PREVALENCE OF *FASCIOLA GIGANTICA* EGGS IN SHEEP AND GOATS AND ON SALAD VEGETABLES IN ZARIA AND ENVIRONS, NIGERIA

BY

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DECLARATION

I declare that the work presented in this thesis entitled '**Prevalence of *Fasciola gigantica* eggs in Sheep and Goats and on Salad Vegetables in Zaria and Environs, Nigeria**' has been performed by me in the Department of Veterinary Public Health and Preventive Medicine under the supervision of Dr B. V. Maikai and Professor J.U. Umoh. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of the thesis was previously presented for another degree or diploma at any University.

Daniel Obinna ESONU …..…….. …….
Signature Date

CERTIFICATION

This thesis entitled **PREVALENCE OF *FASCIOLA GIGANTICA* EGGS IN SHEEP AND GOATS AND ON SALAD VEGETABLES IN ZARIA AND ENVIRONS, NIGERIA** by DANIEL OBINNA ESONU meets the regulations governing the award of Master of Science (M.Sc) degree of Ahmadu Bello University, Zaria and is approved for its scientific contribution to knowledge and literary presentation.

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DEDICATION

This work is dedicated to God Almighty who gave me the wisdom and grace to accomplish this research work and also to the memory of my mother, Late Mrs. Eunice Adamma Esonu who worked tirelessly to see this day come to pass.

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ABSTRACT

Fasciolosis is a worldwide parasitic disease common in ruminants, especially cattle, buffaloes, sheep, goats and swine. It may, however**,** affect humans if they consume contaminated vegetables or raw and improperly cooked liver from an animal infected with *Fasciola gigantica*. To determine the prevalence of *Fasciola gigantica* eggs in sheep and goats and on salad vegetables, four hundred (400) faecal and 180 salad vegetable samples were collected between July and September 2013 within Zaria and environs, Nigeria and examined using the formal ether sedimentation technique and Sodium Chloride flotation methods. The overall prevalence of *F. gigantica* eggs in ruminants was 13.0%, with sheep having a higher prevalence (17.0%) than goats (9.0%). Sheep and goats of greater than two years had significantly ($p<0.05$) higher prevalence of 22.2% and 20.0% of *F. gigantica* eggs respectively as compared to a lower prevalence of 8.1% and 2.4% in the younger ones between 0 to 2 years. The difference in prevalence of *F. gigantica* eggs between the adult and young ruminants was statistically significant ($p<0.05$). The prevalence of *F. gigantica* eggs was significantly ($p<0.05$) higher in animals with poor body condition (34.0%) than those with medium and good body condition with prevalence of 11.3% and 1.8% respectively. Also, higher prevalence of *F. gigantica* eggs was observed in female sheep (24.2%) and goats (12.1%) than in male sheep (10.5%) and goats (1.7%). A significantly ($p=0.001$) higher prevalence (18.5%) of *F. gigantica* eggs was observed in ruminants on non-settled farms than those on settled farms (7.5%). Higher prevalence of *F. gigantica* eggs was observed in ruminants reared by Fulani pastoralists (18.5%) than those on privately owned (8.0%) and Institutional farms (7.0%). The prevalence of *F. gigantica* eggs was 4.8%, 13.7% and 27.0% for ruminants with low, mild and heavy infections of other helminth eggs respectively. No *F. gigantica* egg was seen in sheep and goats that were not infected with other helminth eggs. The overall prevalence of *F. gigantica* eggs on salad vegetables was 2.2%, with lettuce

(*Lactuca sativa*) having a higher prevalence (6.7%) than onions (*Allium cepa*) (3.3%) and carrot (*Daucus carota var sativus*) (3.3%). No *F. gigantica* egg was found on cabbage (*Brassica oleracea var capitata*), tomatoes (*Lycopersicon esculentum*) and cucumber (*Cucumis sativus*). Higher prevalence of *F. gigantica* eggs was observed on salad vegetables sold in Sabon gari market (6.7%), followed by Giwa (3.3%) and Danmagaji markets (3.3%). No *F. gigantica* egg was found on salad vegetables sold in Zaria city, Shika and Samaru market. This study has shown the prevalence of *F. gigantica* eggs in sheep and goats in farms and on salad vegetables at sale within Zaria and its environs. The presence of *F. gigantica* eggs on the vegetables is an indication that the vegetables are potential source of human infection if the snail intermediate hosts are present in the study area.

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CHAPTER ONE

INTRODUCTION

1.1 Background of the Study

Fasciolosis also known as fascioliasis, fasciolasis, distomatosis and liver rot, is an important helminth disease caused by two trematodes *Fasciola hepatica* (the common liver fluke) and *Fasciola gigantica* (giant liver fluke). This disease belongs to the snail-borne trematode zoonoses. In Europe, the Americas and Oceania only *F. hepatica* is a concern, but the distributions of both species overlap in many countries of Africa and Asia (Mas-Coma *et al*., 2005).

The definitive host range is very broad and includes many herbivorous mammals and humans. The life cycle includes freshwater snails from family *Lymnaeidae*, as an intermediate host of the parasite (Torgerson and Claxton, 1999). Adult flukes of both species are localized in the bile ducts of the liver or gallbladder. *Fasciola hepatica* has a cosmopolitan distribution. *Fasciola gigantica* is limited to the tropics and has been recorded in Africa, the Middle East, Eastern Europe and South and Eastern Asia (Torgerson and Claxton, 1999). Mixed infections with both *F. gigantica* and *F. hepatica* may occur in areas where the ecology is conducive for replication of the snail intermediate host (Graber, 1975). The clinical manifestation of Fasciolosis in infected animal has three forms: acute, sub-acute and chronic forms (Troncy, 1989). The principal pathogenic effects are anaemia, hypoalbuminaemia, calcification of the bile ducts and enlargement of the gall bladder. It is also associated with 'black disease', a *Clostridium novyi* type B complicated fatal condition (Urquhart *et al*., 1996).

Humans can become infected after consumption of aquatic plants that contain the infective metacercariae (Markell *et al*., 1999). Thus it is zoonotic (Okewole *et al*., 2000). Several species of aquatic vegetables are known as a vehicle of human infection. Because *F. hepatica* cercariae also encyst on water surface, humans can be infected by drinking of fresh untreated water containing metacercariae (Chen and Mott, 1990). In addition, an experimental study suggested that humans consuming raw liver dishes from fresh livers infected with juvenile flukes could become infected (Taira *et al*., 1997).

Human *F. hepatica* infection is determined by the presence of the intermediate snail hosts, domestic herbivorous animals, climatic conditions and the dietary habits of man (Chen and Mott, 1990). Sheep, goats and cattle are considered the predominant animal reservoirs. While other animals can be infected, they are usually not very important for human disease transmission. On the other hand, some authors have observed that donkeys and pigs contribute to disease transmission in Bolivia (Mas-Coma *et al*., 1998). Among wild animals, it has been demonstrated that the peridomestic rat (*Rattus rattus*) may play an important role in the spread as well as in the transmission of the parasite in Corsica (Mas-Coma *et al*., 1988).

Although in animals, coprological examination, hematological tests and postmortem examination are some preliminary diagnostic and surveillance procedures, intravital diagnosis of fasciolosis is based predominantly on faeces examinations and immunological methods (Urquhart *et al*., 1996). However, clinical signs, biochemical and haematological profile, season, climate conditions, epidemiology situation, and examinations of snails must be considered (Torgerson and Claxton, 1999). In humans, faecal examination is not reliable. The fluke eggs are detectable

in faeces 8–12 weeks post-infection (Torgerson and Claxton, 1999). *Fasciola hepatica* specific-antibodies are recognized using ELISA or Western blot 2-4 week post-infection thereby providing early detection of the infection (Zimmerman *et al*., 1982; Duménigo *et al*., 2000).

1.2 Statement of Research Problem.

Fasciolosis is a worldwide parasitic disease common in ruminants, especially cattle, buffaloes, sheep, goats and swine. It may, however**,** affect humans. Once ingested, parasites migrate through the liver parenchyma to reach the bile ducts. In ruminants, the liver is damaged and the subclinical and chronic disease usually results in decreased production of meat, milk and wool and secondary bacterial infections, fertility problems**,** loss of weight and poor carcass quality and great expenses with anthelmintics (Dargie, 1987; Hillyer and Apt, 1997).

Worldwide losses in animal productivity due to fasciolosis were conservatively estimated at over US$3.2 billion per annum (Spithill *et al*., 1999). In addition, fasciolosis is now recognized as an emerging human disease: the World Health Organization (WHO) has estimated that 2.4 million people are infected with *Fasciola*, and a further 180 million are at risk of infection (Anonymous, 1995).

Previous studies have been based on post mortem and flotation technique. Faecal studies conducted in Saudi Arabia 1986, showed an incidence rate due to *Fasciola gigantica* to be 20%, 14%, 22% and 21% for cattle, buffaloes, sheep and goats respectively (Over *et al*., 1992). An incidence of 34%, 45%, and 23% has been reported in cattle, goat and sheep respectively in Spain (Soulsby, 1982). In a survey carried out in slaughter house in Bauchi by Schillhorn van

Veen (1974), 20.3% of cattle livers were infected with *Fasciola*. Ogunrinade (1983) estimated a 2.5% annual incidence of fasciolosis with annual mortality of 1% in Nigeria. On the Jos Plateau, Synge (1981) found that 31.6% of all adult animals sampled were positive for *Fasciola.* Olupinyo and Ajanusi (2005) found a prevalence of 21% in sheep and 8% in goats in animals for slaughter at Zaria Abattoir.

Food-borne illnesses caused by intestinal parasites are still a public health problem in the developing countries (Del Manso *et al*., 2009). About one third of the world, more than two billion people, is infected with intestinal parasites (Brooker, 2010). Food normally becomes a potential source of human infection by contamination, during production, collection, transport, preparation or processing (Slifko *et al*., 2000). Vegetables are essential for good health, and they form a major component of human diet in every family. They are vital energy contributors which are depended upon by all levels of human as food supplement or nutrient (Oliveria and Germano, 1992). Since vegetables require a moist environment for their growth, particularly those eaten raw and without peeling, it has been demonstrated to be a way for transmission of a wide range of parasites (Al-Binali *et al*., 2006). Various parasites have been associated with vegetables including protozoa and helminthes (Fawzi *et al*., 2004; Daryani *et al*., 2012). According to many studies, there is a strong association between vegetables, especially raw ones and parasitic infections (Ogbolu *et al*., 2009).

1.3 Justification.

Fasciolosis, though an important zoonotic helminthic disease worldwide (Haridy *et al.,* 1999), has been neglected, especially in developing countries like Nigeria where national focus is on

other diseases such as HIV/AIDS. The disease is an up-coming threat among the list of parasitic infections in a large number of countries as a result of environmental changes and man-made modifications; occurring in areas where conditions are suitable for the survival of its intermediate hosts (Haridy *et al.,* 1999).

Human fasciolosis has been reported in developing countries, except Western Europe (Mas-Coma *et al.,* 1999). In humid regions which are under developed, fasciolosis is considered to be the most prevalent infection in large ruminants prevailing up to 90 percent in Africa and Indonesia, ranging to an alarming 100 percent in India (Spithill *et al.,* 1999). Infection in humans occurs when they consume uncooked aquatic vegetables or drink fresh water contaminated with metacercariae (Mas-Coma *et al.,* 1999).

Sheep and goats are among the main supply of meat in both urban and rural areas in Nigeria. A significant proportion of these animals are reared under trans-human husbandry system. In tropical Africa, acute shortage of feeds remains a common occurrence during dry seasons. This compels these animals to graze in areas that are, often, heavily infested with the potential intermediate hosts of the liver flukes. The animals are thus, exposed to the hazards of contracting infections by these helminthes.

The deplorable environmental condition coupled with man-made activities may create an enabling environment for the survival of the intermediate hosts. Therefore finding out the status of *Fasciola gigantica* in ruminants (sheep and goats, which are among the most predominant animal reservoirs) will provide an insight into the status of the infection in these animals in the

study area, which will increase the knowledge of the epidemiology of the disease and may enable us to focus more on how to minimize zoonotic transmission by targeting any point in the transmission cycle. Although infection in humans is through ingestion of metacercariae on vegetables, the presence of *Fasciola* eggs on vegetables will no doubt be a good indicator of contamination in the environment and the potential of human infection through the vegetables if the snail intermediate hosts are present in the area. *Fasciola* eggs on vegetables may also give an indication that source of water or manure used on the vegetables was contaminated with *Fasciola* eggs.

1.4 Aim and Objectives

1.4.1 Aim of the Study

The aim of this study is to determine the prevalence of *Fasciola gigantica* eggs in sheep and goats in farms and on salad vegetables at sale within Zaria and its environs, Kaduna State, Nigeria.

1.4.2 Objectives of the Study

The objectives of the study are to:

1. determine the prevalence of *Fasciola gigantica* eggs in sheep and goats in farms within Zaria and its environs using formal ether sedimentation technique .
2. determine the prevalence of *Fasciola gigantica* eggs on salad vegetables at sale within Zaria and its environs.
3. determine the association between presence of *Fasciola gigantica* eggs and presence of other helminth eggs in sheep and goats in farms within Zaria and its environs.

4. determine the association between *Fasciola gigantica* eggs and age, sex, breed, management practice and body condition of sheep and goats in farms within Zaria and its environs.

1.5 Research Hypotheses

The research hypotheses were advanced in the null form as follows:

1. There are no *Fasciola gigantica* eggs present in sheep and goats in farms within Zaria and its environs.
2. There are no *Fasciola gigantica* eggs present on salad vegetables at sale within Zaria and its environs.
3. There is no relationship between presence of *Fasciola gigantica* eggs and other helminth eggs in sheep and goats in farms within Zaria and its environs.
4. There is no association between *Fasciola gigantica* eggs and age, sex, breed, management practice and body condition of sheep and goats in farms within Zaria and its environs.

1.6 Theoretical Framework

In line with the research hypotheses, the theoretical framework of this study was developed based on the concept that high prevalence of *Faciola* in sheep and goats in Zaria may translate to occurrence of *Fasciola* eggs on vegetables along water banks. Also, occurrence of the eggs on vegetables may give an indication that water bodies used on the vegetables may be contaminated with *Fasciola* eggs.

Previous studies have indicated the occurrence of *Fasciola* in small ruminants such as sheep and goats and the following theories have emanated from such researches:

i. Prevalence is highest in areas where extensive sheep and cattle raising occurs and where dietary practices include the consumption of raw aquatic vegetables (Chitsulo *et al*., 2001).

ii. Transmission occurs through the ingestion of raw, fresh-water vegetation on which the flukes in their metacercariae form are encysted (Chitsulo *et al*., 2001).

iii. The primary definitive hosts of *Fasciola gigantica* are domestic and wild ruminants although infections have been found in a number of mammalian herbivores (Chitsulo *et al*., 2001; WHO, 2007).

iv. Fascioliasis in both small and large ruminants is a serious problem both in terms of economic loss and transmission to humans (Chitsulo *et al*., 2001).

To achieve the goals of this research, the following methodologies were approached:

i. Sedimentation technique was carried out to achieve the set objectives in terms of determining the prevalence of *Fasciola gigantica* eggs in sheep and goats both on intensive and extensive system of management, and on salad vegetables.

ii. The use of flotation technique in which other parasitic eggs and oocysts were identified in sheep and goats and on salad vegetables.

iii. Both the sedimentation and flotation techniques helped us to observe the relationship between the occurrence of *Fasciola gigantica* eggs and other helminth eggs in sheep and goats.

iv. Finally, statistical tests were applied to the stated hypotheses in order to test the dependent and independent variables to determine whether or not there was association between *Fasciola*

gigantica eggs and age, sex, breed, management practice and body condition of the animals sampled.

CHAPTER TWO

LITERATURE REVIEW

2.1 Introduction

Fasciolosis also known as fascioliasis, fasciolasis, distomatosis and liver rot, is an important helminth disease caused by two trematodes *Fasciola hepatica* (the common liver fluke) and *Fasciola gigantica* (giant liver fluke). This disease belongs to the snail-borne trematode zoonoses. In Europe, the Americas and Oceania only *F. hepatica* is a concern, but the distributions of both species overlap in many areas of Africa and Asia (Mas-Coma *et al*., 2005). The definitive host range is very broad and includes many herbivorous mammals and humans. The life cycle includes freshwater snails as an intermediate host of the parasite (Torgerson and Claxton, 1999). Recently, worldwide losses in animal productivity due to fasciolosis were conservatively estimated at over US$3.2 billion per annum (Spithill *et al*., 1999). In addition, fasciolosis is now recognized as an emerging human disease: the World Health Organization (WHO) has estimated that 2.4 million people are infected with *Fasciola*, and a further 180 million are at risk of infection (Anonymus, 1995).

2.2 Etiology

Fasciolosis is caused by two digenetic trematodes *F. hepatica* and *F. gigantica*. Adult flukes of both species are localized in the bile ducts of the liver or gallbladder. *F. hepatica* measures 2 to 3 cm and has a cosmopolitan distribution. *F. gigantica* measures 4 to 10 cm in length and the distribution of the species is limited to the tropics and has been recorded in Africa, the Middle East, Eastern Europe and South and Eastern Asia (Torgerson and Claxton, 1999). In domestic livestock in Japan, diploid (2n = 20), triploid (3n = 30) and chimeric flukes (2n/3n) have been described, many of which reproduce parthenogenetically. As a result of this unclear

classification, flukes in Japan are normally referred to as *Fasciola spp.* (Sakaguchi, 1980). Recent reports based on mitochondrial genes analysis has shown that Japanese *Fasciola spp*. is more closely related to *F. gigantica* than to *F. hepatica* (Itagaki and Tsutsumi, 1998). In India, a species called *F. jacksoni* was described in elephants (Singh *et al*., 1994).

2.3 Geographic Distribution

Human and animal fasciolosis occurs worldwide (Torgerson and Claxton, 1999). While animal fasciolosis is distributed in countries with high cattle and sheep production, human fasciolosis occurs in developing countries except Western Europe. Fasciolosis occurs only in areas where suitable conditions for intermediate hosts exist. Countries where fasciolosis in livestock were repeatedly reported include UK, Ireland, France, Portugal, Spain, Switzerland, Italy, Netherlands, Turkey, Germany and Poland. In Asia, countries like Russia, Thailand, Iraq, Iran, China, Vietnam, India, Nepal, Japan, Korea and the Philippines have recorded cases of fasciolosis, while in Africa, fasciolosis in livestock has been reported in countries like Kenya, Zimbabwe, Nigeria, Egypt, Gambia and Morocco. Other countries include Australia, New Zealand, United States, Mexico, Cuba, Peru, Chile, Uruguay, Argentina, Jamaica and Brazil (GMA NEWS.TV, 2007).

On September 8, 2007, Veterinary officials in South Cotabato, Philippines said that laboratory tests on samples from cows, carabaos, and horses in the province's 10 towns and lone city showed the level of infection at 89.5%, a sudden increase of positive cases among large livestock due to the erratic weather condition in the area (GMA NEWS.TV, 2007).

Studies carried out in recent years have shown human fasciolosis to be an important public health problem (Chen and Mott, 1990). Human fasciolosis has been reported from countries in Europe, America, Asia, Africa and Oceania. The incidence of human cases has been increasing in 51 countries of the five continents (Mas-Coma *et al*., 1999). A global analysis shows that the expected correlation between animal and human fasciolosis only appears at a basic level. High prevalences in humans are not necessarily found in areas where fasciolosis is a great veterinary problem. For instance, in South America, hyperendemics and mesoendemics are found in Bolivia and Peru where the veterinary problem is less important, while in countries such as Uruguay, Argentina and Chile, human fasciolosis is only sporadic or hypoendemic (Esteban *et al*., 1998). In Africa, human cases of fasciolosis, except in northern parts, have not been frequently reported. The highest prevalence was recorded in Egypt where the disease is distributed in communities living in the Nile Delta (Mas-Coma *et al*., 2005).

2.4 Source of Infection for Humans and Transmission

Human *F. hepatica* infection is determined by the presence of the intermediate snail hosts, domestic herbivorous animals, climatic conditions and the dietary habits of man (Chen and Mott, 1990). Sheep, goats and cattle are considered the predominant animal reservoirs. While other animals can be infected, they are usually not very important for human disease transmission. On the other hand, some authors have observed that donkeys and pigs contribute to disease transmission in Bolivia (Mas-Coma *et al*., 1998). Among wild animals, it has been demonstrated that the peridomestic rat (*Rattus rattus*) may play an important role in the spread as well as in the transmission of the parasite in Corsica (Mas-Coma *et al*., 1988). In France, nutria (*Myocastor coypus*) was confirmed as a wild reservoir host of *F. hepatica* (Menard *et al*., 2001).

Humans are infected by ingestion of aquatic plants that contain the infected metacercariae (Markell *et al*., 1999). Several species of aquatic vegetables are known as vehicles for human infection. In Europe, *Nasturtium officinale* (common watercress), *N. silvestris*, *Rorippa amphibia* (wild watercress), *Taraxacum dens leonis* (dandelion leaves), *Valerianella olitora* (lamb's lettuce), and *Mentha viridis* (spearmint) were reported as a source of human infection (Mas-Coma *et al*., 1999). In the Northern Bolivian Altiplano, some authors suggested that several aquatic plants such as bero-bero (watercress), algas (algae), kjosco and tortora could act as a source of infection for humans (Bjorland *et al*., 1995). Because *F. hepatica* cercariae also encyst on water surface, humans can be infected by drinking of fresh, untreated water containing metacercariae (Chen and Mott, 1990). In addition, an experimental study suggested that humans consuming raw liver dishes from fresh livers infected with juvenile flukes could become infected (Taira *et al*., 1997).

2.5 Intermediate Hosts

Snails are the intermediate hosts. Snails of the genus *Lymnaea sp* are widely distributed throughout the world and *L. trunculata* is the most common of them all. In South Africa the most common intermediate hosts are *L. trunculata* (*F. hepatica*), *L. natalensis* (*F.gigantica*) and *L. columella* (*F. hepatica* and *F.gigantica*) (Graczyk *et al*., 1999). Other important *Lymnaea* vectors of *F. gigantica* are *L. auricularis* (Europe, USA. Middle East, Pacific islands), *L. rufescens* and *L. acuminta* (India, Pakistan) and *L. rubiginosa* (Malaysia) (Mas-Coma *et al*., 2005).

2.6 Life Cycle

Eggs are passed in the faeces of the mammalian host, hatch and release motile miracidia. Hatching may take nine days and the optimal temperatures required are 22-26° C. Miracidia have a short life span and must locate a suitable snail within three hours. In infected snails sporocysts, redial stages and cercaria develop. Snails pass the motile cercaria which then attach themselves to plant material, where they encyst and become the infective metacercariae. A minimum of 6-7 weeks is required for miracidia to form metacercaria. Under unfavourable circumstances this may take several months. Following on infection of one snail with one miracidium over 600 metacercariae may be produced. Final hosts ingest the metacercaria and these excyst in the small intestine, followed by migration through the intestinal wall, crossing of the peritoneum and penetration of the liver capsule. The immature flukes migrate through the parenchyma (6-8 weeks period), entering the small bile ducts and finally migrating to the larger bile ducts (and occasionally the gall bladder). Generally the life cycle of both fluke species is the same. The prepatent period of *F. hepatica* is 10-12 weeks and one entire life cycle of *F. hepatica* may be completed in a minimum of 17-18 weeks. *F. hepatica* may live for years (up to 20) in untreated sheep but in cattle it is usually less than one year. For *F. gigantica* most phases of development take longer and the prepatent period is 13 –16 weeks. These parasites are hermatophrodytes, and one adult *F. hepatica* in a bile duct may produce 20 000 eggs per day establishing patent infestation (Figure 2.1).

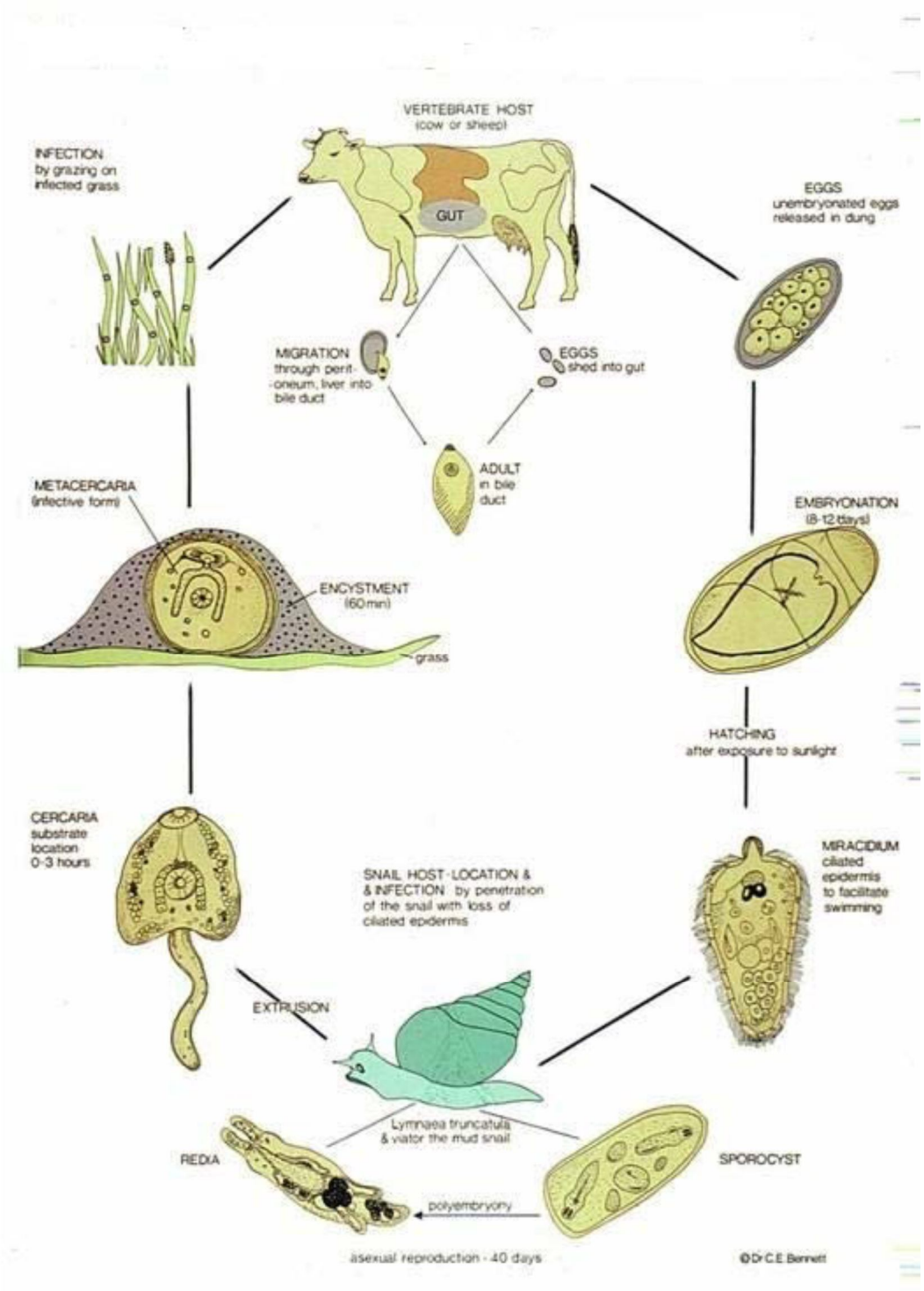


Figure 2.1 Life Cycle of *Fasciola spp* (Courtesy of Dr. Clive Bennett 1999)

2.7 Pathogenesis

The development of infection in definitive host is divided into two phases: the parenchymal (migratory) phase and the biliary phase (Dubinský, 1993). The parenchymal phase begins when excysted juvenile flukes penetrate the intestinal wall. After the penetration of the intestine, flukes migrate within the abdominal cavity and penetrate the liver or other organs. *Fasciola* spp. has a strong predilection for the tissues of the liver (Behm and Sangster, 1999). Occasionally, ectopic locations of flukes such as the lungs, diaphragm, intestinal wall, kidneys, and subcutaneous tissue can occur (Boray, 1969). During the migration of flukes, tissues are mechanically destroyed and inflammation appears around migratory tracks of flukes. The second phase (the biliary phase) begins when parasites enter the biliary ducts of the liver. In biliary ducts, flukes mature, feed on blood, and produce eggs. Hypertrophy of biliar ducts associated with obstruction of the lumen occurs as a result of tissue damage.

2.8 Clinical Signs

Clinical signs of fasciolosis are always closely associated with infectious dose (amount of ingested metacercariae). In sheep, as the most common definitive host, clinical presentation is divided into 4 types :(Behm and Sangster, 1999).

Acute Type I Fasciolosis: infectious dose is more than 5000 ingested metacercariae. Sheep suddenly die without any previous clinical signs. Ascites, abdominal haemorrhage, icterus, pallor of membranes, blood oozing from the natural orifices, weakness may be observed in sheep.

Acute Type II Fasciolosis: infectious dose is 1000-5000 ingested metacercariae. As above, sheep die but briefly show pallor, loss of condition and ascites.

Subacute Fasciolosis: infectious dose is 800-1000 ingested metacercariae. Sheep are lethargic, anemic and may die. Weight loss is dominant feature.

Chronic Fasciolosis: infectious dose is 200-800 ingested metacercariae. Asymptomatic or gradual development of bottle jaw and ascites (ventral edema), emaciation and weight loss.

In blood, anemia, hypoalbuminemia, and eosinophilia may be observed in all types of fasciolosis (Behm and Sangster, 1999). Elevation of liver enzyme activities, such a glutamate dehydrogenase (GLDH), gamma-glutamyl transferase (GGT), and lactate dehydrogenase (LDH), is detected in subacute or chronic fasciolosis from 12-15 week after ingestion of metacercariae (Sykes *et al*., 1980; Anderson *et al*., 1981). Economic effects of fasciolosis in sheep consist in sudden deaths of animals as well as in reduction of weight gain and wool production (Sinclair, 1962; Roseby, 1970). In goats and cattle, the clinical manifestation is similar to sheep. However, acquired resistance to *F. hepatica* infection is well known in adult cattle (Doyle, 1973; Haroun and Hillyer, 1986). Calves are susceptible to disease but in excess of 1000 metacercariae are usually required to cause clinical fasciolosis. In this case the disease is similar to sheep and is characterized by weight loss, anemia, hypoalbuminemia and (after infection with 10,000 metacercariae) death (Boray, 1969). Importance of cattle fasciolosis consist in economic losses caused by condemnation of livers at slaughter and production losses especially due to reduced weight gain (Phiri *et al*., 2006).

In sheep and sometimes cattle, the damaged liver tissue may become infected by the *Clostridium* bacteria *C. novyi* type B. The bacteria will release toxins into the bloodstream resulting in what is known as black disease. There is no cure and death follows quickly. As *C. novyi* is common in the environment, black disease is found wherever populations of liver flukes and sheep overlap (Merck Veterinary Manual, 1998).

2.9 Resistance to Infection

Mechanisms of resistance have been studied by several authors in different animal species. These studies may help to better understand the immune response to *F. hepatica* in host and are necessary in development of vaccine against the parasite. It has been established that cattle acquire resistance to challenge infection with *F. hepatica* and *F. gigantica* when they have been sensitized with primary patent or drug-abbreviated infection (Haroun and Hillyer, 1986). Resistance to fasciolosis was also documented in rats (Milligen *et al*., 1998). On the other hand, sheep and goats are not resistant to re-infection with *F. hepatica* (Chauvin *et al*., 1995; Martínez-Moreno *et al*., 1997). However, there is evidence that two sheep breeds, in particular Indonesian thin tail sheep and Red maasai sheep, are resistant to *F. gigantica* (Roberts *et al*., 1997). No reports concerning the resistance in humans are available.

2.10 Diagnosis

In animals, intravital diagnosis is based predominantly on faeces examinations and immunological methods. However, clinical signs, biochemical and haematological profile, season, climate conditions, epidemiology situation, and examinations of snails must be considered (Dubinský, 1993; Torgerson and Claxton, 1999). Similarly, in humans, faecal examinations are not reliable. Moreover, the fluke eggs are detectable in faeces 8–12 weeks post-infection. In spite of that fact, faecal examination is still the only used diagnostic tool in some countries. While coprological diagnosis of fasciolosis is possible from 8-12 week post-infection (WPI) *F. hepatica* specific-antibodies are recognized using ELISA or Western blot at 2-4 week post-infection (Zimmerman *et al*., 1982; Duménigo *et al*., 2000).Therefore, these methods provide early detection of the infection.

Many recent advances were made in the field of molecular diagnostics. Polymerase Chain Reaction (PCR) tests were developed for the identification of the different snail species, which would be of great advantage in epidemiological investigations. PCR tests, including multiplex PCR and real-time PCR, were developed to diagnose/identify flukes and fluke antigens, and used to study many different aspects of the immunology and epidemiology (Cucher *et al*., 2005).

2.11 Treatment and Prevention

A number of drugs have been used in the control of fasciolosis in animals. Drugs differ in their efficacy, mode of action, price, and viability. Fasciolicides fall into five main chemical groups: (Fairweather and Boray, 1999).

i. Halogenated phenols: bithionol (Bitin), hexachlorophene (Bilevon), nitroxynil (Trodax)
ii. Salicylanilides: closantel (Flukiver, Supaverm), rafoxanide (Flukanide, Ranizole)
iii. Benzimidazoles: triclabendazole (Fasinex), albendazole (Vermitan, Valbazen), mebendazol (Telmin), luxabendazole (Fluxacur)
iv. Sulphonamides: clorsulon (Ivomec Plus)
v. Phenoxyalkanes: diamphenetide (Coriban)

Triclabendazole (Fasinex) is considered as the most common drug due to its high efficacy against adult as well as juvenile flukes and it is used in control of fasciolosis of livestock in many countries. Nevertheless, long-term veterinary use of triclabendazole has caused appearance of resistance to *F. hepatica*. In animals, triclabendazole resistance was first described in Australia, (Overend and Bowen, 1995) later in Ireland (O'Brien, 1998) and Scotland (Mitchell *et al*., 1998) and more recently in the Netherlands (Moll *et al*., 2000). Considering this fact, scientists have

started to work on the development of new drug. Recently, a new fasciolicide was successfully tested in naturally and experimentally infected cattle in Mexico. This new drug is called 'Compound Alpha' and is chemically very similar to triclabendazole (Ibarra *et al*., 2004).

2.12 Control

Efficient control of fascioliasis requires a well planned and executed, integrated control programme designed for each farm, area, country or region (Brunsdon, 1980). The available strategies which can be used individually or in combination are:

· Strategic application of anthelminthics, eliminating the parasites from the host at the most appropriate time for effective prevention of pasture contamination.

· Reduction in the number of intermediate host snails by chemical or biological control.

· Reduction in the number of snails by drainage, fencing and other management practices.

· Reduction in the risk of infection by planned grazing management.

2.12.1 Strategic chemotherapy of ruminants

Seasonal strategic application of effective anthelmintics specific for trematodes, as well timed prophylactic and curative treatments, play an important role in the control of liver fluke infections. Strategic treatments have been developed for several regions of the world based on meteorological data. However, it is advisable to supplement meteorological data with sound epidemiological information in order to improve the timing, and thereby the efficiency, of treatments (Campbell and Rowe, 1986).

2.12.2 Chemical control of snails

The use of molluscicides for the control of snail intermediate hosts is a potential tool for the control of fluke infections (Soulsby, 1982). Before considering chemical control of snails it should be noted that:

· many habitats are topographically unsuitable for the use of molluscicides and it is often very difficult to apply them effectively.

· they are toxic to the environment.

· cooperation between neighbouring properties is required for effective cover.

· regular (at least yearly) application is required because rapid repopulation of snails may occur.

· they are not species-specific and may destroy edible snails highly valued as food in some communities.

· they are expensive.

2.12.3 Biological methods of snail control

Reports from several parts of the world indicate that a number of plants have molluscicidal properties. Planting of these trees and shrubs along streams and irrigation channels can reduce the number of snails in a population. The efficacy of this method for control of flukes has not yet been assessed. The introduction of large numbers of ducks into rice fields after harvest has been used to reduce the snail population. The ducks eat the snails and the fluke species specific to the ducks compete with the fluke species of ruminants in the infection of snails. It is reported that snails infected with duck flukes will not become infected with flukes of livestock. The introduction of edible snail species unsuitable as intermediate hosts into the habitat of the host snails may prevent the flukes from completing their life cycle (Morley and Donald, 1980).

2.12.4 Managemental methods of snail control

The important management methods of controlling fluke infections are:

(a) To prevent snail habitats from developing by regular clearing of drainage channels in vegetation which provide suitable sites for snail development. Good drainage and the building of dams at appropriate sites in marshy and low lying areas may reduce the snail problem.

(b) To keep livestock away from pastures contaminated with metacercariae. This may only be possible when the number of animals involved is small.

(c) Establish proper watering facilities to prevent animals from drinking from lakes, ponds and streams (Morley, and Donald, 1980).

2.13 Situation of Fascioliasis in Nigeria

The existing accounts of the disease in Nigeria are based on local surveys on the incidence of fascioliasis in some areas of the country and often cover only a few months of the year (Ogunrinade and Ogunrinade 1980). For instance, Ferguson (1964), determined the incidence of fascioliasis in Birnin- Kebbi, Maiduguri, Kano and Kaduna. Babalola and Schillhorn van veen (1976), determined the incidence of the disease in Zaria, while Ikeme and Obioha (1973); Uzoukwu and Ikeme (1978) recorded the prevalence rate of fascioliasis in cattle slaughtered in some parts of eastern Nigeria. Economic losses from fascioliasis are enormous. Alonge and Fasanmi (1979) reported that 94% of total liver condemnation in some abattoirs in Northern Nigeria was due to fascioliasis. Ogunrinade (1980) assessed total economic losses due to bovine fascioliasis to be 2 – 3% of the annual output of the livestock industry estimated at □150 – 250 million (3rd National Livestock Development Report, 1978).

In Nigeria, the first incidence of fasciolosis was reported by Burke (1939) when about 3000 goats died of the disease in the then Borno province. In a South-Western State of Nigeria, a gross total liver loss of 8,292kg was observed with about 75% loss of value in 29,952kg of partially condemned livers in a single abattoir over a three-year period (World Bank 2006). Estimating that each of the 36 States and the Federal Capital Territory will record similar losses in at least one abattoir per state, this will translate to huge loss of resources (US$ 5,762,010) for the country. These enormous losses are especially important for a low-income food-deficient country (LIFDC) like Nigeria (World Bank 2006).

In Zaria Kaduna State, cattle population was reported to be 1,144,000 (KDSG, 2008) and about 99% of these cattle were being managed/reared under semi-intensive, extensive system. Most herdsmen graze their herds along the river banks during the prolonged dry season when the upland pasture is poor in quantity and quality. These animals are thus exposed to high risks of *Fasciola* infection (Olumide and Mkpoko, 2001). Sheep and goats are of great social and economic importance in Nigeria due to their contribution in terms of meat, milk, skin, manure and festivities (Olupinyo and Ajanusi, 2005). The Morocco leather from Sokoto Red goat has been rated to be of high grade and has contributed to the Nigerian foreign exchange over the years (Rim Report, 1991). Sheep and goat is the major source of meat in the rural areas (Saidu, 1978). The cost of cattle has increasingly put more demand on sheep and goats for meat. The livestock industry is hampered by diseases that are commonly aggravated by poor management and inadequate nutrition. In particular, parasitic diseases especially helminthosis tend to poise as the biggest problem in livestock development and that a large amount of sheep and goats are being lost annually due to helminthosis (Olupinyo and Ajanusi, 2005).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Area

The study was carried out within Zaria and its environs, Kaduna State, Nigeria. These comprise three Local Government Areas (LGA) namely Giwa, Sabon Gari and Zaria. Zaria is located in the Northern Guinea Savannah zone within latitudes 11^0 7', 11^0 12'N and longitudes 7^0 4'E. It's a medium sized city with an estimated population of 547,000 and a growth rate of 3.5% per annum (Ministry of Economic Development, 1996). It is approximated that about 40-75% of its working population derive their principal means of livelihood from agriculture (ABU, 2000). Agricultural activity in Zaria can be divided into two types: rain-fed (from May to October) and irrigation farming in the dry season (from November to April). It is characterized by a tropical climate, a monthly mean temperature ranging from 13.8 to 36.7^0 C and an annual rainfall of 1092.8mm (Agbogu *et al*., 2006).

Animals reared in this area include cattle, sheep, goats and poultry. The small ruminants reared in this area are mostly managed under the extensive systems. Although this system of management is cheap and less labour intensive, it is characterized by low productivity and high losses due to accidents, diseases and theft (Ajala and Gefu, 2003). Horticulture is the second most prevalent agricultural activity in Zaria with vegetables being mostly produced, but in some cases fruits are sandwiched among cereal crops (Chuo, 2009).

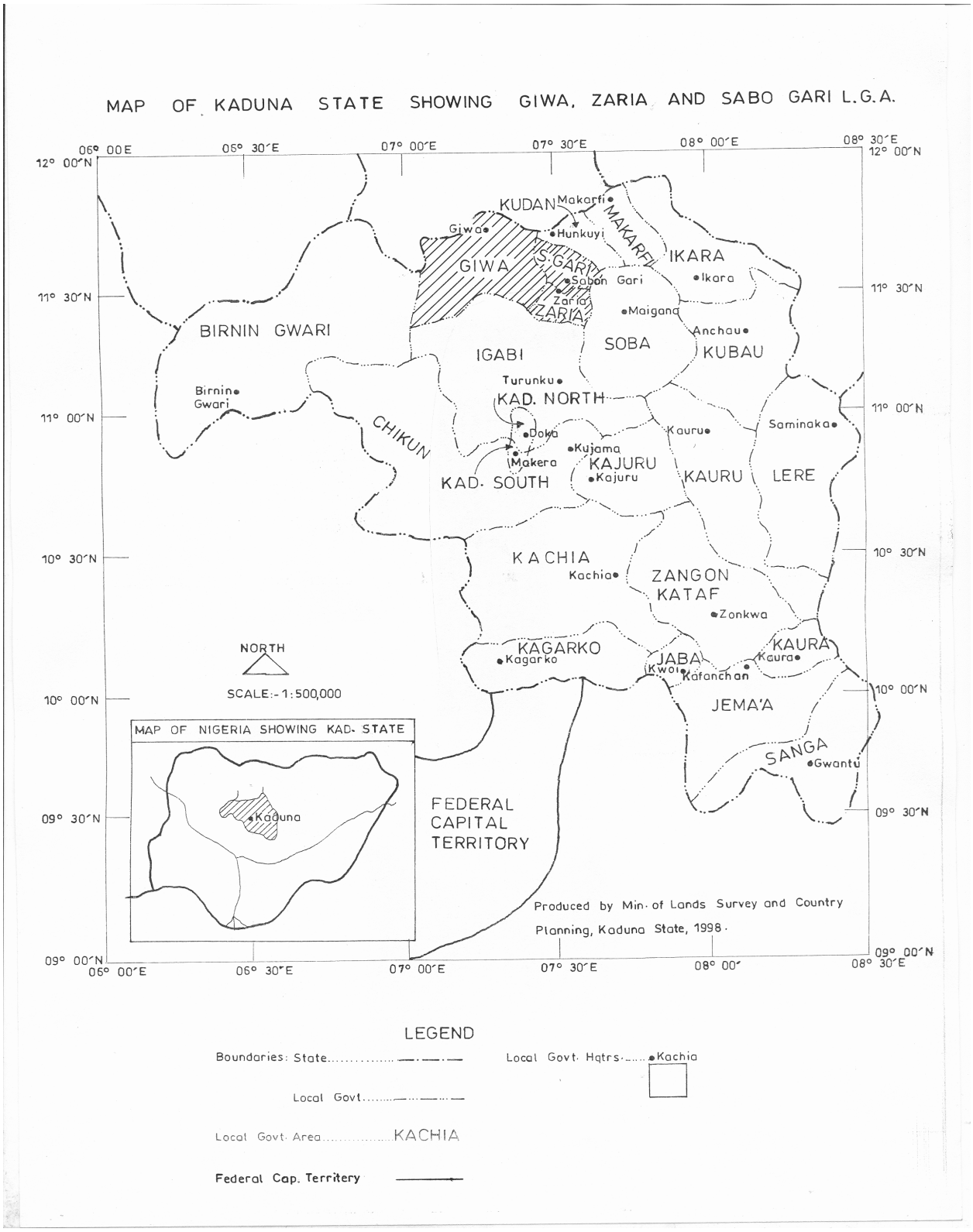


Figure 3.1: Illustrated Map of Kaduna State showing the study areas (Ministry of Lands Survey and country planning, Kaduna State, 1998).

3.2 Study Design.

This was a cross-sectional study in which small ruminants (sheep and goats) in settled farms (private and institutional farms) and non-settled farms (Fulani pastoralists) within Zaria and its environs were selected. For each animal sampled, age, sex, breed and body condition were determined. Ten settled farms comprising five private and five institutional farms and ten non settled farms were identified and used for the study. The farms were selected based on inclusion and exclusion criteria as follows;

Inclusion criteria;

1. Owner's consent and approval.
2. Farm with sheep and goats, and not less than ten (10) animals of each species.

Exclusion criteria;

1. Owner's disapproval.
2. Farm with sheep and goats, but less than ten (10) animals of each species.

Animals within each farm were randomly selected and sampled based on convenience sampling method. Twenty (20) samples were collected from each of the 20 farms; ten samples each from sheep and goats, making a total of 400 samples over a period of three months. For the salad vegetables, 60 samples were collected from each of the three Local Government Areas making a total of 180 samples of salad vegetables.

3.3 Risk Factors of Fascioliasis

Prior to sample collection a structured questionnaire (Appendix I) was used to elucidate some of the risk factors associated with fascioliasis. Some of the factors determined include management

practice (intensive, semi-intensive or extensive), presence of snails on the farm, composition of farm, breed (sheep- Balami, Yankasa, Uda, West African dwarf and goats- Sokoto red, Kano brown, Sahel or desert, West African dwarf), age (dental eruption pattern), sex (male or female).

3.4 Determination of Body Condition

Prior to sample collection, body condition was determined based on the method of Morrical and Hettel (1986) in which body condition was classified as follows:

Condition score 1: Appearance angular and narrow, backbone raised and sharp, hollow behind ribs, tail feels bony, neck bones prominent

Condition score 2: Backbone raised but smooth, ribs are easily felt, tail bone easily detectable, thin neck

Condition score 3: Backbone slightly raised, ribs smooth, can just be felt, and tail bones barely detectable

Condition score 4: Appearance well rounded, backbone can just be felt, ribs are covered, tail firm and rounded

Condition score 5: Appearance very well rounded, backbone barely detectable, ribs cannot be felt, tail fat and broad.

3.5 Sample Collection.

About 10g of faeces was collected directly from rectum of randomly selected animals using clean polythene bag worn over the fingers. The faecal samples were placed in sterile sample bottles. All samples were properly labeled and transported in ice packed cold box to the

Helminthology Laboratory, Department of Veterinary Parasitology and Entomology, Ahmadu Bello University, Zaria.

The salad vegetables used for this study include lettuce, cabbage, tomatoes, carrots, cucumber and onions. One hundred and eighty (180) samples of vegetables were collected; 30 samples from each variety. Ten (10) samples of each variety were collected from each of the three Local Government Areas from retailers. The vegetables were collected at the point of sale at the markets using clean polythene bags and transported to the Helminthology Laboratory, Department of Veterinary Parasitology and Entomology, Ahmadu Bello University, Zaria.

3.6 Laboratory Procedures

3.6.1 Examination of faeces

3.6.1.1 Sedimentation Method:

Detection of fluke eggs was performed using formal ether sedimentation technique as described by Arora and Brij (2010). About 5.0g of faecal sample was thoroughly mixed in 10mls of water and strained through two layers of gauze into a funnel. The filtrate was centrifuged at 2,000 rpm for two minutes. The supernatant was discarded and the sediment resuspended in 10mls of physiological saline. It was again centrifuged and the supernatant discarded. The sediment was resuspended in 7mls of formal saline and 3mls of ether was added. The tube was covered and shaken vigorously. The stopper was then removed and the tube centrifuged at 2,000 rpm for two minutes. Four layers became visible: the top layer being ether, the second is a plug of debris, and third is a layer of formalin and the fourth is the sediment. The plug of debris was detached from the side of the tube with the aid of a glass rod and the liquid was poured off, leaving a small

amount of formal saline for resuspension of the sediment. It was then poured on a clean glass slide and covered with a cover slip and examined under the microscope at 10× magnification for *Fasciola* eggs, which is characterized by its large (190 × 100µm), oval and yellowish colour with a distinct flat operculum (Urquhart *et al*., 1996).

3.6.1.2 Examination of Faeces for other Helminth Eggs:

Detection of other helminth eggs was performed using standard centrifugation faecal examination technique as described by Urquhart *et al* (1996). About 5.0g of faeces was mixed with 10 ml Sodium Chloride flotation solution of 1.20 specific gravity. The mixture was poured through a tea strainer into a beaker, and then the strained solution was poured into a 15-ml centrifuge tube. The tube was filled with the flotation solution until a slight positive meniscus was formed. A coverslip was placed on the tube and the tube was centrifuged at 2,000 rpm for 5 minutes. The tube was removed and allowed to stand for 10 minutes, after which the cover slip was removed and placed on a glass slide and the entire area under the cover slip was systematically examined at 10× magnification. The results were reported as low, mild and heavy infection. Samples with one plus (+) in which 1-9 eggs were seen within a single field on the light microscope indicated low infection, samples with two plus (++) that is when 10-19 eggs are seen within a single field on the light microscope indicated mild infection, while samples with three plus (+++) where 20 eggs or more are seen within a single field on the light microscope indicated heavy infection. Eggs were identified on the basis of their morphological features as described by Urquhart *et al*. (1996).

3.6.2 Examination of salad vegetables

3.6.2.1 Sedimentation Method

About 200g samples of each vegetable variety were washed in distilled water (250ml) in a plastic container for removal of the parasitic ova. The suspension was strained through a sterile sieve to remove undesirable materials. The filtrate was left for 10 hours for sedimentation to take place and the top layer was discarded and the sediment was collected into 30 ml centrifuge tubes and centrifuged at 2000 rpm for 5 minutes, and the supernatant was discarded. The sediment was mixed and a drop was applied on the center of a clean grease-free slide, with clean cover slip placed gently to avoid air bubbles and over flooding. The preparation was examined under microscope using X10 and X40 objectives. The eggs were identified according to Soulsby (1982).

3.6.2.2 Flotation Method

The remaining sediment in the 30 ml centrifuge tubes was shaken with 15 ml Sodium Chloride (specific gravity of 1.20) flotation solution and centrifuged at 2000 rpm for 5 minutes. Additional flotation medium was added to the top of the tube such that a meniscus was formed. Cover slip was placed on each tube for 30 min. The cover slip was removed and carefully placed on a clean grease free glass slide to avoid air bubbles and over flooding. This was then examined under a light microscope (×10) for the presence of helminth eggs. Morphological characteristics of the eggs, as described previously by Soulsby (1987), were used for their identification.

3.7 Data Analysis

Data was analyzed with Statistical Package for Social Science (SPSS) version 17.0. Chi-square was used to determine association between eggs of *Fasciola gigantica* and other helminthes and association between eggs of *Fasciola gigantica* and factors such as age, sex, breed, body condition, and species of animals. Odds ratio (OR) and 95% confidence interval was used on dichotomous variables to test the strength of association. OR values greater than one denotes an association but less than one denotes that the factor may have a protective effect. Percentage based prevalence was calculated using the formula:

$$\text{Prevalence (\%)} = \frac{\text{Number of samples positive}}{\text{Total samples collected}} \times 100$$

CHAPTER FOUR

RESULTS

4.1 Prevalence of *Fasciola gigantica* Eggs in Sheep and Goats

The overall prevalence of *Fasciola gigantica* eggs (plate I) in sheep and goats was 13% (52/400). *F. gigantica* eggs which were characterized by its large, oval and yellowish colour with a distinct flat operculum were seen as described by Urquhart *et al*., 1996. The specific prevalence of *F. gigantica* eggs were 17.0% (34/200) in sheep and 9.0% (18/200) in goats (Table 4.1). There was significant association (OR = 2.07; 95% CI on OR: 1.13 < OR < 3.81) between the prevalence of *F. gigantica* eggs and the species of animals sampled.

Table 4.2 shows the age-based prevalence of *Fasciola* eggs in the species of animals sampled. Higher prevalence of *F. gigantica* eggs were observed in sheep (22.2%) and goats (20.0%) above 2 years of age than in younger ones of sheep (8.1%) and goats (2.4%) that were less than 2 years. There was significant association both in sheep (OR = 0.31; 95% CI on OR: 0.12 < OR < 0.79) and goats (OR = 0.10; 95% CI on OR: 0.03 < OR < 0.35) between the prevalence of *F. gigantica* eggs and age of the animals sampled (Table 4.2).

The highest prevalence of 34.0% (18/53) *F. gigantica* eggs was observed in the poor body condition group (score 2) of sheep and goats, followed by the medium body condition group (score 3) with a prevalence of 11.3% (33/292). The lowest prevalence was recorded in the good body condition group (score 4) which had a prevalence of 1.8% (1/55). There was significant association (p = 0.00) between the prevalence of *F. gigantica* eggs and body condition of the animals sampled (Table 4.3). Also, higher prevalence of *F. gigantica* eggs was observed in

female sheep and goats with respective prevalence rates of 24.2% and 12.1% than in male sheep and goats with rates of 10.5% and 1.7% respectively. Statistical analysis showed that there was significant difference ($p = 0.01$) between the prevalence of *F. gigantica* eggs and sex of animals sampled (Table 4.4).



Plate I: Photomicrograph of *Fasciola gigantica* egg in sheep faecal sample (Light microscope x40 magnification using digital camera)

Table 4.1: Prevalence of *Fasciola gigantica* eggs among sheep and goats in farms within Zaria and its environs, Kaduna State, Nigeria

Species of animal	Number examined	Number positive	Specific rate (%)	Odds ratio (OR)	95% confidence interval on OR
Sheep	200	34	17.0	2.07	1.13 – 3.81
Goats$^{(ref)}$	200	18	9.0	1.00	-
Total	400	52	13.0		

Ref: Reference category

($\square^2$ = 5.659; df = 1; p = 0.017)

Table 4.2: Age-based prevalence of *Fasciola gigantica* eggs among sheep and goats in farms within Zaria and its environs, Kaduna State, Nigeria

Age (yrs)	Number Examined	Number Positive	Specific rate (%)	Odds ratio (OR)	95% confidence interval on OR
***Sheep**					
0-2	74	6	8.1	0.31	0.12 – 0.79
> 2 (ref)	126	28	22.2	1.0	-
****Goats**					
0-2	125	3	2.4	0.10	0.03 – 0.35
> 2 (ref)	75	15	20.0	1.0	-

Ref: Reference category

*($\square^2$ = 6.58; df = 1; p = 0.01)

** ($\square^2$ = 17.73; df = 1; p = 0.00)

Table 4.3: Prevalence of *Fasciola gigantica* eggs among small ruminants with different body conditions in farms within Zaria and its environs, Kaduna State, Nigeria

Body condition	Number examined	Number positive	Specific rate (%)	Odds ratio (OR)	95% confidence interval on OR
Poor	53	18	34.0	27.77	3.55 – 217.49
Medium	292	33	11.3	6.88	0.92 – 51.40
Good$^{(ref)}$	55	1	1.8	1.0	-
Total	400	52	13.0		

Body condition score: Poor = 2, Medium = 3, Good = 4
Ref: Reference category

($\square^2$ = 27.42; df = 2; p = 0.00)

Table 4.4: Sex-based prevalence of *Fasciola gigantica* eggs among sheep and goats in farms within Zaria and its environs, Kaduna State, Nigeria

Sex	Number Examined	Number Positive	Specific rate (%)	Odds ratio (OR)	95% confidence interval on OR
***Sheep**					
Male	105	11	10.5	0.37	0.17 – 0.80
Female(ref)	95	23	24.2	1	-
****Goats**					
Male	59	1	1.7	0.13	0.02 – 0.97
Female(ref)	141	17	12.1	1	-

Ref: Reference category

* ($\square^2 = 6.67$; df = 1; p = 0.01)

** ($\square^2 = 5.45$; df = 1; p = 0.02)

The prevalence of *F. gigantica* eggs were 21.6%, 17.0% and 10.7% for Uda, Yankasa, and Balami breeds of sheep respectively, and 10.4% and 2.7% for Kano Brown and Crosses of goats respectively. There was no statistical significance (p = 0.165) between prevalence of *F. gigantica* eggs and the breed of animals sampled (Table 4.5).

Higher prevalence of *F. gigantica* eggs was observed in ruminants on non-settled farms with prevalence of 18.5% (37/200) than those on settled farms with prevalence of 7.5% (15/200) (Table 4.6). Statistical analysis showed that there was significant difference (p = 0.001) on the prevalence of *F. gigantica* eggs and the category of the farms. Higher prevalence 18.5% (37/200) of *F. gigantica* eggs was observed in ruminants reared by Fulani pastoralist followed by privately owned Farms with prevalence of 8.0% (8/100). The lowest prevalence of 7.0% (7/100) was recorded in Institutional Farms. Statistical analysis showed significant difference (p = 0.005) in the prevalence of *F. gigantica* eggs in ruminants kept in different types of farms (Table 4.7).

The prevalence of *F. gigantica* eggs were found to be 4.8%, 13.7% and 27.0% in ruminants with low, mild and heavy infections of other helminthes respectively. No *F. gigantica* egg was found in animals with negative result for other helminthes (Table 4.8). Other helminth eggs (Plate II-IX) which were identified according to their morphological characteristics had varying prevalence rates as follows: *Dicrocoelium* eggs (3.75%) appeared small, dark-brown and operculate, with a flattened side and contained a miracidium; *Paramphistomum* egg (6.25%) resembles that of *F. hepatica* being large and operculate, but is clear rather than yellow; *Strongyloides* eggs (29.5%) appeared oval, thin-shelled and small, being half the size of typical *Strongle* egg and the eggs were embryonated; *Toxocara* eggs (5.25%) were subglobular with a

thick pitted shell and were almost colourless; *Trichuris* eggs (4.0%) had the characteristic lemon shape with a conspicuous plug at both ends, appearing yellow/brown in colour; *Moniezia* eggs (9.0%) appeared as irregular triangular eggs having a well defined pyriform apparatus; *Strongyle* eggs (70.5%) were large, ovoid and colourless (Table 4.9).

Table 4.5: Breed-based prevalence of *Fasciola gigantica* eggs among sheep and goats in farms within Zaria and its environs, Kaduna State, Nigeria

Breed of animal	Number examined	Number positive	Specific rate (%)	Odds ratio (OR)	95% confidence interval on OR
Uda	37	8	21.6	9.93	1.17 – 84.04
Yankasa	135	23	17.0	7.40	0.96 – 56.69
Balami	28	3	10.7	4.32	0.42 – 43.96
Kano brown	163	17	10.4	4.19	0.54 – 32.55
Crosses of goats$^{(ref)}$	37	1	2.7	1.00	-
Total	400	52	13.0		

Ref: Reference category

($\square^2$ = 9.154; df = 4; p = 0.165)

Table 4.6: Prevalence of *Fasciola gigantica* eggs among ruminants kept on different categories of farm within Zaria and its environs, Kaduna State, Nigeria.

Type of farm	Number examined	Number positive (%)	Specific rate (%)	Odds ratio (OR)	95% confidence interval on OR
Settled	200	15	7.5	0.36	0.19 – 0.67
Non-settled$^{(ref)}$	200	37	18.5	1.0	-
Total	400	52	13.0		

Ref: Reference category

($\square^2$ = 10.698; df = 1; p = 0.001)

Table 4.7: Prevalence of *Fasciola gigantica* eggs among ruminants of different farms within Zaria and its environs, Kaduna State, Nigeria

Type of farm	Number examined	Number positive (%)	Specific rate (%)	Odds ratio (OR)	95% confidence interval on OR
Private	100	8	8.0	0.38	0.17 – 0.86
Institutional	100	7	7.0	0.33	0.14 – 0.77
Fulani$^{(ref)}$	200	37	18.5	1.0	-
Total	400	52	13.0		

Ref: Reference category

($\square^2$ = 10.743; df = 2; p = 0.005)

Table 4.8: Prevalence of *Fasciola gigantica* eggs among ruminants infected with other helminth eggs in farms within Zaria and its environs, Kaduna State, Nigeria

Other helminthes	Number of samples examined	Number positive for *F. gigantica*	Specific rate (%)	Odds ratio (OR)	95% confidence interval on OR
Low infection (+)	145	7	4.8	0.14	0.06 - 0.34
Mild infection (++)	153	21	13.7	0.43	0.22 - 0.83
Heavy infection (+++)$^{(ref)}$	89	24	27.0	1	-
Total	387	52	13.4		

Ref: Reference category

($\square^2$ = 25.92; df = 2; p = 0.000)

Table 4.9 Occurrence of Gastrointestinal parasite eggs in 400 faecal samples from sheep and goats in farms within Zaria and its environs, Kaduna State, Nigeria.

	Number positive (%)	
Parasite eggs	Sheep	Goats
Strongyle	139 (69.5)	143(71.5)
Strongyloides	69 (34.5)	49 (24.5)
Trichuris	7 (3.5)	9 (4.5)
Toxocara	16 (8.0)	5 (2.5)
Moniezia	20 (10.0)	16 (8.0)
Paramphistomum	16 (8.0)	9(4.5)
Fasciola	34(17)	18 (9.0)
Dicrocoelium	10 (5.0)	5(2.5)
Coccidia oocyst	180(90)	187 (93.5)



Plate II: Photomicrograph of *Strongyloides* egg in sheep faeces (Light microscope x40 magnification using digital camera)



Plate III: Photomicrograph of *Strongyle* egg in sheep faeces (Light microscope x40 magnification using digital camera)



Plate IV: Photomicrograph of *Paramphistomum* egg in sheep faeces (Light microscope x40 magnification using digital camera)



Plate V: Photomicrograph of *Toxocara* egg in sheep faeces (Light microscope x40 magnification using digital camera)

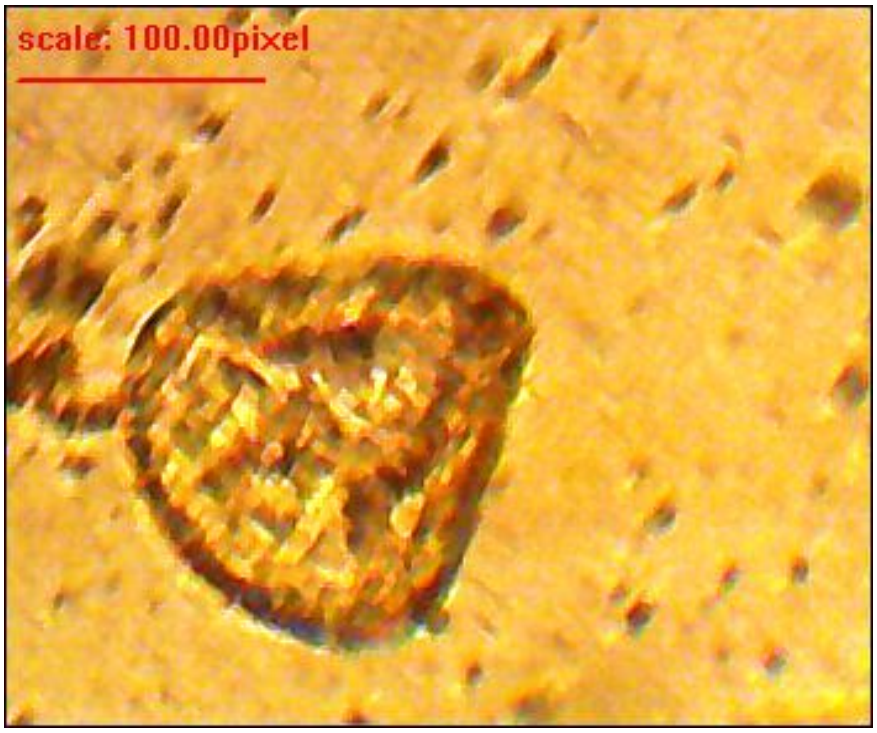


Plate VI: Photomicrograph of *Moniezia* egg in sheep faeces (Light microscope x40 magnification using digital camera)



Plate VII: Photomicrograph of *Dicrocoelium* egg in goat faeces (Light microscope x40 magnification using digital camera)



Plate VIII: Photomicrograph of *Coccidia oocyst* in sheep faeces (Light microscope x40 magnification using digital camera)



Plate IX: Photomicrograph of *Trichuris* egg in goat faeces (Light microscope x40 magnification using digital camera)

4.2 Prevalence of *Fasciola gigantica* Eggs on Salad Vegetables

The overall prevalence of *F. gigantica* eggs on salad vegetables in this study was observed to be 2.2% (4/180). Moreover, the prevalence of *F. gigantica* eggs on salad vegetables sold within Zaria and its environs was 6.7%, 3.3% and 3.3% for lettuce (*Lactuca sativa*), onions (*Allium cepa*) and carrot (*Daucus carota var sativus*) respectively. No *F. gigantica* egg was found on cabbage (*Brassica oleracea var capitata*), tomatoes (*Lycopersicon esculentum*) and cucumber (*Cucumis sativus*) (Table 4.10). Higher prevalence of 6.7% was observed on salad vegetables sold in Sabon Gari market, followed by Giwa and Danmagaji area with prevalence rates of 3.3% each. No *F. gigantica* egg was found on salad vegetables sold in Zaria city, Shika and Samaru market (Table 4.11). Also, prevalence of *F. gigantica* eggs on salad vegetable was observed to be more in Sabon Gari Local Government Area (LGA) (3.3%), followed by Giwa and Zaria LGAs with prevalence of 1.7% each (Table 4.12). There was no significant association ($p = 0.774$) between the prevalence of *F. gigantica* eggs and the areas sampled. The overall prevalence of gastrointestinal parasite eggs observed in this study were 19.4%, 19.4%, 12.2%, 6.1%, 3.9% and 3.3% for carrot, onions, lettuce, tomatoes, cabbage and cucumber respectively (Table 4.13). The following helminth eggs were also observed on the salad vegetables, namely; *Toxocara* (Plate V), *Strongle* (Plate III), *Strongyloides* (Plate II), *Taenia* (Plate X), *Ancylostoma* (Plate XI), *Trichuris* (Plate IX), and *Paraphistomum* (Plate IV).

4.3 Livestock Husbandry Practices

Of the 20 herds sampled and studied, only 5.0% (1/20) practiced zero grazing system while other farms practiced extensive (50%) and semi-intensive (45%) systems of management. A prevalence of 9.3%, 3.8% and 0.0% were observed in animals kept on extensive, semi-intensive

and intensive systems of management respectively. In the farms sampled 80% had other animals such as cattle, donkey, camel or poultry on the farm which we observed a prevalence of 10.3%. Only 45% treat recent animals brought to the farm and also 45% dewormed their animals regularly. A higher prevalence of 9.8% was observed in farms that do not deworm their animals regularly than in those that do deworm their animals in which prevalence rate of 3.3% was observed. A greater majority (90%) of the respondents have found snails and none of the farmers use molluscides. Greater groups (35%) of herd owners use ponds within the vicinity as source of drinking water for the animals and a prevalence rate of 7.5% was observed in this group (Table 4.14).

Table 4.10: Prevalence of *Fasciola gigantica* eggs on salad vegetables at sale in markets within Zaria and its environs, Kaduna State, Nigeria

Vegetable type	Number examined	Number positive	Specific rate (%)
Lettuce (*Lactuca sativa*)	30	2	6.7
Cabbage (*Brassica oleracea var capitata*)	30	0	0.0
Onions (*Allium cepa*)	30	1	3.3
Tomatoes (*Lycopersicon esculentum*)	30	0	0.0
Carrot (*Daucus carota var sativus*)	30	1	3.3
Cucumber (*Cucumis sativus*)	30	0	0.0
Total	180	4	2.2

Table 4.11: Prevalence of *Fasciola gigantica* eggs on salad vegetables sold in different markets within Zaria and its environs, Kaduna State, Nigeria

Market	Number examined	Number positive	Specific rate (%)
Giwa	30	1	3.3
Samaru	30	0	0.0
Zaria city	30	0	0.0
Danmagaji	30	1	3.3
Shika	30	0	0.0
Sabon Gari	30	2	6.7
Total	180	4	2.2

Table 4.12: Prevalence of *Fasciola gigantica* eggs on salad vegetables sold in different Local Government Area within Zaria and its environs, Kaduna State, Nigeria

Local Government Area	Number examined	Number positive	Specific rate (%)	Odds ratio (OR)	95% confidence interval on OR
Giwa	60	1	1.7	0.49	0.04 – 5.57
Zaria	60	1	1.7	0.49	0.04 – 5.57
Sabon Gari[(ref)]	60	2	3.3	1.0	-
Total	180	4	2.2		

Ref: Reference category

($\square^2 = 0.511$; df = 2; p = 0.774)

Table 4.13 Occurrence of Gastrointestinal parasite eggs in 180 vegetable samples sold in markets in Zaria and its environs, Kaduna State, Nigeria.

	Number positive (%)					
Parasite eggs	Carrot	Onions	Lettuce	Tomatoes	Cabbage	Cucumber
Toxocara	11	16	5	4	0	0
Strongyle	9	4	2	1	2	3
Strongyloides	8	4	7	4	1	1
Taenia	2	2	1	0	1	0
Ancylostoma	1	1	1	0	0	0
Trichuris	0	1	1	0	0	0
Coccidia oocyst	3	5	3	2	3	2
Fasciola	1	1	2	0	0	0
Paramphistomum	0	1	0	0	0	0
Total	35 (19.4)	35 (19.4)	22 (12.2)	11 (6.1)	7 (3.9)	6 (3.3)

Table 4.14 Risk factors associated with *Fasciola gigantica* eggs in sheep and goats in the farms sampled

Factors	Number examined (%)	Number positive (%)	Odds ratio (OR)	95% confidence interval on OR
Management systems				
Extensive	10 (50.0)	37 (9.3)	-	-
Semi-intensive	9 (45.0)	15 (3.8)	-	-
Intensive	1 (5.0)	0 (0.0)	-	-
Animals on farms				
Other animals	16 (80.0)	41 (10.3)	0.92	0.45-1.89
Only sheep/goat	4 (20.0)	11 (2.8)	1.00	-
Regular Deworming				
*Yes	9 (45.0)	13 (3.3)	0.36	0.19-0.70
No	11 (55.0)	39 (9.8)	1.00	-
Snails on farm				
Yes	18 (90.0)	50 (12.5)	3.06	0.72-13.10
No	2 (10.0)	2 (0.5)	1.00	-
Regular use of Molluscides				
Yes	0 (0.0)	0 (0.0)	-	-
No	20 (100.0)	52 (13.0)	-	-
Source of water				
*Well	4 (20.0)	4 (1.0)	0.25	0.07-0.91
Borehole	2 (10.0)	3 (0.8)	0.38	0.09-1.60
Tap	5 (25.0)	8 (2.0)	0.41	0.14-1.22
Pond	7(35.0)	30 (7.5)	1.29	0.52-3.19
River	2 (10.0)	7 (1.8)	1.00	-

* Significant association



Plate X: Photomicrograph of *Taenia* spp. egg on salad vegetable (Light microscope x40 magnification using digital camera)



Plate XI: Photomicrograph of *Ancylostoma* egg on salad vegetable (Light microscope x40 magnification using digital camera)

CHAPTER FIVE

DISCUSSION

The prevalence of *Fasciola gigantica* eggs was more in sheep than in goats. The variation in the prevalence among species may be explained by the fact that sheep have indiscriminate type of grazing behaviour while goats are selective grazers which reduced the chance of exposure to infective stage of *F. gigantica* which is commonly found on grasses around marshy areas. Also sheep are social animals hence they graze along with cattle in the fadamas and this may increase the chances of infection. These results agree with the findings of Henok and Mekonnen (2011) who reported prevalence of fasciolosis as 14.6% and 8.8% in sheep and goats, respectively at Hirna town, Ethiopia. It also agrees with the findings of Olupinyo and Ajanusi (2005) who found a prevalence of 21% in sheep and 8% in goats in animals for slaughter at Zaria Abattoir.

This study indicated higher prevalence of *F. gigantica* eggs in adult (above 2years) sheep and goats and lower rate of infection in young animals (less than 2years). This could be due to the fact that young animals are usually kept indoors or around the home and are not allowed to go far with adult animals for grazing, so reducing their chances of exposure to infective parasitic stages when compared to adults.

Higher infection with *F. gigantica* eggs was observed in poor body condition sheep and goats followed by medium body condition and lowest prevalence of *F. gigantica* eggs were recorded in good body condition animals. These findings could suggest the importance of fasciolosis in causing weight loss and emaciation as a characteristic sign of the disease. Besides, the high prevalence of *F. gigantica* eggs in animals with poor condition could be justified by the fact

given by Devendra and Marca, (1983) who indicated animals of poor body condition are vulnerable to parasitic diseases. The significant variation in the prevalence of *Fasciola* infection in relation to body condition could be further explained by the fact that as the body condition improves, infection with *Fasciola* decreased because *Fasciola* worms are known to suck blood and tissue fluid and even damage the parenchyma of the liver due to the migrating immature worms (Marquardt *et al*., 2000). Moreover, cholangitis and liver cirrhosis induced in chronic fasciolosis could reduce bile flow to the duodenum and hence reduced lipid emulsification, digestion and absorption of fatty acid and lipid soluble vitamins (Gargili *et al*., 1999).

More females of sheep and goats were infected compared to males. This may be attributed to the immunosuppressive effect of reproductive hormones of the female animals during pregnancy and lactation period. The prevalence of *F. gigantica* eggs was observed to be lower in settled farms such as private farms and institutional farms than in non-settled farms such as the Fulani pastoralist. This variation could be due to the migratory habits of the nomads to marshy areas in different seasons of the year increasing the chances of exposure to the infective stages of these helminthes.

This study has shown that *F. gigantica* eggs are found on salad vegetables sold in markets within Zaria and its environs. The higher contamination rate in Sabon-gari may be attributed to unhygienic handling of these salad vegetables as it had more retail outlets, and is the most populated out of the six markets. The market also offers more varieties of vegetables at affordable prices hence a higher patronage by consumers. It may also be that because of the higher demand in Sabon Gari, adequate potable water is often not made available within the

market for washing of the fresh vegetables before selling to consumers. In this research study, lettuce had the highest contamination. This agrees with the findings of Maikai *et al*., (2012) and Uga *et al*. (2009), whose works both showed that contamination was high in leafy vegetables followed by root and fruity vegetables. Lettuce is a readily available and affordable salad vegetable compared to the other types of vegetables. As a result, handling of lettuce is always so poor because in each retail outlet, there is more quantity of the produce to be sold out to consumers.

The recovery of eggs of *Fasciola gigantica* on salad vegetables points to the fact that the source of water or manure used on the vegetables maybe contaminated with *Fasciola* eggs. Though the infective stage of *Fasciola* is the metacercariae, finding these eggs on the vegetables is still of significance as it gives an indication of poor hygienic practices. The presence of *F. gigantica* eggs on vegetables is an indication of a high degree of faecal contamination and inadequate washing. It also indicates a high potential for human infection through the vegetables if the snail intermediate hosts are available in the sampled areas. This study may have reemphasized the importance of fasciolosis as a possible zoonotic infection to consumers of contaminated salad vegetables. Likewise, some of the other helminth eggs found on the vegetables are also of public health significance.

CHAPTER SIX

CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

Fasciolosis is an important helminth disease and one of the major obstacles for livestock development in Nigeria causing remarkable direct and indirect losses in different parts of the country where its occurrence is closely linked to the presence of biotypes suitable for the development of snail intermediate host. This study has shown the presence of *Fasciola gigantica* eggs in sheep and goats reared within Zaria and its environs. There is an association between the presence of *F. gigantica* eggs and other helminthes eggs in sheep and goats raised in farms within the study area. Also it was observed that there is an association between the presence of *F. gigantica* eggs and species, sex, age of the animals, as well as management practice and body condition scores. It has shown that *F. gigantica* eggs were more prevalent in ruminants with poor body condition scores than those with medium and good body condition scores. It can be inferred from this study that *F. gigantica* is still of considerable importance in sheep and goats in the study area. Considering the fact that majority of small ruminants die of acute *Fasciola* infections, the observed 13% prevalence is of significance. The presence of *Fasciola* eggs on the vegetables is an indication that the vegetables are potential sources of human infection if the snail intermediate hosts are present in the study areas. Additionally, some of the other helminth eggs recovered from the vegetables are of zoonotic importance.

6.2 Recommendations

The following are hereby recommended;

1. Farmers who rear sheep and goats should improve provision of feeds to their animals so that the animal can have good body condition that confers some level of resistance against *F. gigantica* infection.

2. Farmers should routinely treat their animals with the appropriate antihelmintics and awareness should be created on the prevention and control methods of fasciolosis.

3. Inhabitants of Zaria should be informed and educated with regard to food safety and improvement on sanitary conditions in the local vegetable markets.

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APPENDIX I

DEPARTMENT OF VETERINARY PUBLIC HEALTH AND PREVENTIVE MEDICINE, FACULTY OF VETERINARY MEDICINE, AHMADU BELLO UNIVERSITY, ZARIA

QUESTIONNAIRE

PREVALENCE OF *FASCIOLA GIGANTICA* EGGS IN SHEEP AND GOATS IN FARMS WITHIN ZARIA AND ITS ENVIRONS, KADUNA STATE, NIGERIA.

(I) FARM DATA

1. Date of survey: ----------
2. Location: ----------
3. Type of farm: (a) Settled (b) Non- settled
4. If settled, (a) Private (b) Institutional
5. Type of management system practiced?
   (a) Extensive (b) Semi-intensive (c) Intensive
6. Presence of other animals grazing on the farm? Yes ( ) No ( )

   If yes, specify:

   ----
7. Do you treat animals before they first enter the farm? Yes ( ) No ( )
8. If yes, type of treatment:

   antibiotic + dewormer( ) antibiotic only( ) dewormer only( )

Others (specify): ----------

9. Do you deworm your animals regularly? Yes ( ) No ( )

10. Frequency of routine treatment per year: once ( ) twice ( ) thrice ( )

11. Have you found snails in your farm? Yes ( ) No ( )

12. Do you use molluscides regularly? Yes ( ) No ( )

13. Source of water for animals:
    (a) Well (b) Borehole (c) Tap (d) Stream (e) Pond
    (f) Dam (g) River

Others (specify): ----------

(II) ANIMAL DATA

1. Species of animal: sheep( ) goat( )

2. Breed of animal:----------

3. Sex of animal sampled: male ( ) female ( )

4. Estimated age of animals in years:
   < 1yr ( ) 1 – 2yrs ( ) 2 – 3yrs ( ) 3 – 4yrs ( ) > 4yrs ( )
5. Body condition score:
   (a) 1( ) (b) 2( ) (c) 3( ) (d) 4( ) (e) 5( )