a mean (± SEM) of 1.74 ± 0.08 for amphistome. The mean infection intensities of *F. gigantica* in cattle were light (97.2%), moderate (2.4%) and severe (0.4%) whereas all infections with amphistomes were light. A significantly positive, but very weak, correlation ($r = 0.038$) was obtained between the epg of *F. gigantica* and amphistome ($p < 0.001$) (Figure 7.1).

The prevalence and distribution of infections according to origin of the cattle are displayed in Figure 7.2. Single *F. gigantica* infections were detected only in the Central district (1.83%; 95% CI: 1.36, 2.40%) whereas no infections were detected in livestock originating from all other districts. Consequently, mixed infections were only observed in the Central district, with 0.51% (95% CI: 0.28, 0.86%) of livestock containing dual infections. This represented only 0.16% of all cattle tested. Infection by amphistomes were detected in 7.94% (95% CI: 6.95, 9.02%) of the cattle sampled from the Central district.

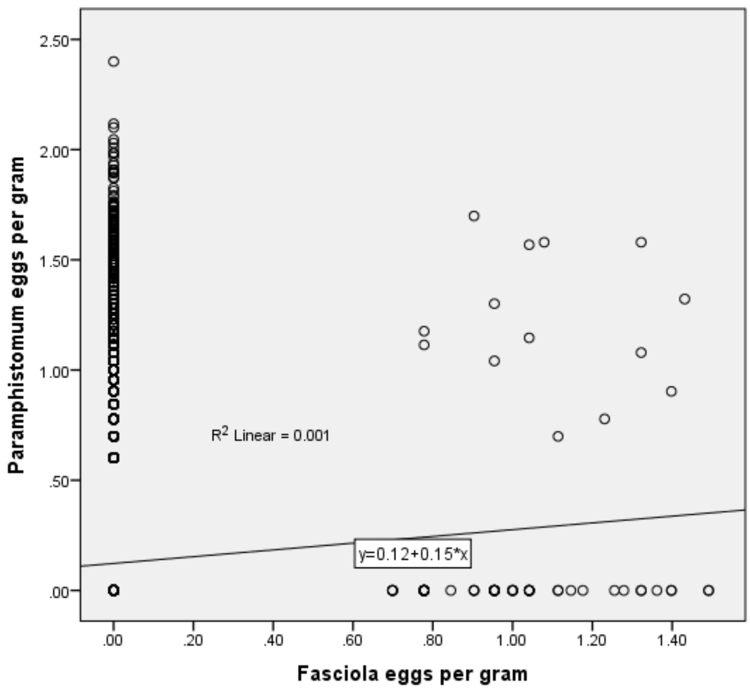


Figure 7.1 Correlation between *Fasciola gigantica* and amphistome epg in cattle from six districts in Botswana

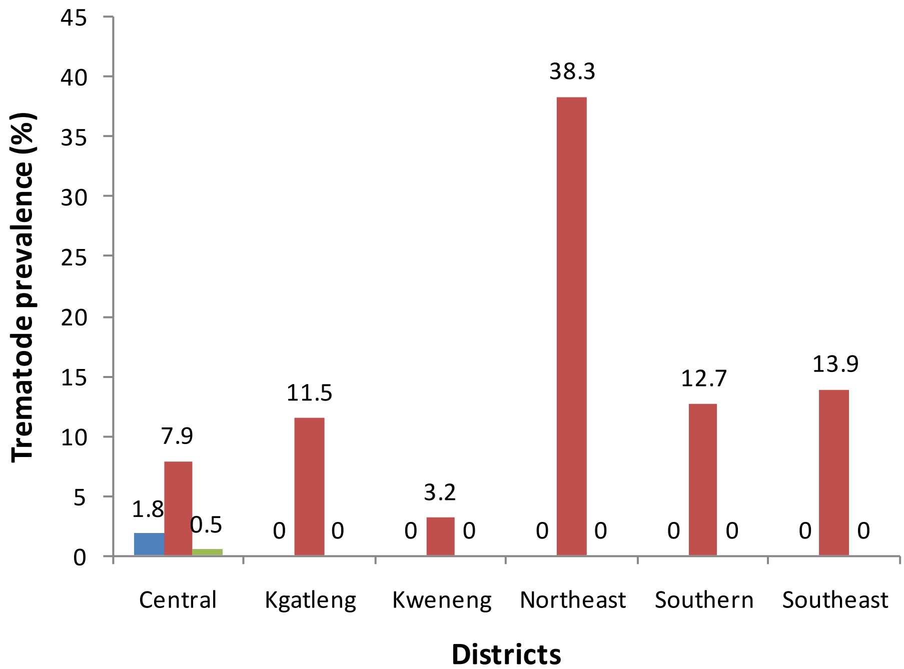


Figure 7.2 Prevalence of *Fasciola* (blue), amphistome (red) and mixed infection between *Fasciola* and amphistome (green) according to district of origin in Botswana

The variation between single and dual trematode infections according to the geographic origin of cattle was significant (χ^2 (5) = 30.34, p < 0.001), with only the Central district recording an association of infection between the two trematodes.

Few adult cattle (0.10%; 95% CI: 0.05, 0.20%) had mixed infections compared with, 0.06% (95% CI: 0.02, 0.13%) of weaners and no infection in calves 0.00% (95% CI: 0.00, 0.04%). There was no significant difference in concurrent infections between age categories (χ^2 (2) = 2.27, p (0.32) > 0.05). The association between mixed infection and gender of cattle was also not significant [χ^2 (1) = 2.83, p (0.09) > 0.05]. However, females had a higher prevalence (0.14%; 95% CI: 0.07, 0.24%) than males (0.02%; 95% CI: 0.00, 0.08%).

Amphistomes were detected in cattle from all the six study districts (11.59%). The North-east district had the highest prevalence of 38.28% while the other districts had a relatively similar prevalence (8.45 to 13.94%) with the exception of Kweneng district which had the lowest prevalence of 0.42%. These differences were significant, (χ^2 (5) = 444.79, p < 0.001). The OR showed that cattle from the North-east district were 12 times more likely to have amphistome infections than cattle from Kweneng district, and cattle from the other districts were 3 to 4 times more likely to have infection than cattle from Kweneng district. The communal sector (10.33%; 95% CI: 9.69, 10.99%) had a significantly higher prevalence than the commercial sector (1.26%; 95% CI: 1.04, 1.52%), (χ^2 (1) = 43.05, p < 0.001).

The prevalence of amphistomes also varied between age groups, (χ^2 (2) = 66.19, $p <$ 0.001 with adult cattle having a higher prevalence (7.04%; 95% CI: 6.51, 7.60%) than weaners (3.90%; 95% CI: 3.50, 4.33%) and calves (0.65%; 95% CI: 0.49, 0.84). Adults (OR = 3.06) and weaners (OR = 2.71) both had a higher risk of infection with amphistomes than did calves. The prevalence in female cattle (8.15%; 95% CI: 7.59, 8.75%) was significantly higher (χ^2 (1) = 18.89, $p < 0.001$) than that in males (3.44%; 95% CI: 3.06, 3.84%).

There were also significant differences in the prevalence of amphistomes between breeds, (χ^2 (6) = 201.43, $p < 0.001$). The Brahman crosses had the highest prevalence (7.03%; 95% CI: 6.50, 7.59%), with the lowest prevalence in the Nguni and Simmental breeds (0.06%; 95% CI: 0.02, 0.13% and 0.01%; 95% CI: 0.00, 0.06%), respectively. Tswana crosses were more likely (OR = 5.34; 95% CI: 2.15, 13.29) to be infected than the Nguni breed (referent). Tswana and pure Brahman had similar risk of infection with amphistomes to the Nguni breed (both OR = 1.02).

7.4 Discussion

Infections with trematode are some of the most common parasitic problems of cattle and other ruminants in Africa, with *Fasciola* and amphistomes reportedly the most frequently found trematodes (Keyyu *et al*., 2005b; Pfukenyi *et al*., 2005a; Phiri *et al*., 2006a; Yabe *et al*., 2008). In accordance with this observation, the findings from the present study have indicated the presence and extent of *F. gigantica* natural infection and its association with amphistomes infection in cattle in Botswana. This study has revealed that *F. gigantica* was less common, occurring in only one district in Botswana. In contrast, amphistomes were detected in cattle originating from all the six districts sampled in this study.

The observation of a positive association between *Fasciola* and amphistome infections has been reported elsewhere in Africa (Keyyu *et al*., 2005b; Phiri *et al*., 2006a; Yabe *et al*., 2008; Fromsa *et al*., 2011) and other continents (Szmidt-Adjide *et al*., 2000; Mage *et al*., 2002; Arias *et al.*, 2011). This parasitic alliance may be explained by the fact that both trematodes utilize the same snail intermediate hosts (Szmidt-Adjide *et al*., 2000; Hordegen, 2005; Yabe *et al*., 2008) and follow a similar life cycle. This would suggest that the increase in prevalence and geographical distribution that has been reported for *Fasciola* may also occur for amphistomes (Gordon *et al.*, 2013). The observed positive, although weak association, in this study, based on faecal egg counts, was an indication that an increase in *F. gigantica* egg production was associated with an increase in amphistome egg production.

The present findings concur with those of a similar study in Zambia (Phiri *et al*., 2006a), which the authors suggested was evidence of a mutually inclusive relationship. In contrast, in Indonesia (Asia), another trematode, *Echinostoma revolutum*, competes with *F. gigantica* inside the intermediate host snail, *L. rubiginosa*, by displacing them from the snail hosts. This potential of *Echinostoma* to interfere with the transmission of *Fasciola* has led to the trematode being used for biological control of bovine fasciolosis, unlike *Paramphistomum* (Copland and Skerratt, 2008; Suhardono and Copeman, 2008). However, in the current study in Botswana, the prevalence of concurrent infection of *F. gigantica* and amphistomes was lower than that reported in Zambia (34.6% and 66%) by Phiri *et al*. (2006a) and Yabe *et al*. (2008), respectively, in Ethiopia (8.08%) by Fromsa *et al*. (2011) and Spain (20%) by Arias *et al*. (2011). The extremely low overall frequency of dual infections could be ascribed to the absence of *F. gigantica* in cattle from five of these districts included in the current study.

The overall prevalence of infection with *F. gigantica* (0.74%) was significantly lower than that of amphistomes (11.59%). These results demonstrated that amphistomes could be the predominant trematode parasite of cattle in Botswana. Further, the fact that bovine amphistomes were present throughout the Central district when compared with *Fasciola* (restricted to the Tuli Block within the Central district) and in all the districts studied is an indication that the rumen fluke has a wider distribution than the liver fluke in Botswana. However, faecal egg output from both trematodes manifested predominantly as low intensities of infection. The higher prevalence of amphistomes compared to *F. gigantica* could probably be attributable to the presence of, and the adaptability of the flukes to, an array of snails that act as intermediate hosts and the fact that this group includes several species of trematodes affecting cattle (Urquhart *et al*., 1996; Pfukenyi *et al*., 2005a).

The difference between infections might also have resulted from the density of metacercariae on the pastures or from the specific development of metacercariae in the bovine definitive host (Szmidt-Adjide *et al*., 2000). This variation in epg could also be suggestive that the various snail intermediate hosts of amphistomes survive better and are widespread in most areas in Botswana. The mean faecal egg counts were higher for amphistomes (1.74 ± 0.08) than for *F. gigantica* (0.08 ± 0.01). The variation in egg counts might also be due to the fact that Paramphistomids are very prolific egg layers whereas Fasciolids are not (Dorchies, 2006).

Age and gender differences were, in general, not significantly associated with the prevalence of dual infections. However, infections recorded in adult cattle were higher than in weaners, and no mixed infections were detected in calves. Also, female animals had higher infections than males. The prevalence and trematode *epg* reported in this study rank among the lowest in Africa. The real prevalence of these trematode infections may be higher than that reported here as coprological techniques have a reported lower sensitivity than serological tests (Hillyer, 1999; Spithill *et al.*, 1999; Dorchies, 2006; Awad *et al*., 2009).

A satisfactory alternative method for investigating associations and burdens of the two parasites may be through parasite counts as reported by a study in Zambia (Yabe *et al*., 2008). These authors found a stronger correlation between the two trematodes using parasite counts. As a result, the use of fluke counts may provide a better guide for determination of the relationship between *F. gigantica* and amphistomes than with the faecal egg counts (Yabe *et al.*, 2008). However, the use of parasite counts is more time consuming than egg counts. Some of the limitations of faecal egg counts include fluctuations in the faecal egg output induced by the pathophysiological changes in the definitive host (Boray, 1969). Faecal egg counts may also be influenced by a low parasitic burden which leads to a low egg output resulting in egg being shed at a level below that which is detectable (Dorchies, 2006). The time of the day of faecal sampling or acquired immunity by cattle in chronic infections can also result in reduced egg production capacity and hence faecal shedding (Yabe *et al*., 2008). Therefore a positive diagnosis could be considered conclusive evidence of an active infection and eggs being released onto the pastures, with subsequent infection of intermediate host snails (Dorchies, 2006).

This study has highlighted a lower prevalence and restricted distribution of *Fasciola* infections (Chapter 4) and a higher prevalence and more widespread distribution of amphistomes infections in Botswana. The higher prevalence of the rumen fluke compared with the liver fluke was in agreement with recent studies in Cambodia (Dorny *et al*., 2011), Ethiopia (Fromsa *et al*., 2011; Melaku and Addis, 2012), France (Szmidt-Adjide *et al*., 2000; Mage *et al*., 2002), Nigeria (Nnabuife *et al*., 2013), Spain (Diaz *et al.*, 2007; Arias *et al*., 2011; González-Warleta *et al*., 2012), Tanzania (Keyyu *et al*., 2005b; Keyyu *et al*., 2006) and Zambia (Phiri *et al*., 2006a; Yabe *et al*., 2008). However, in these latter studies the prevalence of both flukes was higher than that detected in the current study in Botswana. The higher prevalence of amphistomes in cattle may be attributed to the lack of effective treatment against these parasites (Mage *et al*., 2002; Keyyu *et al*., 2005b) since most drugs used in Botswana, including broad-spectrum anthelmintics, are effective mainly against gastrointestinal nematodes and in some cases *Fasciola* spp., but not amphistomes.

Data from this investigation has shown that the Kweneng district, which is more arid than the other districts included in this study, had a significantly lower prevalence than the other five districts. In general, the type of management had an influence on the prevalence of amphistomosis, with communal farms having a significantly higher prevalence than commercial farms. This observation is in accordance with other studies which found a high prevalence of flukes in livestock reared under traditional systems (Keyyu *et al*., 2005b). This may be the result of communal farms rarely treating their livestock with anthelmintics. The higher prevalence might be also due to the common grazing pastures and watering points, which might have lead to contamination of the pastures and ingestion of infective metacercariae.

A significant increase in the prevalence of amphistomes infection with age was noticed, with adult animals having the highest prevalence and calves, the lowest. This may be due to greater opportunities for exposure to the parasite during grazing or a lack of anthelmintic treatment of adult animals in communal grazing areas. The higher prevalence in adults has also been reported by others (Keyyu *et al*., 2005b; Pfukenyi *et al*., 2005b; Keyyu *et al*., 2006; Arias *et al*., 2011). Pfukenyi *et al*. (2005b) in Zimbabwe, reported that a higher prevalence in adult cattle may be associated with immunity developed against the pathogenic effects of the immature flukes, limiting the intensity of re-infection while not inhibiting egg production by mature flukes in these animals.

Other studies have reported that previous exposure and host age provide some protection against re-infection (Phiri *et al*., 2006a) although infection may not be eliminated, resulting in adult cattle acting as reservoirs of infection and sources of eggs which contaminate pastures and infect younger animals (Rolfe *et al*., 1991; Pfukenyi *et al*., 2005b; Phiri *et al*., 2006a). In this study, gender differences were observed, with female cattle displaying a significantly higher prevalence of amphistome infections than males, and this was consistent with the findings of Szmidt-Adjide *et al*. (2000) in France. A higher, but not significantly different, prevalence of amphistomes in females than males has also been reported in a similar study by Phiri *et al*. (2006a). This difference could be confounded by age, since most male cattle are young adults, they would have grazed the pastures for a shorter time than older females. Male animals are generally sold at a younger age (3 to 4 years) whereas females are kept for breeding for a longer period.

A difference in breed susceptibility to amphistome infection was observed in this study. The Tswana and Brahman cross breeds showed a significantly higher prevalence of amphistomosis than other breeds. It was expected that cross breeds would have a lower prevalence than pure (exotic) breeds, due to hybrid vigour, as has been shown in previous studies where cross-bred cattle have high resistance to internal parasites, including flukes (Praya, 2003). However, the findings of this study were mixed, with Tswana crosses and Brahman crosses showing a higher susceptibility to infection and consequently limited resistance to infection. The lowered prevalence in the native Tswana and purebred Brahman cattle could be attributed to acquired immunity whereas the lowest prevalence of amphistomes shown by Nguni may be ascribed to both natural and acquired resistance developed by this indigenous breed.

A study in South Africa found similar results, that native breeds exhibit a lower prevalence to fluke infection (Ndlovu *et al*., 2009) than exotic breeds. However, most studies have indicated that communal cattle, which graze contaminated pastures, regardless of breed, are more likely to have a higher prevalence of flukes than cattle with less access to contaminated pastures (Tembely *et al*., 1988; Spithill *et al*., 1999; Phiri *et al.*, 2005a; Keyyu *et al*., 2006; Suon *et al.*, 2006; Munguía-Xóchihua *et al*., 2007; Yildrim *et al*., 2007). This is further supported by the findings of Arias *et al*.(2011) who observed a higher prevalence of mixed infection with *Fasciola* and amphistomes in Rubia Gallegas cattle which grazed natural or cultivated pastures than in Friesians which were stabled and had limited access to pastures. The lack of detection of infection in the Simmental breed in this study could possibly be due to the small number of cattle sampled or developed resistance since this breed is also reared under communal management.

The presence of infection with adult amphistomes has generally been regarded as relatively innocuous in cattle in many countries (Mage *et al*., 2002; Hordegen, 2005; Rieu *et al*., 2007) and as result has not been thoroughly evaluated in spite of the damage produced by the infection (Diaz *et al*., 2006). Therefore, there is limited literature on the pathological effects of amphistomes in cattle (Phiri *et al*., 2007a).

However, recent reports have demonstrated that amphistomes can induce clinical disease when numerous immature flukes are ingested, since they cause destruction and inflammation of the gastrointestinal tract leading to digestive upsets, impaired absorption and appetite depression, resulting in diarrhea, anorexia, weakness, decline in production and possibly death of the host, mainly in younger animals (Spence *et al*., 1996; Hordegen, 2005; Rieu *et al*., 2007). Thus, the pathogenicity of amphistomosis should not be ignored even though it is milder than that of fasciolosis.

Amphistomosis, nevertheless, is still a neglected and generally underestimated disease with inadequate information about its effects in most areas of the world despite the regular recovery of the flukes in slaughtered cattle and their reported detrimental impact on ruminant health (Mage *et al*., 2002; Keyyu *et al*., 2005b; Phiri *et al*., 2007a). However, the economic importance of amphistomosis has been highlighted through its associated lowering of productivity (Rieu *et al*., 2007; Yabe *et al*., 2008) and dual infection between *Fasciola* and amphistomes may exacerbate losses associated with fasciolosis (Yabe *et al*., 2008).

This study has revealed the existence of dual infections with *Fasciola* and amphistomes in cattle in Botswana. The positive association between the two trematode infections would mean that for control programmes to be effective, this co-infection needs to be considered. Further extensive epidemiological research, to determine the impact of concurrent infections and the possible cross-immunity between these two trematode infections is warranted.

CHAPTER EIGHT

8 General Discussion

The investigation reported in this thesis was designed to determine the epidemiology of *F. gigantica* infection in cattle and the geographical distribution of the intermediate host snail, *L. natalensis*, in Botswana, and thereby provide information to help design appropriate control programmes for bovine fasciolosis. Fasciolosis is a disease that does not have pathognomonic signs nor does it usually result in severe clinical signs, hence very little research has been undertaken on the disease in the country. Prior to the study reported in this thesis, decisions were based on a few laboratory reports on the prevalence from individual areas, and there was no definite national level prevalence or information on the possible impact of the disease on livestock in the country.

8.1 Prevalence

The overall prevalence of bovine fasciolosis in Botswana based on abattoir data (including both the retrospective and prospective studies) was 0.12%. The prevalence from abattoirs in the south (Lobatse, MITI and MSA) was 0.02% and that from abattoirs in the north of the country (Selibe-phikwe, Tonota and Francistown) was 0.05%. In the south, condemned livers were recorded at Lobatse export abattoir and MSA local council abattoir whereas MITI local abattoir did not record any condemnations. In the north, condemned livers were recorded only at the Francistown export abattoir. The positive cattle slaughtered at the MSA in Gaborone were all from the north whereas the exact origins of those slaughtered at Lobatse abattoir could not be verified. However, the majority of condemned livers due to fasciolosis were from cattle originating in the north of Botswana, highlighting the geographical distribution of this parasite and its intermediate host.

The lower prevalence of fasciolosis reported in southern compared with northern Botswana could be associated with the fact that abattoirs in the south are supplied principally by cattle from relatively drier areas, with the bulk of the animals from the west and south-west and some from the south and south-east of the country. In contrast, abattoirs in the north get their supply of cattle from areas with higher rainfall, including central, eastern margin and north-eastern parts of the country. These findings are, therefore, suggestive that rainfall could have a direct effect on the occurrence of fasciolosis as stated by Kithuka *et al*. (2002).

The prevalence obtained from the coprological examination was 0.74%, and was significantly higher than that recorded from the liver inspections (0.03%). This observation was much lower to similar studies carried out in other African countries, including Egypt (Kuchai *et al*., 2011), Zimbabwe (Pfukenyi *et al*., 2006) and Zambia (Phiri *et al*., 2005a). This could be attributable to the low sensitivity associated with abattoir studies, since the condemnation of livers is based only on gross pathological damage but may be due to real differences in the distribution of flukes. The pattern of distribution of bovine fasciolosis reported in the present study in the Tuli Block ecological zone of the Central district is linked to the higher rainfall received by this area, the greater number and larger water bodies that exist in the eastern part of the country and the higher livestock density as a result of commercial beef cattle ranching practised in the area. These findings are in accordance with those reported in studies from other countries with similar environmental conditions (Kithuka *et al*., 2002; Phiri *et al*., 2005b; Pfukenyi *et al*., 2006).

The age of an animal is an important determinant of *F. gigantica* infection in the bovine definitive host, and the prevalence generally tends to increase with age (Gonzalez-Lanza *et al*., 1989; Spithill *et al*., 1999). In this study significant differences were also found between the three age categories, with calves having the lowest prevalence and adults the highest. Similar findings have been documented in other countries including Australia, Spain, Vietnam, Kenya, Tanzania and Zimbabwe (Baldock and Arthur, 1985; Gonzalez-Lanza *et al*., 1989; Holland *et al*., 2000; Waruiru *et al*., 2000; Keyyu *et al*., 2005b; Pfukenyi *et al*., 2006). This higher infection level in adults is supposedly the result of increased likelihood for infection as a result of prolonged exposure time of pasture grazing (Schillhorn Van Veen *et al*., 1980; Waruiru *et al*., 2000; Keyyu *et al*., 2005b; Pfukenyi *et al*., 2006).

Gender is another factor with a notable impact on the prevalence of bovine fasciolosis. This study detected a significant difference between male and female animals, with females showing a higher prevalence. These findings were analogous to those reported previously in other parts of the world (Asanji and Williams, 1984; Ducommun and Pfister, 1991; Phiri *et al*., 2005a; Yildrim *et al*., 2007; Kuchai *et al*., 2011). The higher prevalence observed in female cattle has been attributed to the stress associated with pregnancy and parturition (Spithill *et al*., 1999) and the system of livestock raising that, in most situations, allows the retention of females for a longer period for breeding than with male cattle (Phiri *et al*., 2005a).

Another noticeable outcome was some evidence of dissimilarity in susceptibility to infection with *F. gigantica* in different breeds of cattle. The Brahman crosses had a higher prevalence than purebred Brahman, whereas the Nguni breed showed no evidence of infection at all. This lack of infection in the Nguni could be a characteristic of inherent immunity by this native breed or a combination of both innate and acquired resistance to fasciolosis (Molina, 2005). The egg counts were, however, generally low in infected cattle in this study regardless of breed, gender or age. Resistance to fluke infection by Nguni cattle has also been reported recently in a study in South Africa, which detected lowered epg in this breed than in cattle of Brahman or Angus breeds (Ndlovu *et al*., 2009). However, other studies have found contrasting results, with traditional breeds showing a higher prevalence than exotic cattle (Tembely *et al*., 1988; Kato *et al*., 2005; Keyyu *et al*., 2006). Moreover, some investigations did not find any variations between different breeds of cattle (Sánchez-Andrade *et al*., 2002; Yildrim *et al*., 2007; Yeneneh *et al*., 2012). These differences could be associated with different opportunities for exposure to the parasite and different management and husbandry practices adopted between countries and regions.

8.2 Economic Assessment

In the current study it was noted that the severity of hepatic pathology induced by infection was not directly related to the number of flukes infesting a particular liver with, and therefore the severely affected livers had less flukes than moderately affected ones. Similar findings have been reported by Yilma and Mesfin (2000) in Ethiopia. These hepatic lesions were likely to be associated with the resultant fibrotic effects which are believed to obstruct the passages of flukes within the liver (Yilma and Mesfin, 2000; Mungube *et al*., 2006). The overall degree of pathogenicity (of approximately 30 flukes per liver) recorded in the present study was moderate, and less than the high pathogenicity (greater than 50 flukes per liver) reported by a similar study in Ethiopia (Yilma and Mesfin, 2000).

In addition, this investigation has revealed the presence and extent of *F. gigantica* infections in cattle and the associated economic importance of the disease. The economic impact of bovine fasciolosis, which was the first to be done in Botswana, was assessed based solely on direct losses from the condemnation of livers at selected abattoirs in the country. The results indicated that only modest financial losses are currently incurred as a consequence of *Fasciola*-infected livers. The losses are much lower than those recorded in similar studies from Africa and other countries around the world, which have reported estimates of substantial annual financial losses of millions of (US) dollars. However, the losses estimated in the current study are an underestimate of the real losses as only condemned livers were evaluated. Adding the losses associated with clinical and subclinical infection on productivity would, most likely, increase the size of these losses.

The findings from this study suggest that fasciolosis in cattle is not of clinical importance and thus is a livestock disease of lower economic significance in Botswana. Nevertheless, although the economic ramifications of the disease may be negligible at national level, impacts at a herd or farm level, especially on commercial beef cattle enterprises in the Tuli Block, could be significant. In most countries in Africa and in other developing nations, the disease has remained one of the most common diseases, in terms of prevalence and distribution, and therefore is of significant economic importance. However, since this study excluded the wetlands of Botswana in the north and north-west of the country, which are potential high risk areas for fasciolosis, it would be assumed that had they been included, the results might have been different.

It is also imperative to emphasize that almost all the farmers who were interviewed did not have any knowledge about this disease. It is important to ensure that livestock owners in Botswana are aware of fasciolosis, since livestock production, in particular beef production, is of fundamental economic significance to the country.

8.3 Snail distribution

Lymnaea natalensis is an established and well known major intermediate host snail of *F. gigantica* in tropical Africa and Asia, and has been shown to be the most abundant and pervasive fresh water snail in Africa (Frandsen and Christensen, 1984; Brown and

Kristensen, 1989; Dar *et al*., 2004; Moema *et al*., 2008). This study, however, did not detect this intermediate host snail in any of the potential habitats sampled including the Tuli Block area, where fasciolosis was recognized. Consequently, the present research work was not able to establish the natural infection of *L. natalensis* with *F. gigantica* in the Tuli Block, as other investigations have done. Similar findings, where intermediate host snails could not be recovered in areas positive for fasciolosis, have been reported elsewhere (Alves *et al*., 2011). In contrast, most studies around the world have reported a direct link between the detection of fasciolosis and the existence of specific lymnaeid snails (Mzembe and Chaudhry, 1979; Knapp *et al*., 1992; Tembely *et al*., 1995; Mage *et al*., 2002; Coelho and Lima, 2003; Pfukenyi *et al*., 2006; Phiri *et al*., 2007b; Walker *et al*., 2008) .

Despite the failure to detect natural infection in the intermediate host, the identification of infection with *F. gigantica* in the bovine definitive host, through coprological examinations and examination of livers was sufficient evidence to highlight the presence of the parasite in the Tuli Block cattle raising areas. In fact, previous studies have demonstrated that the essential determinant in the distribution and establishment of *F. gigantica* is the pre-existence of its intermediate host snail (Walker *et al*., 2008). The distribution of the intermediate host snail is heavily dependent upon the availability of moisture in the environment, and with the existence of semi-arid conditions in most parts of Botswana, it would be expected that moisture remains a limiting factor in the survival of *L. natalensis*. The lack of moisture that lead to the scarcity and in some situations total absence of aquatic plants in potential habitats, during the study period, would have resulted in a large decline in the intermediate host population. The presence of aquatic vegetation is essential for providing a suitable habitat for the survival, growth and reproduction of snails.

8.4 *Fasciola* co-infection with Amphistomes

Fasciolids and Paramphistomids are reported to be the most common trematode helminthoses infesting cattle in Africa (Keyyu *et al*., 2005b; Pfukenyi *et al*., 2005a; Phiri *et al*., 2006a; Yabe *et al*., 2008; Fromsa *et al*., 2011). Concurrent natural infections with these flukes are common because they utilize the same intermediate host snails (Szmidt-Adjide *et al*., 2000; Hordegen, 2005; Yabe *et al*., 2008). Thus, the prevalence of these snail-borne parasitoses is influenced to a greater extent by how plentiful and efficient the vector snails are than by the mere abundance of infected animals in an area (Pfukenyi *et al*., 2005a).

In this study, the presence and extent of natural *F. gigantica* infection and its mutual infection with amphistomes in Botswana has been revealed. The prevalence of infection with *Fasciola* spp. was lower than that reported elsewhere in Africa and was restricted to the Tuli Block area in the Central district whereas evidence of infection with amphistomes was present in the six districts surveyed and at a higher prevalence (Chapter 7). Consequently, dual trematode infection was only present in the Tuli Block area. The association of infection between the two trematodes was measured based only on the faecal egg count, and was found to be positive but weak. This positive relationship is indicative of a mutual parasitic alliance between the two trematodes. These findings are in accordance with those from similar studies conducted in other parts of the world (Szmidt-Adjide *et al*., 2000; Mage *et al*., 2002; Keyyu *et al*., 2005b; Phiri *et al*., 2006a; Yabe *et al*., 2008; Arias *et al*., 2011; Fromsa *et al*., 2011). However, the prevalence in the current study was much lower than that reported from these other studies.

The overall prevalence of *F. gigantica* of 0.74% was lower than that of amphistomes of 11.59%. The findings could be an indication that amphistomes is a predominant and more widespread trematode than *F. gigantica* in cattle in Botswana. This variation in the prevalence of the two flukes could also be suggestive of a more widespread distribution and better survival of the diverse intermediate host snails of the rumen fluke than those of the liver fluke. Both flukes showed modest egg output in faeces, regardless of age, gender or breed of animal, which may be an indication of a low fluke burden and therefore lowered egg production by the cattle. The low faecal egg counts may also be an underestimation of the actual trematode infection due to the limitation of detecting eggs by the coprological technique alone. The higher prevalence of amphistomes could also be ascribed to the paucity of knowledge on the rumen fluke by most farmers, leading to insufficient treatment against this trematode.

8.5 Limitations

The most striking limitation of the present study was not being able to visit the livestock farms on a monthly basis to collect faecal samples as initially planned due to logistical challenges. Consequently, it was not possible to determine if there was a monthly and/or seasonal variation in the presence of bovine fasciolosis in Botswana. It is well established, by most authors, that the prevalence of infection with *Fasciola* spp. is higher during the wetter than the drier months of the year, but there is also evidence that infection may still be acquired in all months of the year in some parts of the world (Amato *et al*., 1986; Morel and Mahato, 1987; Rangel-Ruiz *et al*., 1999; Yilma and Mesfin, 2000; Maqbool *et al*., 2002; Phiri *et al*., 2005b; Keyyu *et al*., 2006; Mungube *et al*., 2006; Pfukenyi *et al*., 2006; Adedokun *et al*., 2008; Kuchai *et al*., 2011).

This study only used examination of faeces for the diagnosis of infection. Coprological examination is considered by many investigators to be less sensitive and generally inadequate, without the incorporation of more reliable serological methods. Future expansion of the study to include a serological component would be of benefit.

The collection of faecal samples during ante-mortem inspection, for comparison with the postmortem inspection of livers of slaughtered cattle at the abattoirs, was not possible due to logistical issues. In many cases, it was difficult to trace the exact origin of slaughtered cattle due to the movement or transportation of cattle throughout the country (other than from FMD infected areas). This was a major problem in the south of the country where the abattoir survey detected evidence of *F. gigantica* infections whereas the faecal samples from the field survey did not indicate any infections.

Another important limitation was the lack of success in obtaining the intermediate host snail because of the drought that prevailed during the study period. This dry period most likely destroyed the snail population that could have been present prior to the time of undertaking the study. Consequently, the study could not detect natural infection of snails with *F. gigantica*.

8.6 Future directions

The present study has provided beneficial statistics on the prevalence of infection with *Fasciola* spp. in cattle in Botswana. However, it is necessary to carry out further surveillance studies covering wider areas, including the North-west district where wetlands of Botswana exist, and sampling more animals to provide further evidence on the distribution and impact of this parasite. Moreover, sampling on a monthly basis would help ascertain whether or not a monthly and/or seasonal pattern exists and would determine if the intermediate host snail is present in 'normal' rainfall years.

The definitive diagnosis of infection with *F. gigantica* in live animals is usually accomplished by parasitological examination through faecal detection of fluke eggs. In spite of this, diagnosis by coprological examination alone is generally regarded as having low sensitivity. Therefore, there is a need to conduct sero-epidemiological studies by utilizing serological methods or immune diagnostic tests, such as the ELISA and Western immunoblot, for the detection of antibodies or antigens in infected animals. The sero-diagnostic tests are more sensitive and as a result are capable of providing accurate diagnosis of *F. gigantica* infection in cattle. These tests are also considered to be of some significance in estimating fluke burdens and predicting the success of any chemotherapeutic interventions. Immune diagnosis has been suggested to be helpful in the diagnosis of early infections and in mapping the presence of infection in animals and humans (Hillyer, 1999).

The development of a predictive model for fasciolosis, such as a geographic information system (GIS) risk based model, should be undertaken to map the areas of potential risk and to fully elucidate the distribution of bovine fasciolosis in Botswana.

Finally, a thorough economic analysis should be undertaken to quantify both the direct and indirect costs of the disease through measuring its impact on animal production and productivity.

APPENDICES

Appendix 1

Cattle in a crush for faecal sampling

Appendix 2

Potential snail habitats: moist (left) and dry (right)

Appendix 3

Cattle grazing potential snail habitats

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