INVESTIGATION INTO *FASCIOLA* INFECTION IN SNAILS AND CATTLE ON THE MAMBILLA PLATEAU, TARABA STATE, NIGERIA

By

GODWIN FIHDILL MANGBON

DEPARTMENT OF VETERINARY MEDICINE

FACULTY OF VETERINARY MEDICINE

AHMADU BELLO UNIVERSITY, ZARIA, NIGERIA

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BY

Godwin Fihdill MANGBON (DVM. MAIDUGURI, 2006)
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DEPARTMENT OF VETERINARY MEDICINE,
AHMADU BELLO UNIVERSITY, ZARIA
NIGERIA.

SEPTEMBER, 2014

DECLARATION

I hereby declare that the work in this thesis titled "**Investigation into *Fasciola* infection in snails and cattle on the Mambilla Plateau, Taraba State, Nigeria**'' has been carried out by me in the Department of Veterinary Medicine under the Supervision of Prof. S.O. Okaiyeto, Prof. L.B.Tekdek and Dr A.J. Natala. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this thesis has been previously presented for another degree or diploma at any institution.

Godwin FihdillMANGBON	……..	…….
Name of student	Signature	Date

CERTIFICATION

This thesis titled "**INVESTIGATION INTO *FASCIOLA* INFECTION IN SNAILS AND CATTLE ON THE MAMBILLA PLATEAU, TARABA STATE, NIGERIA.**" By Godwin Fihdill MANGBON meets the regulationsgoverning the award of the degree of Master of Science, Veterinary Medicine of theAhmadu Bello University Zaria and isapproved for its contribution to knowledge and literary presentation.

PROF. S.O. OKAIYETO	…….	……
Chairman Supervisory Committee	Signature	Date
PROF. L.B. TEKDEK	……..	……..
Member supervisory committee	Signature	Date
DR. A.J. NATALA	…….	……..
Member Supervosory Committee	Signature	Date
PROF. L.B. TEKDEK	……..	……..
Head of Department of Veterinary Medicine	Signature	Date
PROF. A.Z. HASSAN	……..	……
Dean, School of Postgraduate Studies Ahmadu Bello University, Zaria	Signature	Date

DEDICATION

This piece of work is dedicated to the Almighty God, the one who fetches water with a basket to disgrace the bucket, and to my Dear Wife and Mother of my childrenDr Peace Godwin Mangbon.

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ABSTRACT

The objectives of this study were to investigate the occurrence of *Fasciola* infection in cattle from field and slaughter slab sampling, to survey water bodies for presence of snails involved as intermediate hosts in the transmission of *Fasciola* and to ascertain the knowledge of cattle owners on the mode of transmission of the infection on the Mambilla plateau, Sardauna LGA of Taraba state. This study was conducted between June, 2013 and November, 2013. A total of 515 cattle of both sexes and ages were sampled from Gembu and Nguroje slaughter slabs. Liver samples were examined for the presence of flukes while gallbladder and faecal samples were collected and analysed for the presence of *Fasciola* species eggs from each of the animals. Adult flukes were found in 92 (17.9%) liver samples while *Fasciola* eggs were present in the gallbladders of 147 (28.5%) and faecal samples of 141 (27.4%) of slaughtered cattle. In the field survey, faecal samples were collected from 1000 cattle and analysed. Two hundred and eighty samples (28%) were positive for *Fasciola*speciieseggs. The occurrence of *Fasciola* species infection from both field and slaughter slab survey had no significant association with age, sex and geographical location of slaughter slabs ($p>0.05$). There was however, a significant ($p<0.05$) association between detection of adult *Fasciola*speciesin the liver and the presence of eggs in the gallbladder. Histopathological findings in the liver revealed marked extensive haemorrhages, necrosis and congestion of the central vein while sloughing of bile duct epithelium accompanied by fibrous connective tissue infiltration were also seen.Forty eight (48) aquatic habitats in eleven wards of Sardauna L.G.A. that served as drinking points for cattle were sampled for fresh water snails. From the aquatic habitats surveyed for fresh water snails, 158 *Bulinus* species were sampled from one pond which

contained many of that species of snails. None of them was positive for larvae of *Fasciola* species. Fifty (50) questionnaires were administered to herdsmen and cattle owners to ascertain their knowledge of the mode of transmission of the infection. It revealed that none (100%) of them knew how the infection isbeing transmitted. This study has revealed that active infection with *Fasciola* species is prevalent in cattle on the Mambilla plateau but little is known about its mode of transmission in that area.

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CHAPTER ONE

1.0 INTRODUCTION

1.1 Preamble

Fasciolosis is a helminthicdisease of zoonotic importance worldwide (Haridy *et al.* 1999) and iscaused by the parasites*Fasciola hepatica and Fasciola gigantica* which infect mainly sheep, cattle, goats, swine, horses, other herbivores and accidentally man and therefore are of economic importance (Yamaguti, 1958;Boray, 1997, 1999; Daniel and Mitchell, 2002; Mas-Coma *et al.*,2005).The two species are widely distributed across the globe (Esteban *et al*., 1998; Torgerson and Claxton, 1999;Spithill *et al*., 1999; Baig *et al*., 2005; Chen *et al*., 2006; Le *et al*., 2008;). *Fasciola hepatica* is found mostly in temperate regions where sheep and other ruminants are raised. It originated in the European continent and gradually spread to other continents (Boray, 1969). *Fasciola gigantica* is restricted mainly to tropical areas such as Africa, South America, South-east Asia, Southern Europe and Hawaii and also in the former USSR (McCarthy and Moore, 2000). The two species of flukes show a wide distribution in African and Asian continents and have common characteristics. The geographical distribution of *Fasciola gigantica* overlaps with *Fasciola hepatica* in many African and Asian countries and sometimes in the same country, although in such cases the ecological requirement of the flukes and their snail host are distinct (Soliman, 2008). Furthermore, Human fasciolosis has also been reported in developing countries, except Western Europe (Mas-Coma *et al*., 1999). Fasciolosis results in economic losses in livestock productionthrough mortalities, reduced fertility, abortions, slow growth and reduction of milk and meat production, infected livers and withered carcasses (Phiri *et al.,*

2006). Its peak prevalence is duringthe rainy season with possible complications associated with *Clostridium novyi* (Mas-Coma *et al.,* 2005).

Several factors account for the prevalence of fasciolosis, for instance, wet lands, marshy areas and stagnant water favor the multiplication of the intermediate host (snail). Free grazing in those areas allows the *Fasciola*to complete its lifecycle by passing from the snail to the final host (cattle) (Iqbal *et al.,* 2007). In some advanced countries prevalence of fasciolosis may be up to 75 percent (Spithill and Dalton, 1998). In humid regions which are under developed, fasciolosis is considered to be the most prevalent infection in large ruminants being up to 90 percent in Africa and Indonesia, and an alarming 100 percent in India (Spithill *et al.,* 1999). The prevalence rate of fasciolosis due to *Fasciola gigantica* in many parts of Africa has been determined mainly at slaughter as reported by several workers such as Malek (1980) who reported a prevalence rate of 45% and 30% to 90% in Cameroun and Ethiopia respectively. In Kenya, Cheruiyot (1987) reported a prevalence rate of 19.8% while Keyyu*et al.* (2003) reported prevalence of 28.4% to 63.8%. Chambers (1987) in Zimbabwe reported a prevalence of 50% while in Zambia Phiri *et al.*(2005) reported prevalence rate of 53.3% at meat inspection and 48.7% at coprologic examination. In Nigeria, Schillhorn Van Veen (1980) reported a 65% prevalence rate. This arose a lot of interest as many other reports have recently been documented. Adedokun *et al*. (2008) reported a prevalence of 47.4% in Ibadan while Umar *et al.* (2009) in jalingo reported a prevalence of 14.1%. Sugun *et al*. (2010) in Bauchi, Ardo *et al.*(2013) in Adamawa and Aliyu *et al.*(2014) in Zaria also reported prevalences of 76.9%, 21.8% and 14.5% respectively.Infection in humans occurs when they consume uncooked aquatic vegetables or drink fresh water contaminated with metacercariae (Mas-Coma *et al.,* 1999).

The intermediate host for *Fasciola* is the fresh water snail of the genus *Lymnaea* (Bargues *et al*., 2001; 2003). Species of the freshwater snails from the family Lymnaeidae are well known for their role as intermediate hosts in the life cycle of *Fasciola gigantica*; however, throughout the years an increasing number of other molluscan intermediate hosts of *Fasciola gigantica* have been reported based on laboratory tests (Soliman, 2008). There are many ecological factors affecting snail population including temperature, light, hydrogen ion concentration (pH), vegetation, depth and current of the water, chemical composition of the soil, and snail population competition. It has been reported that the *Lymnae*species of snails that serve as intermediate hosts of *Fasciola gigantica* can bedistinguished from those that serve as intermediate hosts of*Fasciola hepatica*, both morphologically and based on their habitat requirements (Soliman, 2008). *Fasciola gigantica* is the most common liver fluke in sub-Saharan Africa (Wamae *et al.*, 1998) likely due to the widespread distribution of its intermediate host *Lymnaea (Radix) natalensis* (Hubendick, 1951). On the other hand owing to a more limited distribution of its intermediate host *Lymnaea (Galba) truncatula* (Hubendick, 1951), *Fasciola hepatica* can exist in zoonotic foci which are more restricted to cooler regions of Africa (Walker *et al*., 2008; Abunna *et al*., 2010; Kanyari *et al*., 2010). Nonetheless, actual or potential overlap of both species of *Fasciola* can occur especially where snail-habitats converge, for example, with increasing altitude as in the Mambilla Plateau (Malone *et al.*, 1998).

1.2 Statement of Research Problem

Cattle play an important role in the economy of Nigeria (Biu and Adindu, 2004; Biu and Babagana, 2004). However parasitic diseases such as fasciolosis which are also of zoonotic importance constitute a major economic problem by lowering productivity of cattle in

addition to losses incurred from condemnation of affected organs at slaughter (Mc Manus and Smyth, 1986; Cook, 1989; Biu and Adindu, 2004). A significant rise in bovine fasciolosis has been reported in the last few years (Mitchell, 2002) which is believed to be due to global warming hence determining a different distribution of the snail *Galba truncatula* which is a required intermediate host for *Fasciola hepatica* (Kenyone*et al.,* 2009; Mas-Coma*et al.,* 2009). A very new aspect of the pathogenicity which was recently uncovered showed thatsome helminth parasitesnotably*Fasciola* species impaired their hosts immune response thereby increasing susceptibility to infection and also affecting the sensitivity of immunologically based diagnostic tests (Claridge*et al*., 2012). Fasciolosis caused by *Fasciola gigantica* is the most economically important helminthic disease of animals in Northern Nigeria (Schillhorn van Veen *et al*., 1980). It has been calculated that bovine fasciolosis represents a median loss of € 299 (nearly ₦ 62,192) per infected cow in Europe(Schweizer *et al.*,2005).

The wide host range of *Fasciola*species and the different habitats of their intermediate hosts make it difficult to define the exact points of infection from parasitological survey alone hence study of the distribution of its associated snail intermediate hosts have also been necessitated (Walker *et al.,* 2008).

There has been no documented study on bovine fasciolosisknown to be conducted on the Mambilla Plateau.Therefore this study was designed to establish the status of *Fasciola*species infectionas well as the habitats and distribution of the snailintermediate hosts for its transmission and to also determine the level of knowledge of cattle owners about fasciolosis on the Mambilla Plateau.

1.3 Justification

On the Mambilla Plateau, cattle are kept on demarcated grazing lands and nomadic practice is absent. These grazing lands are mostly hills surrounded by valleys containing slow and fast flowing streams and rivers which serve as the only source of drinking water for cattle. In the dry season, some of the slow flowing streams are blocked to form artificial dams while the rivers are reduced in volume, hence leaving behind stagnant water bodies which also serve as sources of drinking water for cattle herds, other animals and humans and possibly the habitat of snail intermediate hosts. Incidences of *Fasciola* infection have been recorded in cattle from these herds brought for slaughter to the slaughter slabs.

During the dry season, which is just about three months (November to January), pasture on the hills get dry and withered such that animals resort to competitive browsing on the available pastures around the valleys and fadama areas, hence exposing them to infection when they browse on metacercariae-infested pasture.

Another practice that is likely to predispose both cattle and humans to infection with *Fasciola*speciesis the dry season irrigation farming of vegetables in the valleys and some marshy areas on the plateau and the use of cow dung carried from the hilltops to the valleys as organic manure and also the use of water bodies in the valleys which are either stagnant or slow flowing for irrigation thereby providing a conducive environment for hatching of *fasciola* eggs and survival of the resulting miracidia. The complex nature of the lifecycle and the epidemiology of this snail borne disease coupled with the system of grazing and dry season farming on the Mambilla plateau present challenges for identifying

the exact points where infections are picked up and also makes the institution of effective measures to control or manage the disease at herd or individual level a very difficult task.

Though fasciolosis ranks high among all the zoonotic diseases caused by helminthes worldwide (Haridy*et al*., 2002) it has been so neglected in the public health arena when compared to the attention given to other helminthic diseases (WHO, 2010). It is believed to affect as many as 17 million people worldwide (Hopkins, 1992) with 91.1 million individuals at risk of the infection (Keiser and Utzinger, 2005).

The Mambilla Plateau borders the Republic of Cameroun to the East and also extends to border an important wild life reserve to the North; the Gashaka Gumti National Park which is the largest wildlife park in West Africa. Like elsewhere in Taraba State, the lowland areas surrounding the Mambilla Plateau are also prone to infectionswith *Fasciola* species.

Considering the fact that Mambilla Plateau has a temperate climate and accounts for a greater percentage of the cattle population in Taraba State, the need to investigate the status of fasciolosis, assess the knowledge of cattle owners and herdsmen on its mode of transmission and to clearly define the specific habitat and distribution of intermediate hosts (snails) on the Mambilla Plateau is of vital importance. The findings of this study will enable the prevailing authorities to put in place an effective controlmeasureagainst infection with *Fasciola* species and their snail intermediate hosts and educatecattle owners and herdsmen in Taraba state and Nigeria at largeon the mode of transmission of *Fasciola* species and how to prevent their cattle from contacting it.

1.4 Aim of the Study

To investigate the occurrence of *Fasciola*infection in cattle on the Mambilla Plateau, Sardauna Local Government area of Taraba Stateand presence of snails involved asintermediate hosts in water bodiesand to ascertain the knowledge of cattle owners on the mode of transmission of the Fasciola species infection.

1.5 Objectives of Study

The objectives of this study are to:

i. Evaluate the presence of *Fasciola* infection in cattle on the Mambilla Plateau using slaughter slabs and fieldsamplings
ii. Survey water bodies for presence of snails involved as intermediate hosts in the transmission of *Fasciola* on the Mambilla Plateau
iii. Identify the animal risk factors involved in *Fasciola* infection
iv. Assess the knowledge of cattle owners on the mode of transmission of fasciolosis using questionnaires

1.6 Research Questions

i. Arecattle on the Mambilla Plateau infected with*Fasciola* species?
ii. What are the habitats anddistribution of the snailintermediate hosts in the transmission of this parasite?
iii. What are the animal risk factors involved in *Fasciola*infection?
iv. Are cattle owners knowledgeable about the mode of transmission of *Fasciola* species?

CHAPTER TWO

2.0 LITERATURE REVIEW

Fasciolosis poses an increasing threat among the parasitic infections in many countries around the world as a result of environmental changes and man-made modifications; occurring in areas where conditions are suitable for the survival of its intermediate hosts (Mas-Coma*et al.,* 2009). Considering the worldwide spread, occurrence and zoonotic nature, fasciolosis has emerged as a major global and regional concern due to its effect on all domestic animals and with infection being most prevalent in regions with intensive sheep and cattle production (WHO, 2007; 2008).

2.1 Historical Background of *Fasciola*Species Infection

The earliest known reference to the liver fluke (and liver rot) was made in 1379 by Jean de Brie in France (Borror, 1971) in his treatise on the proper management of sheep. Since then *Fasciola hepatica* has been prevalent in all parts of the world. The first sketch of an adult *Fasciola* isolated from a ram liver was made by Francesco Redi in the sixteenth century (Redi, 1668).Fasciolosis appeared in the form of an epidemic throughout Europe, with the worst hit areas being Holland and Germany. The majority of infections occurred in animals that were pastured in the vicinity of areas that had stagnant water and poor drainage. *Fasciola* hepatica was identified by Linnaeus Carolus (Linnaeus,1758). *"Fasciola"* in Latin means fillet or small bandage and "hepatica" meaning liver.

The earliest appearance of *Fasciola* hepatica in animals dates back to the Roman Iron Age. This was discovered during an excavation undertaken (1996-1999) in a valley in Central

Germany (Dittmar and Teegen, 2003). The excavation was carried out in a graveyard containing skeletons of horses, cattle and humans. Eggs thought to bethose of *Fasciola hepatica* were seen in soil samples taken from the pelvic area of a single human skeleton and a bovine. Snail shells belonging to the genus *Lymnaea* were also recovered from the excavation area (Dittmar and Teegen, 2003).

2.2 Taxonomy and Classification of *Fasciola* Species

Fasciola belongs to the invertebrates and further classification is as follows:

Kingdom: Animalia

Phylum: Platyhelminthes

Class: Trematoda

Subclass: Digenea

Order: Echinostomida

Suborder: Echinistomata

Superfamily: Fascioloidea

Family: Fasciolidae

Genus: Fasciola

Species: *Fasciola hepatica* (Linnaeus, 1758) and *Fasciola gigantica* (Cobbold, 1856).

The adult mature and gravid fluke is flat with its body shaped like a leaf. The size ranges from 25 to 30 mm and 8 to 15 mm in length and width respectively, depending upon the species. The adult parasitizes the liver/or gallbladder of the final hosts (Andrews, 1999). The fluke has an elongated anterior end known as a cephalic cone which has an oral and ventral sucker. The intestines are highly branched and present throughout the body. The male and female reproductive organs are present near the posterior sucker in the center of the body. The female reproductive tract is a dense ovary and is located just above the testes and is linked to a short convoluted uterus that opens in to a genital pore above the ventral sucker. The vitellaria are highly dispersed and divided in the lateral and posterior region of the body. *Fasciola gigantica* and *Fasciola hepatica* parasites are very similar to each other, varying in length and width. In addition, the cephalic cone of *Fasciola hepatica* is shorter than *Fasciolagigantica*. The shape of their eggs is also very similar (Soulsby, 1982). *Fasciola hepatica* measures approximately 150µm x 90µm while *Fasciola gigantica,* approximately 200µm x 100µm (Dunn, 1978).

2.3 Life Cycle of *Fasciola*Species

The life cycle of *Fasciola* species (Figure 2.1) consists of a wide range of final hosts, made up of both domestic and wild mammals (Reinhard, 1957; Boray, 1969). The final host is mainly a vertebrate, including a diverse group of herbivorous mammals such as cattle, buffalo, goat, sheep, horse, zebra, donkey and rabbits. Sometimes humans can also serve as accidental hosts. Furthermore, infection has also been reported in birds (Vaughan*et al*., 1997). The adult worms reside in the hepatic bile ducts of the definitive host where they lays eggs which congregate in the gallbladder and are released with bile into the small intestine (duodenum) and subsequently passed out in faeces. These eggs are further washed

into freshwater (lakes, pools and canals) and hatch under suitable conditions (22–25^{0}C for 9–15 days), releasing a free-swimming miracidium, which burrows into the flesh of an appropriate snail within 8 hours (Mas-Coma *et al*., 2009). Upon penetration of the snail's tissues, the miracidium sheds its ciliated cover and moves to the liver of the snail through its blood vessels. Within 3 weeks of snail penetration, the miracidium develops to the sporocyst stage, which in turn develops to redia. The redia finally develops to cercariae which arereleased from the snail's hepatopancrease at about 6 weeks after infection as free-swimming and straight-tailed (WHO, 2007).Cercariae released from the snail into water locate and attaches to aquatic vegetation where it encysts as infective metacercariae.One miracidium can produce over 600 metacercariae through asexual multiplication(Parthenogony) in the molluscan intermediate host.Humans and animals acquire infection by ingestion of aquatic plants such as watercress, mint, springwater that are contaminated with metacercariae (Markell and Voge, 1999; Mas-Coma *et al.*, 1999; Rondelaud *et al*., 2000; Collins *et al.*, 2000). It has been reported that cercariae of *Fasciola*species can be floating on water and still encyst thus humans can become infected when they drink water contaminated with metacercariae (Chen and Mott, 1990;Aksoy *et al.*, 2005).An experimental study by Taira *et al.* (1997) suggested that consumption of raw or semi-cooked liver parasitized with juvenile flukes also predisposed humans to the infection.After ingestion by a susceptible host, the metacercariae (depending on the number ingested) excysts in the duodenum of the host and burrow through it into the coelom where they wander until they are able to locate the liver. They then penetrate and migrate in the liver parenchyma for 6-8 weeks, before settling in the biliary passages where

they grow to sexual maturity. The adult worms begin producing eggs which are passed out in the faeces 3–4 months after ingestion of metacercariae.The cycle is then repeated.

Juvenile flukes from excysted metacercariae that do not locate the liver remain in ectopic locations either in the peritoneal cavity or they penetrate other organs and tissues such as the lungs, diaphragm, kidneys, intestinal wall and subcutaneous tissue (Boray 1969; Chen and Mott, 1990).

The life cycle of *Fasciola gigantica* is similar to that of *Fasciola hepatica* but with slight differences which include different species of snail as intermediate host, less ability of itsmetacercariae to withstand dryness or harsh conditions and the fact that its developmental process takes longer period compared to *Fasciola hepatica* (Arora and Brij, 2010).

The intermediate hosts of liver flukes are hermaphroditic freshwater snail species, belonging to the Family Lymnaeidae inhabiting water bodies (Mas-coma *et al*., 2005) from which immature stages (cercaria/metacercaria) are made available for definitive hosts or aquatic plants.

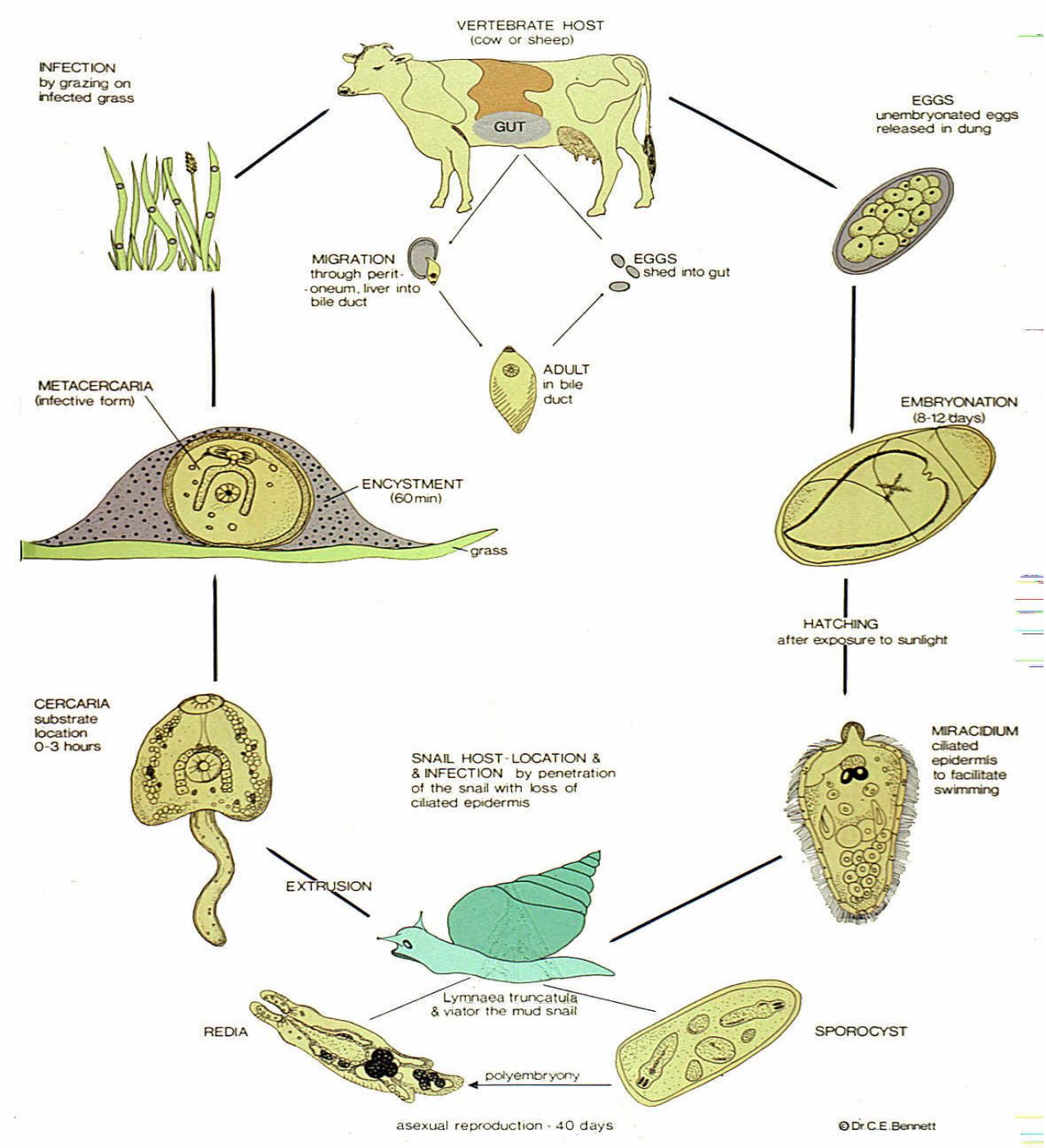


Figure 2.1: Life Cycle of *Fasciola* species. Culled from Dr Clive Bennett (1999;2013)"The *Fasciola* home page"

2.4 Snails as Intermediate Hosts

The intermediate host for *Fasciola*species is the fresh water snail of the genus *Lymnaea* (Bargues *et al*., 2001; 2003). In Europe, Asia, Africa and North America *Fasciola hepatica* is transmitted via *Lymnaea truncatula;* whereas in North America and in Australia *Lymnaea bulimoides* and *Lymnaea tomentosa* are the main intermediate hosts respectively. Other species like *Lymnaea viator* and *Lymnaea diaphena* transmit fasciolosis in South America and *Lymnaea columnella* in the USA, Australia, Central America and New Zealand (Dunn, 1978; Soulsby, 1982; Njau *et al*., 1989; Urquhart, 1996; Yilma and Malone, 1998). Fasciolosis in humans and animals in Brazil is mostly transmitted by *Lymnaea columnella*, which serves as the most important intermediate host (Coelho and Lima, 2003).

The most important intermediate host for *Fasciola gigantica* is *Radix auricularia*. However, other species are also known to harbour the fluke including *Lymnaea rufescens* and *Lymnaea acuminata* in the Indian Subcontinent; *Radix rubiginosa* and *Radix natalensis* in Malaysia and in Africa respectively; and the synonymous *Lymnaea cailliaudi* in east Africa. In Nigeria, the most important genus is *Lymnaea natalensis* which is now called *Radix natalensis* (Adedokun*et al*., 2008). Placement of this species in the genus *Radix* was confirmed by Correa *et al*. (2010).This organism is widely distributed geographically in Nigeria (Ukoli and Asamu, 1979; Thomas and Tait, 1984) and in Africa (Soliman, 2008).

2.5 Morphology of *Fasciola*Species

Due to overlap between *Fasciola hepatica* and *Fasciola gigantica* it is difficult to identify the prevalent species accountable for infection in the tropics. As the distribution patterns of both *Fasciola* species overlap, the emergence of an intermediate form has been reported (Lotfy and Hillyer, 2003). This may be the reason that infections diagnosed due to *Fasciola gigantica* are less compared to *Fasciola hepatica* (Marcilla *et al.*, 2002). The similarity in morphometry of the three species of *Fasciola (Fasciola hepatica, Fasciola gigantica* and the yet to be named intermediate form) is very close (Srimuzipo *et al*., 2000; Terasaki *et al*., 2000). Earlier, morphometric studies on adult flukes or their eggs were used to identify the different species. These were isolated from various domesticated animal hosts (Bergeon and Laurent, 1970). Valero *et al.* (2005) and Periago *et al.* (2006) used the Computer image analysis system (CIAS) technique to measure the variations in morphometry between different populations of *Fasciola.* Previously this technique was performed on morphometry of both pure species of *Fasciola* adults in cattle from different regions (Valero *et al*., 2001; Valero *et al*., 2002). In Egypt, Periago *et al.* (2006) used the CIAS technique to investigate inter and intra specific morphological diversity by analyzing specific morphological characteristics. Ashrafi *et al*. (2006) concluded that among various morphometrical parameters, ventral sucker on the posterior (VS-P), body length (BL)/ body weight (BW), pharynx and oral sucker size can serve as a valuable taxonomic feature for species differentiation by using CIAS technique. Moreover, Lotfy *et al.* (2002) reported that fluke length, testes length and length of the area behind the testes were the most striking dimensions to distinguish the two species. Morphologic, morpho-anatomic and morphometric information can certainly differentiate between species; however these

approaches are inapplicable when the intermediate form is involved. Therefore, in such cases the soluble protein isoelectric focusing (IEF) method has been considered as a useful tool for differentiating the two species (Lee and Zimmerman, 1992; Lotfy *et al.,* 2002). In endemic areas, where there was overlapping of the two *Fasciola* species, the molecular markers provided a better tool for species differentiation (Le *et al.,* 2008). Specific differentiation of various species can only be made by a morphological study of adult flukes and by using molecular tools (Periago *et al*., 2008). In general, fasciolids can be differentiated by morphometric techniques (Ashrafi *et al*., 2006), but flukes with intermediate characters can cause misunderstanding (Itagaki *et al.,* 2005a; 2005b). The existence of two species may result in hybrids through crossbreedingespecially in local areas (Lotfy and Hiller, 2003). This phenomenon has necessitated the use of molecular methods to recognize the existing species (Semyenova *et al.,* 2003, 2005, 2006; Lin *et al*., 2007; Le *et al*., 2008).

2.6 Economic Importance of *Fasciola*Species Infection

In Veterinary Medicine, fasciolosis is considered a great limiting factor in the production potential of small and large ruminants resulting in enormous economic loses in the livestock market (Phiri *et al*., 2006). An economic loss of over 200 million US Dollars annually has been estimated to be caused by fasciolosis in the agriculture budget world-wide (Ramajo *et al.,* 2001) and up to 600 million animals are said to be affected according to articles reported a decade ago (Global plan to combat neglected tropical disease) (WHO, 2007). Apart from this it affects productivity of large ruminants world-wide by causing up to 20 percent loss in body weight, decreased milk and beef production and decreased fertility in cattle (Torgerson and Claxton, 1999). Furthermore, detrimental effects of

fasciolosis in sheep consist of reduction of weight gain and wool production as well as sudden deaths of animals (Sinclair, 1962; Roseby, 1970). Foreyt and Todd (1976; 1982) considered fasciolosis as a major factor of liver condemnation and reduced feed intake resulting in reduced livestock productive efficiency.

In the United States cattle and sheep producers bear annual economic losses of $30,000,000, as a result of direct and indirect effects of fasciolosis. Direct losses are attributed to infected livers at slaughter (Malone, 1986). In England, the direct losses of 600,000 bovine livers represented approximately £1 million / US$1.7 million loss (Haseeb *et al*., 2002). While, indirect losses include reductions in average daily weight gain, reduced milk yield (Bell, 2005; Schweizer *et al*., 2005; Phiri *et al*., 2006) and reduced herd performance in cow-calf operations (Malone *et al.*, 1982; Dargie, 1986). In France, treatment of*Fasciola*-infected dairy cows enhanced conception rates by 23 percent (Mage, 1989). An infection of up to thirty adult *Fasciola* affects the quality and quantity of wool growth in sheep (Dargie, 1986; Clarkson, 1989). In Africa, losses due to fasciolosis were valued to an estimated total of US$201 million. In the tropics, more than 85% of the livestock are owned by small holder mixed farmers and in sub-Saharan Africa in which Nigeria belongs, livestock products contribute over 25% of the GDP (Ogborn, 1999)excluding contributions made by manure and animal draught power used for ploughing and transport as components of many farming systems. All these are greatly impeded when these animals are infected with Fasciola species. Thereforemeasurement and evaluation of economic effects of fasciolosis should not be restricted to losses due to liver condemnation alone.(Hammond and Sewell, 1974; Schillhorn van Veen, 1980; Malone *et al.*, 1982).

Alonge and Fasanmi (1979) reported that 94% of total liver condemnation in some abattoirs in Northern Nigeria was due to fasciolosis.Similarly, Ogunrinade and Ogunrinade (1980) estimated an annual financial loss of US$ 1.03 million as a direct result of mortality due to fasciolosis to be 2-3% of the annual output of the livestock industry which was about 150- 250million Naira. In the same vein, Ibironke and Fasina (2010) in a three year survey of causes of liver rejection reported a gross total liver loss of 8,292 kg with about 75% loss of value in 29,952 kg of partially condemned livers in a single abattoir. When this was extrapolated over the 36 states and the Federal Capital Territory based on theassumption that each will record similar losses in at least one abattoir per state, it translated to a loss ofUS$ 5,762,010 for the whole country. In Taraba state, Umar *et al.* (2009) working in the Jalingo abattoir alone reported a financial loss of ₦5,433,400. This not considering loses from other abattoirs and slaughter slabs within the state.Animal productivity worldwide was affected by fasciolosis with an estimated loss of approximately US$3.2 billion per annum (Spithill *et al.,* 1999).

2.7 Pathogenesis of Fasciolosis

Upon ingestion by a susceptible host, the metacercariae excysts in the duodenal lumen and becomes an immature fluke, it then bores through the intestinal wall and migrates to the liver. The immature fluke feeds on and destroys the liver parenchymal cells hence resulting in widespread hemorrhage and hepatic insufficiency (Boray, 1967). The immature flukes slowly grow into adult and reside in the hepatic bile duct, and since they are hermaphroditic, fertilization and cross fertilization occurs as they attain sexual maturity in the next two months.

There are two phases of infection, the parenchymal or migratory phase and the biliary phase.

The parenchymal or migratory phase is marked by the penetration of immature flukes through the intestinal wall into the peritoneal cavity. *Fasciola* possess affinity for the liver tissue (Dubinský, 1993; Behm and Sangster, 1999) although some of them end up in ectopic locations such as the lungs, diaphragm, intestinal wall, kidneys and subcutaneous tissues (Boray, 1969; Chen and Mott, 1990). During fluke migration mechanical destruction of the tissues occur leading to inflammatory activities around the migratory tracks. When they finally locate the bile ducts, they mature and produce eggs. This is referred to as the biliary phase which results in hyperplasia of the bile ducts. The eggs of flukes are un-embryonated and are released after several weeks of development of the parasite. These eggs are passed from bile ducts into the intestines and are defecated with faeces. The development of the egg is completed after it is deposited in freshwater or in a moist environment.

2.8 Clinical Signs of Fasciolosis

Clinical signs of fasciolosis are always closely associated with infective dose (amount of ingested metacercariae). In cattle, clinical presentation is divided into 4 types (Dubinský, 1993Behm and Sangster, 1999) namely: acute type 1 fasciolosis, acute type 2 fasciolosis, sub-acute fasciolosis and chronic fasciolosis.

Acute Type I Fasciolosis occurs when an animal ingests more than 5000 metacercariae, which may lead to its sudden death without showing any clinical signs(though slight weakness and abdominal haemorrhage may be seen). In Acute Type II Fasciolosis

infection occurs when the animal ingests about 1000-5000 metacercariae. In this case the animal dies and the common signs include; pallor of mucous membranes, loss of condition and ascites. Sub-acute Fasciolosis occurs with the ingestion of 800-1000 metacercariae. Weakness and anemia may occur but weight loss is the dominant feature with death resulting if treatment is not instituted quickly. Chronic Fasciolosis occurs when 200-800 metacercariae are ingested. This is prolonged and asymptomatic except for gradual development of bottle jaw (ventral edema), especially if the host immunity is compromised. (Behm and Sangster, 1999).

In all types of fasciolosis, anemia, hypoalbuminemia, and eosinophilia may be observed (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 weeks after ingestion of metacercariae (Anderson *et al*., 1981 and Sykes*et al*., 1980).Other signs of fasciolosis in sheep include sudden deathas well as reduction in weight and wool production. (Sinclair, 1962 and Roseby, 1970). In goats and cattle, the clinical manifestation is similar to that of sheep. However, acquired resistance to *Fasciola hepatica* infection is well known in adult cattle (Doyle, 1973; Haroun and Hillyer, 1986). Calves are susceptible to disease but an excess of 1000 metacercariae are usually required to cause clinical fasciolosis. In this case the disease is similar to that of sheep and is characterized by weight loss, anemia, hypoalbuminemia and (after infection with 10,000 metacercariae) death (Boray, 1969). Importance of cattle fasciolosis consist of 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 *Clostridium novyi* type B with the release of toxins into the bloodstream resulting in infectious necrotic hepatitis(Black disease) and death ensuesif there is no treatment. As *Clostridium novyi* is common in the environment, Black disease is also common wherever populations of liver flukes and sheep, goats and cattle overlap (Radostits *et al.*, 2006).

2.9 Resistance of Cattle to Infections with *Fasciola*Species

Resistance to fasciolosis is an important aspect in sheep, goats and cattle (Roberts *et al*., 1997; Milligen *et al.,* 1998). Cattle have been shown to acquire resistance to infection with *Fasciola hepatica* and *Fasciola gigantica* when they are sensitized with primary, patent or drug-abbreviated infection (Haroun and Hillyer, 1986; Adeoye and Fashiyi, 1986). Patent infection refers to an infection in a definitive host that results in the appearance of reproductive products of the parasites such as eggs and larvae (Richard *et al.,* 2012), while primary infection refers to the first time that an organism is exposed to a pathogen. During a primary infection, the body has no innate defenses against the organism;antibodies develop later with subsequent exposure to the parasite (Stuttgard and Thiemie, 2005). Resistance to fasciolosis was also documented in rats (Van Milligen *et al.,* 1998). On the other hand, sheep and goats are not resistant to re-infection with *Fasciola hepatica* (Chauvin *et al.,* 1995 and Martinez-Moremo *et al*., 1997). However, there is evidence that two sheep breeds; the Indonesian thin tailed sheep and the red maasaai sheep, are resistant to *Fasciola gigantica* (Wamae, 1996; Roberts *et al.,* 1997).

2.10 Diagnosis of Fasciolosis

The most common method of diagnosing liver fluke infection has been faecal sedimentation for egg counts (Happich and Boray, 1969). This method of coprological

examinations may not be sufficient to identify infections in animals and humans when flukes are migrating and often damaging the liver. It may also be ineffective when the parasitic burden is too small for detection therefore, immunonological methods have to be relied upon for early and prompt diagnosis (Hillyer, 1988; Martinez-Moreno *et al.,* 1997; O'Neill *et al.,* 1998; Hillyer, 1999) but unfortunately many of these immunological diagnostic kits are experimental and not readily available for use in regular practice. Other procedures that are based on detection of antigens in sera, milk or in faeces (Torgerson and Claxton, 1999) have also been used for diagnosing *Fasciola* infection. Also, clinical signs and some changes in hepatic enzyme levels and haematological profiles may be an aid but changes in these parameters are not specific for parasitic infection. Climatic conditions, epidemiology of the flukes and the study of snails and their habitat can also be taken into account (Dubinsky, 1993). Immunodiagnosis provides earlier detection of fasciolosis within a month of infection (Zimmerman *et al.,* 1982; Duménigo *et al.,* 2000) but parasitological examination is still commonly used, though it can only give indications about 8-10 weeks post infection. In humans, a condition known as pseudo-fasciolosis or false fasciolosis may sometimes be encountered which may not be from an actual infection but due to eggs observed in the faeces as a result of recent ingestion of infected livers containing eggs (Hillyer, 1999). In addition, biochemical and haematological examinations of human sera are useful adjuncts in diagnosis, as are ultrasonography and radioisotope thermoelectric generators (RTGs) of the abdominal cavity, biopsy of liver and gall bladder punctuate.

2.10.1Faecal egg counts(Coprological Technique)

The principle behind this technique revolves around the fact that flukes have heavy eggs which do not float on water therefore it is necessary to use either liquids of high density for flotation, sedimentation or sieving techniques. Moreover, due to the low fecundity of adult worms, application of the concentration method becomes necessary (Salem*et al.,* 2007). The following techniques are applied in the faecal egg count method of diagnosis:

2.10.1.1 Flotation method

The flotationmethod is a qualitative test based on the separation of eggs from faecal material and concentrating them by means of a flotation fluid with an appropriate specific gravity. It can be used in conjunction with the McMaster technique to detect low numbers of eggs (when present below the McMaster sensitivity of 50 eggs per gram of faeces). It is widely used, though it has been modified according to the parasites being investigated. Modifications are related to the weights and specific gravities of the faecal samples, the volume of the liquid depending on its density and the surface on McMaster slide that the sample is being examined on. It has been stressed that the ratio between the weight of faecal samples and specific gravity must not exceed 1:15 and egg counting must be done on the whole surface of the McMaster slide (Cringoli*et al.,* 2004). Iodomercurate of potassiumis the best liquid for fluke egg per gram (epg) counts;unfortunately, it is not environment friendly thus it has been banned in many countries.

2.10.1.2 Sedimentation method

This technique appears to be more accurate and sensitive than flotation techniques (Boray, 1969). Cup sedimentation using tap water seems to be simpler and cheaper than the flotation technique in some cases but it is more time consuming. It is possible to improve on this method by four times sedimentation of 10grams of faeces before the final examination of sediment in a McMaster slide on the bottom of the chambers or with the use of formol-ether which is a high density liquid to reduce the number of sedimentations. The sensitivity of this method was reported to be up to 33.3% (Conceicao*et al.,* 2002). To improve the accuracy of this technique, it is advisable to increase the sample from 10grams to 30grams of faeces especially when manipulating low egg- shedding; this gives a better sensitivity (Rapsch*et al.,* 2006).

2.10.1.3 Sieving techniques

An original four-sieve-technique is accurate for the quantitative and qualitative assessment of the presence of eggs of *Fasciola hepatica* in the faeces (Girao and Ueno, 1994). Samples are homogenized with tap water then slowly passed through four sieves of respective openings: 174, 96, 87 and 65 mm which are on top of each other. They are slowly washed under running water discarding the first 3 sieves one after the other. Material retained on the last sieve (65mm) is transferred to an edged Petri dish and examined. The sensitivity of this method is high giving room for counting 1 egg per gram and even less according to the weight of the fecal sample.

2.10.2Reliability and limitations of coprological techniques

There is a reluctance to use egg per gram counts for identification of fluke infection due to limited accuracy, and false negative results related to a sensitivity of 30 % (Happich and Boray, 1969).However this may be improved as indicated by several researchers using sedimentation or sieving methods when the amounts of faecal samples are increased to between 30 and 50grams. Coproscopy can be very efficient in case of repeated sampling, resulting in sensitivity of approximately 92% (Rapsch*et al.,* 2006). Nevertheless,*Fasciola* egg per gram counts may be falsely negative during the migration phase since immature worms passing through the parenchyma do not lay eggs. Moreover, with low parasitic burdens the output is small and egg laying may be irregular and pass undetected. Flukes are not prolific egg layers so the number of eggs is always very low. It has been reported that if there are less than 20 flukes in bile ducts, the sensitivity of epg counting methods is too low to show eggs in the faecal sample. Being aware of these limitations, a positive result is a definite proof of an active infection and the foci of infection for the herd with eggs being released onto pasture and infecting the snails. Moreover, coprological examination gives additional information on other parasites, which may be of great value in an overall approach to a Bovine Health Management Programme (Dorchies, 2007).

2.10.3 Hepatic Enzymes

Before the development of immunological diagnostic techniques many researchers studied extensively damage to hepatic tissues and to bile duct epithelium with their resultant effects on the hepatic enzymes such as glutamate dehydrogenase (GLDH) and glutamateoxaloacetate aminotransferase and Gamma-glutamyl transferase. These

enzymesindicate damage to hepatic cells and bile duct epithelium.Their levels are increased during the migratory phase of *Fasciola*species from eight to twelve weeks post infection(Anderson *et al*., 1981). This is followed by a decrease and frequently interrupted by brief increases, between twelve to twenty weeks post infection (Wensvoort and Over, 1982). Many researchers have dealt with the kinetics of these enzymes during *Fasciola* infection illustrating the ongoing changes during infection (Gaasenbeek*et al.,* 2001). Unfortunately when parasitic burdens are very low the dosage of this enzyme is not useful (Salem*et al.*, 2007).

2.10.4 Serological Diagnosis

Several methods are described, most of them relate to the use of ELISA test and their variations but counter-electrophoresis, haemagglutination and indirect immunofluorescence tests may also be used. The majority of tests are applied to the detection of blood antibodies and the latest to the detection of antigen in faeces or milk (Dorchies, 2007).

2.10.4.1 Serological tests for *Fasciola* species

ELISA tests are currently used by vast majority of investigators.An indirect haemaglutination test has been very well adapted to bovine fasciolosis (Levieux*et al.,* 1992a; Levieux*et al.,* 1992b). It is reliable, cheap and easy to use. Moreover, it is applicable for sheep, horses, man and other susceptible hosts because it does not necessitate a specific conjugate for each species.It involves the detection of antigens and antibodies in blood. It is also applicable on milk and faecal samples.

2.10.4.1.1 ELISA detection of antigens in blood

Detection of circulating antigens is a very good way to confirm the presence of living parasites in bile ducts. Several trial results have been published and they seem to be very promising, some of them were able to detect the presence of infection in animals as early as six days after infection (Leclipteux*et al.,* 1998).

2.10.4.1.2 ELISA detection of antibodies in blood

This is currently the most frequently used assay with somatic f2-antigen (Institut Pourquier, Montpellier, France) with a crude excretory/secretory (ES) antigen or with purified/recombinant antigen. ES products are produced by various methods. Briefly, *Fasciola hepatica* freshly recovered from an infected animal is incubated at various temperatures, in saline or buffer, for a set time. It seems that ES products are very similar whatever the mode of recovery (Hillyer, 1998). Purified antigens obviate the occurrence of non-specific positive reactions: Cathepsin L1, a cysteine proteinase from gut epithelial cells of flukes cleave immunoglobulin and inhibit antibody-mediated attachment of eosinophils to newly excysted juveniles. This enzyme is present in ES products and may be isolated (Smith *et al.,* 1993). ELISA antibody tests are reliable, with high sensitivity and specificity of up to 95% and 98.2 % respectively with the use of f2-antigen (Reichel, 2002), and up to 98% and 96% respectively with the use of Excretory/ Secretory antigen containing predominantly fluke cysteine protease (Salimi-Bejestani*et al.,* 2005). They allow detection of early infection, sometimes after the first week of infection (Arias*et al.,* 2006) and false positive results are limited when using purified antigen. On pooled sera, herd prevalence of infection as low as 5 per cent,can be detected using f2-antigen ELISA

(Reichel *et al.,* 2005). Nevertheless, positive results do not determine whether or not live flukes are present: detectable antibodies may persist long after treatment or after the natural death of liver flukes. A positive result indicates that the animal is, or has been, infected by the parasite, antibodies detected in natural infection may or may not be related to an active infection. Moreover it may be assumed that nonspecific polyclonal stimulation of B lymphocytes induces production of specific antibodies, for example, a *Toxocara vitulorum* antigen consisting of five polypeptides (137.7 kDa, 81 kDa, 75 kDa, 48 kDa and 21.6 kDa) has been found to be cross reactive with *Fasciola gigantica* (Abdel-megeed and Abdel-rahman, 2004).

2.10.4.2 Application of serological tests for *Fasciola* to bulk tank milk

The detection of infected herds from bulk milk samples appears more difficult than by use of blood ELISA. Bulk milk ELISA results are consistently lower than the corresponding bulk serum ELISA results. Using a commercial kit with f2-antigen, it has been demonstrated that only bulk milks from herds with infection prevalence of at least 60 per cent were identified (Reichel *et al.,* 2005). With another test it was possible to identify herds in which more than 25 per cent of the cows were infected (Salimi-Bejestani *et al*., 2005).

2.10.4.3 Detection of *Fasciola* copro-antigens

This is an ultra-sensitive capture method, using monoclonal antibody (mAbMM3), produced by immunisation of mice with a 7 to 40 kDa purified and O-deglycosylated fraction of *Fasciola hepatica* ES products (Mezo*et al.,* 2004). This ultra-sensitive method is also very specific with no cross-reaction even for animals infected by *Dicrocelium*

lanceolatum. It has to be stressed that the identification of antigen in sera or in faeces would be a very useful tool to control the parasitism allowing selective treatment of infected animals. Risks of anthelmintic resistance and residues would be decreased if these tests were available and inexpensive.

2.10.5 Pathology

Lesions produced by *Fasciola*occur mostly in the liver although occasionally, the parasite may lodge in other tissues such as the lungs and kidneys where they are usually found within abscesses (Jones and Hunt, 1983).

2.10.5.1 Gross Pathology

Gross pathological lesions depend on the stage and severity of the infection (Burger, 1992). The disease is characterized by anaemia due to severe liver damage caused by immature flukes tunneling through and feeding on the liver parenchyma which results in hemorrhages, extensive hepatic fibrosis, hyperplastic cholangitis, irregular outline, pallor and firmness of the liver with the ventral lobe being most affected and reduced in size (Urquhart*et al*., 1987; Biffa*et al*., 2006 and Talukder*et al*., 2010). Urquhart*et al*. (1987) further observed that the fluke eggs sometimes provoke a granuloma-like reaction that can lead to ectasia and stenosis of the affected ducts. Thickening of the bile duct and fibrosis of the portal area and sometimes evidence of calcification are mostly observed in the chronic stage of the infection (Okaiyeto*et al*., 2012).

2.10.5.2 Histopathology

Many workers have noted varying lesions in livers affected with *Fasciola* species (Uzoukwu and Ikeme, 1978; Phiri, 1997 and Okaiyeto*et al*., 2012). While some have reported that lesions produced by migrating stages of liver flukes include hemorrhagic tracts of erythrocytes and degenerating hepatocytes mixed with variable numbers of eosinophils, polymorphs and mononuclear cells (Singh and Parihar, 1988), others have described lesions in the bile ducts and periportal areas showing hyperplastic changes with variable increase in the peri-ductular connective tissue. Uzoukwu and Ikeme, (1978) reported marked interlobular fibrosis, proliferation of bile ducts and peri-biliary fibrosis with numerous micro- abscesses containing *Fasciola* larvae or eggs found accompanying cellular reactions and calcifications. Recently, a study was carried out on the changes associated with chronic *Fasciola*infection in a farm of eleven (11) pregnant Friesian cattle in a dairy farm in Niger state, Nigeria by Okaiyeto*et al*. (2012) in which one of the cows died in the process of parturition and post-mortem examination was conducted. The histopathological changes reported in the liver in this study were characterized by fibroblasts mixed with lymphocytes and few mononuclear cells in the areas previously migrated by the young flukes. Areas of atrophy, necrosis and fatty changes were also reported.

2.11 Treatment of Fasciolosis

Several benzimidazoles, salycilanilides, nitrophenols and halogenated carbons have been demonstrated as having a good efficacy either for adult fluke in the bile ducts, or on immature and adult flukes in the liver, or on all stages. Clinicians have to adapt their

prescriptions to suit the system (beef cattle, dry cows, dairy or suckler cows) and the period of infection (presence or not of early/late immature or adult flukes) considering the fact that not all compounds are equally effective against all stages of development of *Fasciola* as drugs differ in their efficacy, mode of action, price and availability. Triclabendazole (TCBZ), a benzimidazole derivative, has been the drug of choice for treatment of liver fluke infections in livestock for more than 20 years and is also used to treat human fasciolosis (Fairweather, 2005). In contrast to most other fasciolicides, triclabendazole has high efficacy (approximately 99%) against both the mature adult worms in the bile duct and the migratory immature flukes, which is significant since the immature flukes cause the most tissue damage during their migration through the liver (Fairweather & Boray, 1999). In humans, the safest and most effective drug used is Triclabendazole (Savioli *et al.,* 1999; Ishii *et al.,* 2002) although Nitazoxanide and Bithionol have also been used with successin treating *Fasciola* infection in humans (Rossignol *et al.,* 1998; Ramachandran, 2000). In the same struggle to do away with these flukes and their devastating effect on man and livestock, an Egyptian drug made from myrrh, has been investigated as an oral treatment of parasitic ailments, including fasciolosis and schistosomiasis called Mirazid (Soliman*et al.,* 2004).Mirazid® proved to be a promising alternative fasciolicidal drug and although its mode of action is still being investigated,the fluke's tegument seems to be the main target of Mirazid® and myrrh volatile oil. When tried, the results suggested that Mirazid® fasciolicidal activity can be further reinforced by increasing its content of myrrh volatile oil(Massoud *et al.,* 2013)

In the treatment and control of animal fasciolosis (Overend and Bowen, 1995; O'Brien, 1998; Mitchell *et al*., 1998 and Fairweather and Boray, 1999) the use of halogenated

phenols (Bithionol, Bitin), hexachlorophene (Bilevon), Nitroxynil (trodax®).), Salicylanilides (closantel (flukiver®, supaverm®), Rafoxinide (flukanide, Ranizole)), Benzimidazoles (triclabendazole (fasinex®), Albendazole (vermitan®, valbazen®), Mebendazol (Telmin®), Luxabendazole (fluxacu®). Phenoxyalkanes (diamphenetide (coriban®)), Praziquantel and Ivermec (ivomec super®) has been employed buttriclabendazole is still the most effective drug for use in animals. Constant use of these drugs may result in resistance (Moll *et al*., 2000). In New South Wales, resistance was found to rafoxanide, closantel and nitroxynil. In western England and in Wales, resistance to salycilanilide has also been described. Resistance is manifested by the immature but rarely by the adult flukes (Fairweather & Boray, 1999). This may be the reason why resistance to clorsulon has not yet been recorded. Loss of efficacy of triclabendazole in naturally infected sheep has been reported in many countries which include Australia (Overend and Bowen, 1995), Ireland (Lane, 1998; O'Brien, 1998), Scotland (Mitchell *et al.,* 1998) and the Netherlands (Moll *et al.,* 2000). As with nematodes, there is no reversion to susceptibility after switching to other drugs (Borgsteede *et al.,* 2005). The spread ofTriclabendazole resistance has also fuelled the search for novel fasciolicidal compounds. A Triclabendazole derivative, designated compound alpha, has proven to be highly active against immature and adult *Fasciola hepatica* (Ibarra *et al*., 2004; Fairweather, 2005) and has recently been shown to disrupt the tegument of flukes from a triclabendazole -resistant isolate (McConville *et al.,* 2007). Other experimental fasciolicides such as artemether and OZ78 have also been reported to have activity against triclabendazole - resistant flukes (Keiser *et al.,* 2007).

For the treatment of acute fasciolosis, it is essential to choose a product highly effective against the juveniles that damage the liver parenchyma. For chronic disease, a compound active against the adult fluke is required. Product safety is an important consideration as hepatic detoxification mechanisms are already impaired. Flukicides can be used therapeutically for treatment against the disease or prophylactically to prevent outbreaks. Some bind to plasma proteins (e.g. closantel) or erythrocytes (clorsulon), thereby extending their period of protection (Fairweather and Boray, 1999). All flukicides either have milk-withholding periods or are prohibited from use in animals providing milk for human consumption and so the best time to treat dairy cattle is at the drying off stage. Triclabendazole is a compound specifically for use against *Fasciola hepatica* in sheep (10 mg/kg) and cattle (12 mg/kg). Higher doses are required for the control of *Fasciola gigantica* in buffalo.

The general strategy for control using anthelminthics proposed by FAO recommended treatment at the end of a period of ecologically reduced activity of the parasite and snail intermediate host. This is followed by treatment one to two months after the expected peak of infection in host and an additional treatment in heavily infected areas or where infection may be acquired throughout the year (Morel and Mahato, 1987; Rai *et al.,* 1996). If the source of infection is herbage and water at the fringes of rivers, most reports in Nigeria recommend strategic treatment with anthelmintics about September and again in about February to treat infection acquired from July to December (Schillhorn Van Veen *et al.,* 1980; Okaiyeto *et al*., 2011).

2.12 Prevention and Control of Fasciolosis

Much of the literature on the control of *Fasciola gigantica* is based on successes and failures of methods used in temperate countries to control *Fasciola hepatica* (Spithill *et al*., 1999). These include strategic anthelmintic treatment, grazing management, application of molluscicides, and fencing off or draining swampy areas. The relevance of these approaches to control of *Fasciola gigantica* in the tropics must be questioned and control options developed on the basis of a sound understanding of the mode of transmission of the parasite. Relatively little is known about the epidemiology of *Fasciola gigantica* and this hampers the development of control strategies for tropical production systems in areas where the infection is endemic. These are often areas where irrigated rice is cultivated intensively. Although regional recommendations have been made for some developing countries (FAO, 1994), in most areas of tropical Asia and Africa there are no routinely used control programs for fasciolosis (Spithill *et al*., 1999). To be appropriate for the low-income, smallholder-based systems of most of Africa, control measures for *Fasciola gigantica* should be low-cost, readily available and applicable with little disruption to existing agricultural practices. Snail control using molluscicides, feeding management and biological control are alternatives that may complement the use of anthelmintics. Realistic control options will only be effective if there is a sound understanding of when and how transmission of the infective stages of the parasite is taking place and widespread adoption of measures to control the infection will ultimately depend on farmers being convinced that the benefits from control justify the costs of implementing them (Williams and Loyacano, 2001).

2.12.1 Prevention and control in ruminants

Several control methods against ruminant fasciolosis are available and can either be used independently or as a combination of two or more of them. These methods involve reduction in the number of intermediate snail hosts by chemical or biological means, strategic application of anthelmintics, reduction in the number of snails by drainage, fencing and other management practices and reduction in the risk of infection by planned grazing management (Roberts and Suhardono, 1996).Specific aim of the control programmes may be to prevent the build-up of parasites in the environment and to avoid areas of heavily contaminated pasture (Brunsdon, 1980). Various attempts have been made at controlling fluke infections, the most successful appears to be routine deworming of the animals using anthelmintics and this is best done at the end of the rainy season, shortly before the rains and in the middle of the rains. Triclabendazole and clorsulon have high efficacy against both mature and immature *Fasciola gigantica* (Suhardano *et al.,* 1991). Strategic deworming of ruminants at sufficient period during the year prevents the occurrence of the disease and faecal contamination of pasture (Okaiyeto *et al*., 2011).

An alternative or additional advance in the control of animal fasciolosis would be the development of anti-*Fasciola* vaccines. Given the current political climate regarding the deposition of agro-chemical residues, together with more onerous legislation governing the development of new anthelmintic drugs, it is increasingly likely that the future control of helminthic infections may rely heavily on this approach. Although a range of vaccines against *Fasciola hepatica* infections have been developed by several laboratories (reviewed by McManus & Dalton, 2006) none are commercially available at present. However, since the incidence of animal fasciolosis is increasing in Europe and

anthelmintic resistance continues to spread, it is more likely that a vaccine will offer the level of protection that is comparable with the existing anti-fluke drugs hence, becoming more financially attractive to pharmaceutical companies (Dalton & Mulcahy, 2001).

2.12.2 Prevention and control in Humans

Control of human fasciolosis depends on reducing infection in animals and preventing the contamination of edible aquatic plants with infective metacercariae. This could be achieved by introducing good control programmes that involve widespread drug treatment of humans and animals coupled with educating the affected farming communities on aspects of parasite transmission and the importance of separating animals from areas where food is grown. Since infection is highly age-related, with highest prevalence in children between 8 and 11 years old, drug treatment should be particularly focused towards children in high-risk areas (local schools being a focal point) (Mas-Coma*et al.*, 2004). Mas-Coma. *et al.* (1995; 2004) also reported that in highly endemic areas, prevention of human fasciolosis can be ensured by avoiding the consumption of aquatic plants containing metacercariae.

2.12.3 Snail Control

The control of the snail intermediate host offers a great deal in controlling liver flukes. They can be controlled chemically by the use of molluscicides such as copper sulphate (CuSo4) solution at1part per million (ppm) for destruction of the snail and their eggs (Berger, 1991). It can also be applied with sand extender at 10-35kg per hectre (Soulsby, 1982), and livestock should not be allowed to graze until after rainfall due to its toxicity (Taylor, 1964; Berger, 1991). However, this approach has not achieved widespread acceptance; furthermore, certain parts of the pasture can be difficult to access with

insufficient amount of the chemical. Moreover, as a result of high biotic potential of snails, pasture can quickly become re-infected, making repeated applications necessary, thus adding to expense and increasing risk of contamination. Therefore, slow release formulations combined with snail attractant if made available may reduce some of the potential risks to the environment.

2.12.3.1 Natural Molluscicides.

There has been an increasing interest in the exploitation of plants with natural molluscicidal activity. Some Eucalyptus spp have molluscicidal effect in situ (Hammond *et al.,* 1994). The latex of *Euphorbiales* spp has been shown to kill some snail species that are intermediate hosts to flukes (Singh and Agarwal, 1992). Unfortunately, there appears to be no work quantifying the molluscicidal effect of these plants in the field.

2.12.3.2 Management and Biological Control of Snails

Reducing snail abundance through improved pasture, either by drainage or fencing off areas prevents infection of grazing areas. However, this approach is expensive and is not always cost effective (Wilson *et al*., 1982). The *Lymnaeid* snails are vulnerable to predators such as arthropods, amphibians, reptiles, birds and rodents. Generally, snails exist in equilibrium with their predator species, although intensive duck and goose husbandry has been shown to eliminate *lymnaeid* snails and effectively control fasciolosis (Levine, 1970; Rai *et al.,* 1996; McDonnell *et al.,*2009). Certain *sciomyzid* fly larvae have been shown to predate *lymnaeid* snails and this has been proposed as a method for the biological control of snails in Ireland (Berg, 1952; Over, 1982).

2.12.3.3 Physical control of Snails

Irrigation projects can provide the snail with ideal habitats. Regular clearing of vegetation from drainage channels may reduce silting and blockages that normally support snail-contaminated herbage. Seepages from irrigation channels often harbor large snail colonies in low-lying areas; adequate drainage would prevent accumulation of fresh water snails for extended periods in wet low-lying areas. Draining marshy pastures and building dams may also reduce snail habitats and increase grazing areas (Boray *et al.*, 1985).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Location

This study was carried out on the Mambilla Plateau of Taraba State which lies on latitude $5^0$31'' and 7°18'' North and on the longitude $10^0$18'' and 11° 37'' East. It stands at about 1850 meters above sea level and covers an area of over 1,389 square kilometers (3,625 sq m) (Fidelis, 2007) withan annual rainfall of over 1850 millimetres (http://www.onlinenigeria.com/links) which spans up to nine months (March to November) of the year (Lenshie*et al.,* 2012). It has a temperate climate with lush pasture and green vegetation. Day time temperatures hardly exceeds 25 °C (77.0 °F) making it the coldest plateau in Nigeria. The access route up the Plateau is adorned by natural springs that bore through rocks with powerful water falls. The landscape is so mountainous and undulating that flat lands are scarcely seen. Some mountains on the plateau and around it are over 2,000 meters (6,562 ft) above sea level making them the highest mountain ranges in Nigeria (Physical map of Nigeria. Freeworldmaps.net. 2011) and in West Africa.Some rivers, notable among them the Donga River and Taraba River, both have their sources on the Mambilla Plateau. The Plateau is bordered on the North by Nigeria's largest game reserves, the Gashaka Gumti National Park (The Gashaka Primate Project, 2011) and on the East by the Republic of Cameroun.

Agriculture is concentrated in the valleys while the highlands have been extensively grazed. The practice is such that mixed cropping is done in the rainy season while in the dry season, varieties of vegetables are farmed using streams and water bodies in the valleys

for irrigation and cow dung used as organic manure. It is tsetse fly free and harbours over one million herds of cattle (Lenshie*et al.,* 2012).Trading in cattle is done on designated days of the week in the various cattle markets located in Gembu, Nguroje, Mayo- Ndaga, Dorofi and Mbamnga. The practice is that cattle are moved from all over the plateau to such markets where buyers purchase either to stock their farms or to transport them down to the Eastern and Southern parts of the country.Some buyerstransport them across the border into Cameroun. Other cattle owners from the neighboring Cameroun also bring in their cattle to the market to sell. The plateau also has three Federal Animal Control Posts namely; Mayo- Ndaga, Nguroje and Dorofi Animal Control Posts.



Fig. 3.1 Map of Taraba State showing Sardauna Local Government (Mambilla Plateau). Culled from http://www.cometonigeria.com

3.2 Sample Size

Sample size was calculated using the formula by Mahajan (1997)

$n=Z^2Pq/d^2$

Where q= complementary probability (1-p)

n= minimum sample size

P= prevalence of Bovine fasciolosis from previous studies (52.3%) by Adedokun *et al.* (2008)

d= desired absolute precision 0.05

Z = appropriate value for the standard normal deviate set at 95% confidence interval (1.96).

Therefore $n= \frac{1.96^2 \times 0.523 \times 0.477}{0.05^2}$

$= 383$

However the sample sizes were extrapolated to 515 and 1000 for cattle sampled at slaughter and those sampled on farms respectively. This was done to give a better representation of the population of cattle within the study area during the period under study.

3.3 Study Design and Sampling Procedure

The study animals were the breeds of cattle indigenous to the Mambilla Plateau (the Adamawa gudali and few red Bororo) which have been interbred extensively and span the whole length and breadth of the plateau.

This study involved four components. The first component was sampling cattle which were slaughtered in Gembu and Nguroje slaughter slabs in which both ante-mortem physical examination and post-mortem examinations were carried out on each of the animals slaughtered. The second component was the field sampling that also involved taking of faecal samples from live animals on farms in the various Wards forcoprological analysis. The third component involved identifying snail habitats and examining snails for larval *Fasciola* infection in the water bodies on the Mambilla plateau while the fourth component was the administration of questionnaires assessing the knowledge of cattle owners and herdsmen on the mode of spread of fasciolosis on the Mambilla plateau.

3.3.1 Sampling at Gembu and Nguroje Slaughter slabs

This was carried out from June to October 2013 and involved collection of faeces and gallbladder of each slaughtered animal and the examination of dissected liver tissues for presence of immature flukes. Gembu and Nguroje slaughter slabs were used and all cattle that were brought to these slabs for slaughter within this period of survey were sampled. Willingness of the slaughter house management and cattle owners to participate in the survey was sought. Five hundred and fifteen (515) cattle were sampled. For each animal, a thorough physical examination was conducted at ante mortem which included taking rectal temperatures, resting respiratory and pulse rates. Other examinations conducted

werechecking for ocular, nasal, oral and vaginal discharges. The mucous membranes of the eye, mouth and the vulva were also examined as well as the hair coat and skin, then the age was estimated by dentition according to the principle that there are four pairs of temporary incisors or milk teeth which are usually replaced by permanent ones one pair at a time at a certain fairly predictable interval during the first three to four years of the cattle's life by a process of wearing (Pace and Wakeman, 2003).

At post mortem, gallbladder for each slaughtered animal was also collected. Dissected liver tissues were also inspected thoroughly for presence of migratory immature and mature flukes and infected liver tissues were collected.Fecal samples, gallbladder and affected liver tissues collected were all taken to the Mambilla Baptist Hospital Laboratory for furtherexamination.

3.3.2 Field Sampling in eleven Wards in Mambilla Plateau

This was undertaken from June to November 2013. It involved the collection of faecal samples from a total ofone thousand (1000) live cattle randomly selected from different herds in each of the eleven (11) wards of the Mambilla plateau, Sardauna Local Government Area. Cluster sampling was employed with each of the Wards serving as a cluster,then a random number generation method of cattle owners and location of herds was done. Number of cattle sampled per Ward was based on their percentage to the total number of cattle in the study area. The age of each animal was estimated by dentition and by information from the herdsmen or cattle owners. Cattle estimated to be less than two years old (<2 years) were categorized as young, those from two years to five years (2-5

years) were categorized as young adults while those above five years old (>5 years) were categorized as adults.

3.3.3 Survey of water bodies for Snails and larval stages of*Fasciola*species

Water bodies to be sampled were selected based on visualizing presence or absence of snails regardless of density (Madsen and Chimbari, 2003), visual evidence of hoof prints and cow dung or actual presence of cattle at such designated points and based on information offered by herdsmen or cattle owners as points where cattle accessed water and grass. Forty eight (48) selected freshwater sites within the eleven (11) Wards were surveyed for aquatic snails. Sites chosen included a variety of streams, marshes and pools to cover as wide an area as possible. Using collecting sieves and snail scoops 158 snails were collected. The presence and numbers of each genera of snail was recorded according to field identification keys of Brown using morphological features such as form, shape, number of whorls and direction of coiling (Brown, 1994).

3.3.4 Questionnaire Administration to assess knowledge of cattle owners on mode of transmission of *Fasciola* species

This involved the administration of a set of questionnaires to assess knowledge of cattle owners on the mode of transmission of *Fasciola* species on the Mambilla plateau. Fifty (50) questionnaires were administered to various cattle owners at random in each of the eleven (11) Wards and their responses were also documented accordingly. The data collected included information on the sex and age of respondents and the size of their herds, the system of management and the means by which number of cattle in the farm is increased, information on the sources of water and feeds for the animals during the rainy

and dry seasons,information on the general awareness of cattle owners and herdsmen on *Fasciola* infection evidenced by their knowledge of its local name and their level of knowledge on its mode of transmission. The questionnaire was administered in Mambilla and Fulfulde languages as the common languages of communication within the study area.

3.4 Collection and Handling of Samples

Faecal samples were collected from the rectum of each animal using gloved hands and dropped into clean leak-proof, labeled polythene bags, separate for each animal. Whole gallbladders were also collected and dropped into labeled polythene bags. Affected liver tissues collected from the slaughter slabs were dropped into labeled bottles containing 10% formalin. Collected snails were dropped into labeled bottles containing 10% formalin. These were all transported to the Mambilla Baptist Hospital (MBH) Laboratory in a clean, cold flask.

3.5 Laboratory Analyses:

Faecal samples were analyzed for *Fasciola* eggs and egg count conducted using the technique described by Urquhart *et al*.(1996).10 grams of faeces was measured into a beaker and 40 mls of normal saline was added into it and stirred thoroughly with a tongue blade until a homogenous mixture was obtained and filtered through a tea strainer into another beaker. The particulate faecal matter that was retained by the filter was discarded while the filtrate was transferred into a labeled test tube, centrifuged and spun at 3000 rotations per minute for 5 minutes. Afterwards the supernatant was carefully decanted and the sediment stirred again thoroughly with normal saline and recentrifuged. This was repeated three times and then a pipette was used to transfer part of the sediment onto a

microslide, covered with a coverslip and viewed under x100 magnificcations.10 different parts of each sample were viewed and the results obtained was inferred on that particular sample.

Bile collected from the gall bladder was examined for *Fasciola*eggs using the method described by Thienpont *et al.* (1979). This was carried out by mixing bile with equal volumes of normal saline and straining through a tea strainer and centrifuging at 3000 rotations per minute for 15 minutes. The clear supernatant was decanted and the sediment recentrifuged. This was repeated three times and the sediment was examined under a microscope at x100 magnifications.

Gross pathology of affected parts of liver and bile duct were also recorded. Affected liver tissues were examined whole for generalised liver fluke infection. Incisions were made in different parts of the liver and examined for presence of flukes in the parenchyma, and the bile ducts were opened to check for adult flukes. The liver was then cut into slices using a sharp knife and pressed to squeeze out flukes from its tissue and smaller bile ducts,then fixed in 10% buffered neutral formalin solution to be processed for histopathological examination using the method ofArora and Iyer (1973).

The fixed tissues were washed in running water after which they were dehydrated through a series of ascending grades of alcohol, cleared in two changes of chloroform and finally embedded in hard paraffin. Sections were cut at 5 to 6 microns by using a rotary microtome. All sections were stained routinely with Harris Haematoxilin and Eosin for detailed histopathological examinations at x400 magnification.

Collected snails were identified according to field identification keys of Brown (1994). The opening of the lymnaeid snail is on the right when the snail is held with the spires pointing away from the viewer. When the apex of the spires is facing the viewer the spires turn clockwise.To examine for the presence of cercaria or other larval stages of *Fasciola* species, the snails were crushedand used to make smears on clean dry grease-free glass slides, and examined under the microscope at a magnification of × 400.

3.6 Data Analyses

Data obtained from this study were analysed and presented as percentages and tables,otherswere expressed as mean $\pm$ S.E.M. Chi- square and fisher's exact test were also used where necessary. Pearson correlation was also employed to correlate the occurrence of the flukes in the liver of the cattle to the presence of their eggs in the gallbladder. Values of $P< 0.05$ were considered significant.

CHAPTERFOUR

4.0 RESULTS

4.1 Sampling in Gembu and Nguroje Slaughter Slabs

4.1.1 Ante-mortem findings

Ante-mortem clinical evaluation in cattle slaughtered at Gembu and Nguroje slaughter slabs in Mambilla Plateau,Sardauna LGA ofTaraba State is shown in Table 4.1.The rectal temperatures, respiratory and pulse rates of all (515) animalswere within normal range with average values of38.51^{0}C, 37 cycles per minute and 62.5 beats per minuterespectively. Muco-purulent nasal discharges accompanied with serous ocular dischargeswere observed in 4 (0.7%) of the animals. Pallor of mucous membrane was also observed in 51(9.9%)cattle all of which turned out to be positive for *Fasciola*infection. Only 1(0.2%) cattle had an icteric mucous membrane. Mild tick infestation was noticed on 384 (74.6%)of cattle. Ruffled hair coat and patches of alopecia was observed on 11 (2.1)while 4(0.7%) of them had exudative pustular dermatitis with extensive scab forming crusts which adhered firmly to the affected skin and was further held in place by hair fibresand removedcrusts revealed moist hyperaemic areas. There was also heavy dandruff on the hair coats which was ruffled. Other conditions observed on physical examination were 6 (1.2%) animals with fractured limbsand 5 (0.9%) animals that were obviously pregnant.

Table 4.1Ante-mortem clinical signs observed in cattle at Gembu and Nguroje slaughter slabs

Description	Observed clinical signs	Frequency of sign	Sign specific (%)
Mucous membrane	Pallor	51	9.9
Mucous membrane	Jaundiced	1	0.2
Discharges	Occullo-nasal	4	0.7
Haircoat and skin	Ruffled hair coat and patches of alopecia	11	2.1
	Pustular dermatitis with scabs	4	0.7
	Tick infestation	384	74.6
Other conditions	Fractured limbs	6	1.2
	Pregnancy	5	0.9
Total		476	92.4

4.1.2:The Prevalence of *Fasciola* speciesinfection in the liver, gallbladder and faeces of cattle slaughtered at Gembu and Nguroje slaughter slabs.

Table 4.2 shows the prevalence of *Fasciola* species infection in the liver, gallbladder and faeces of cattle slaughtered at Gembu and Nguroje slaughter slabs on the Mambilla Plateau. A total of 515 cattle were slaughtered,made up of 364 (70.7%) inGembu slaughter slaband 151 (29.3%) in Nguroje slaughter slab. At the Gembu slaughter slab, the prevalence of *Fasciola* species infection were 17.6%, 26.7% and 29.7 in the liver, gallbladder and faeces respectivelyand for Nguroje slaughter slab, the prevalence of *Fasciola* species infection were 18.5%, 33.1% and 21.9%in the liver, gallbladder and faeces respectively. The overall prevalence of *Fasciola*species infection in the liver, gallbladder and faeces of cattle slaughtered at Gembu and Nguroje slaughter slabs were 17.9%, 28.5% and 27.4% respectively. The differences in prevalence between sites of *Fasciola* infection were not significant ($p > 0.05$).

Table 4.2The Prevalence of *Fasciola* speciesinfection in the liver, gallbladder and faeces of cattle slaughtered at Gembu and Nguroje slaughter slabs.

Abattoir Location	No. of Animals Slaughtered		Liver		Gallbladder		Faeces	
	No.per slaughter slab	(%)	No. Affected	(%)	No. Affected	(%)	No. Affected	(%)
Gembu	364	70.7	64	17.6	97	26.7	108	29.7
Nguroje	151	29.3	28	18.5	50	33.1	33	21.9
Total	515	100.0	92	17.9	147	28.5	141	27.4

LIVER	$X^2 = 0.06712$,	p= 0.7956
BILE	$X^2 = 2.1870$,	p = 0.1392
FAECES	$X^2 = 3.2790$,	p = 0.0702

4.1.3:Distribution of *Fasciola* species infection in the liver, gallbladder and faeces of cattle slaughtered in Gembu and Nguroje slaughter slabs based on sex.

Sex distribution of *Fasciola*species infection in the liver, gallbladder and faeces of cattle slaughtered in Gembu and Nguroje slaughter slabs on Mambilla Plateau is shown in Table 4.3.The prevalence was higher in gallbladder of males (35.6%) than in the gallbladder of females (27.9%) while it was low in the liver of both males and females at values of 17.8% and 17.9% respectively. The prevalence was also higher in the faeces of males (33.3%)than in the faeces of females (26.8%).These differences were not significant ($p>0.05$).

Table 4.3 Sex distribution of *Fasciola*species infection in the liver, gallbladder and faeces of cattle slaughtered in Gembu and Nguroje slaughter slabs on Mambilla Plateau

Sex	Animals Slaughtered	Liver		Gallbladder		Faeces	
	No. of cattle sampled (%)	No. Affected	(%)	No. Affected	(%)	No. Affected	(%)
Male	458.7	8	17.8	16	35.6	15	33.3
Female	47091.3	84	17.9	131	27.9	126	26.8
Total	515100.0	92	17.9	147	28.5	141	27.4

LIVER $X^2 = 0.0002503$ p = 0.9874
BILE $X^2 = 1.1890$ p = 0.2756
FAECES $X^2 = 0.8794$ p = 0.3484

4.1.4:Distribution of *Fasciola* species infection in the liver, gallbladder and faeces in cattle slaughtered in Gembu and Nguroje slaughter slabs based on age.

Age distribution of *Fasciola* species infection in the liver, gallbladder and faeces in cattle slaughtered in Gembu and Nguroje slaughter slabs on the Mambilla Plateauis shown in Table 4.4. Considering hepatic infection, the prevalence was highest in the age group of <2 years (50%) and lowest in the age group of >5 years (16.3%). Considering *Fasciola* infection in the gallbladder, prevalence was highest in the age group of <2 years (50.0%) and lowest in the age group of 2-5 years (24.0%). However, in faeces the prevalence was highest in the age group of >5 years (24.9%) and lowest in the age group of <2 years (0.00%). These differences were not significant ($p>0.05$).

Table 4.4 Age distribution of *Fasciola*species infection in the liver, gallbladder and faeces in cattle slaughtered in Gembu and Nguroje slaughter slabs on the Mambilla Plateau.

Age(Years)	Total no. of Cattle sampled	Liver		Gallbladder		Faeces	
		No. Affected	(%)	No. Affected	(%)	No. Affected	(%)
<2	2	1	50.0	1	50.0	0	0.0
2-5	200	40	20.0	47	24.0	63	31.0
>5	313	51	16.3	99	31.6	78	24.9
Total	515	92	17.9	147	28.5	141	27.4

LIVER $X^2 = 2.5550$ $p = 0.2787$
FAECES $X^2 = 2.7850$ $p = 0.2484$

4.2 Field Survey of *Fasciola*Species Infection in Cattle in Eleven Wards in Mambilla Plateau, Sardauna Local Government Area of Taraba State.

4.2.1 Distribution of *Fasciola*species infection in cattle sampledin eleven Wards ofMambilla Plateau.

The Prevalence of *Fasciola*species infection in faeces of cattle sampled from eleven Wards in Mambilla Plateauis shown in Table 4.5. A total of 1000 cattle were sampled on farm.The prevalence was highest in Magu Ward (44.0%) while it was lowest in Gembu B Ward (21.1%).Prevalence for other Wards was Mbamnga Ward (25.6%), Gembu A Ward (24.0%), Warwar Ward (24.4%), Ndumyaji Ward (24.1%), Kabri Ward (28.3%), Kakara Ward (37.5%), Titong Ward (23.1%), Mayo-Ndaga Ward (25.7%) and Nguroje Ward (29.4%). The overall prevalence of *Fasciola* species infection in cattle sampled from the eleven Wards was 28.0%. The difference in prevalence was not significant ($p>0.05$).

Table 4.5The Prevalence of *Fasciola*species infection infaeces of Cattle sampled on field in eleven Wards in Mambilla Plateau.

Location(Wards)	No. of cattle sampled per Ward	No. infected	Percentage (%)
Gembu B	19	4	21.1
Mbamnga	43	11	25.6
Gembu A	25	6	24.0
Warwar	41	10	24.4
Magu	25	11	44.0
Ndum-yaji	29	7	24.1
Kabri	166	47	28.3
Kakara	72	27	37.5
Titong	134	31	23.1
Mayo-Ndaga	136	35	25.7
Nguroje	310	91	29.4
TOTAL	1000	280	28.0

$X^2 = 9.865$ $p = 0.4524$

4.2.2 Sex based distribution of cattle infected with *Fasciola* species in eleven wards in Mambilla Plateau

Sex distribution of *Fasciola* species infection in Faeces of cattle sampled on field in eleven Wards of Mambilla Plateauis shown in Table 4.6. More females 557 (55.7%) were sampled than males 443 (44.3%). The prevalence of *Fasciola*species infection was higher in females (30.3%) than in the males (25.1%). These Differences in prevalence among sexes was not significant (P>0.05).

Table 4.6Sex based distribution of cattle infected with *Fasciola* species in eleven wards in Mambilla Plateau

Sex	Total no. of Cattle sampled		No.of cattlePositive	
	No.	(%)	No.	(%)
Male	443	44.3	111	25.1
Female	557	55.7	169	30.3
Total	1000	100.0	280	28.0

$X^2 = 3.4180$ $p = 0.0645$

4.2.3 Age based distribution of cattle infected with *Fasciola* species in eleven wards in Mambilla Plateau

Age distribution of *Fasciola* species infection in faeces of cattle sampled on field in eleven Wards of Mambilla Plateau is shown in Table 4.7. The prevalence of *Fasciola*infection was highest in cattle within the age group of >5 years (31.6%) while it was lowest in cattle within the age group of <2 years (23.5%). The prevalence of infection in cattle within the age group of 2-5 years old was 26.2%. These Differences in prevalence among the different age groups was not significant (P>0.05).

Table 4.7Age based distribution of cattle infected with *Fasciola* species in eleven wards in Mambilla Plateau

Age (years)	Total no. of Cattle sampled		No. of Cattle positive	
	No.	(%)	No.	(%)
<2	98	9.8	23	23.5
2-5	535	53.5	141	26.4
>5	367	36.7	116	31.6
TOTAL	1000	99.0	280	28.0

$X^2 = 4.0850$ $p = 0.1297$

4.3 PATHOLOGICAL LESIONS OBSERVED

4.3.1 Gross pathological lesions

The infected livers were slightly enlarged with rounded edges and numerous petechialhaemorrhagic patches scattered over the parietal surface. Upon incision, extensivehemorrhagemarking migratory tracts of immature flukes wasnoted(Plate I).Hypertrophy of the bile duct with emergence of adult Fasciola species observed (PlateII). The bile duct produced a gritty sound when cut throughand adult flukes were seen ranging from 10 to over 50 in number (Plate III).

4.3.2 Histopathological changes

The histopathological examination of the liver revealed massive necrosis and atrophy. The necrotic lesions indicated by karyorhexis and karyolysis of nuclei and many hepatocytes were seen without nuclei (Plate IVA).Numerous mononuclear cells were also seen (Plate IV B). Branches of portal and hepatic veins were congestedwith some containing thrombi (Plate V).Sloughing off of bile duct epithelium into the lumenleading to production of granular mucosa was noticed(Plate VI) as was infiltration offibrous connective tissuesin the muscularis of the bile duct (Plate VII).

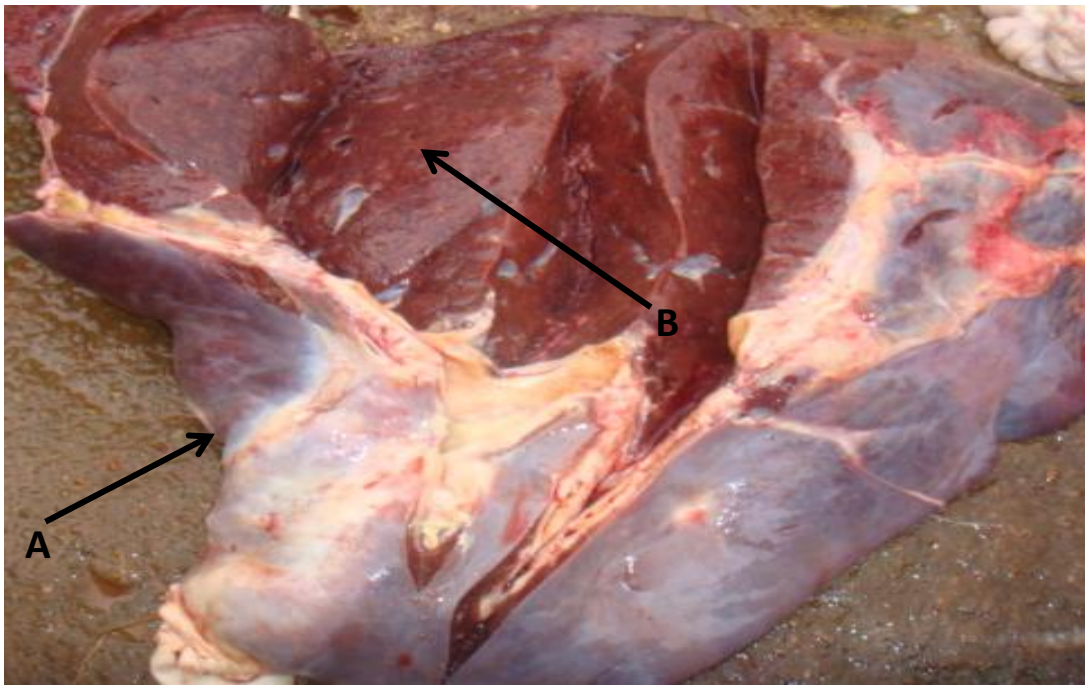


Plate I:Liver of Cattle from Gembu slaughter slabshowing
(A)Rounded edges
(B)Extensivehaemorrhage within parenchyma.

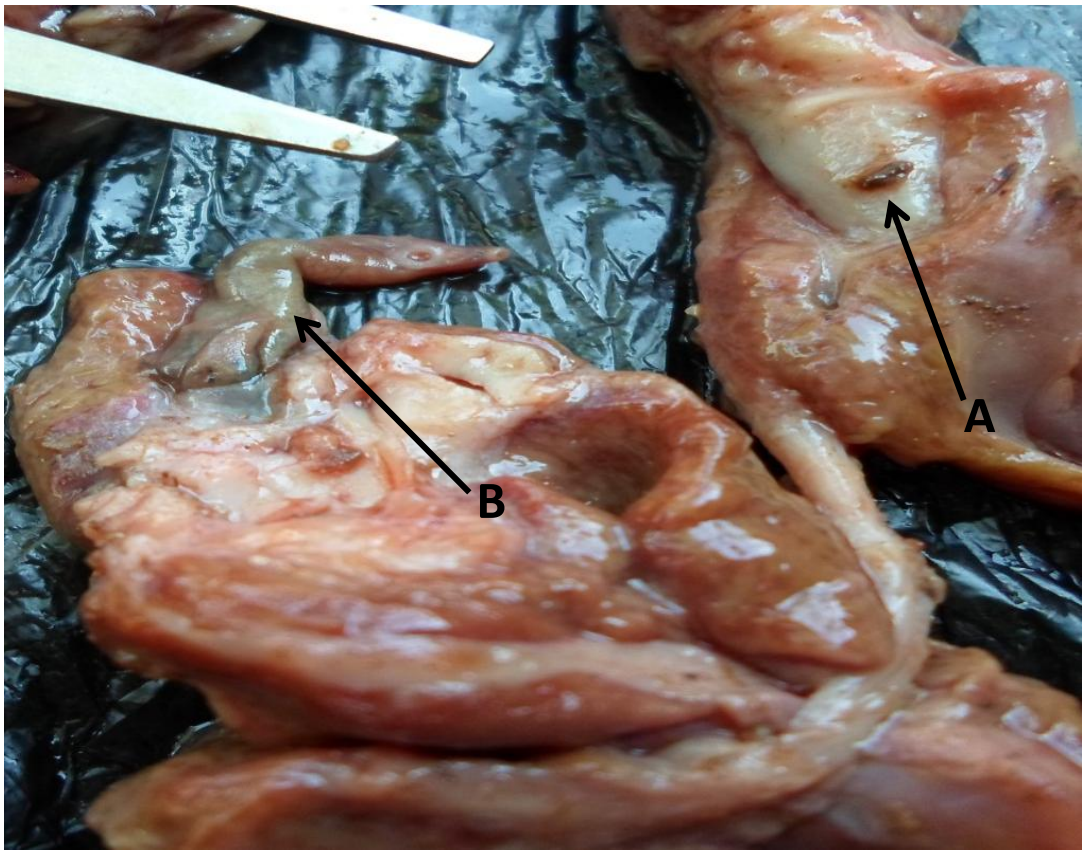


Plate II:Liver of Cattle slaughtered in Gembu slaughter slab showing
(A) Hypertrophiedbile duct due to accumulation of flukes
(B) Adult *Fasciola*species emerging from a bile duct.

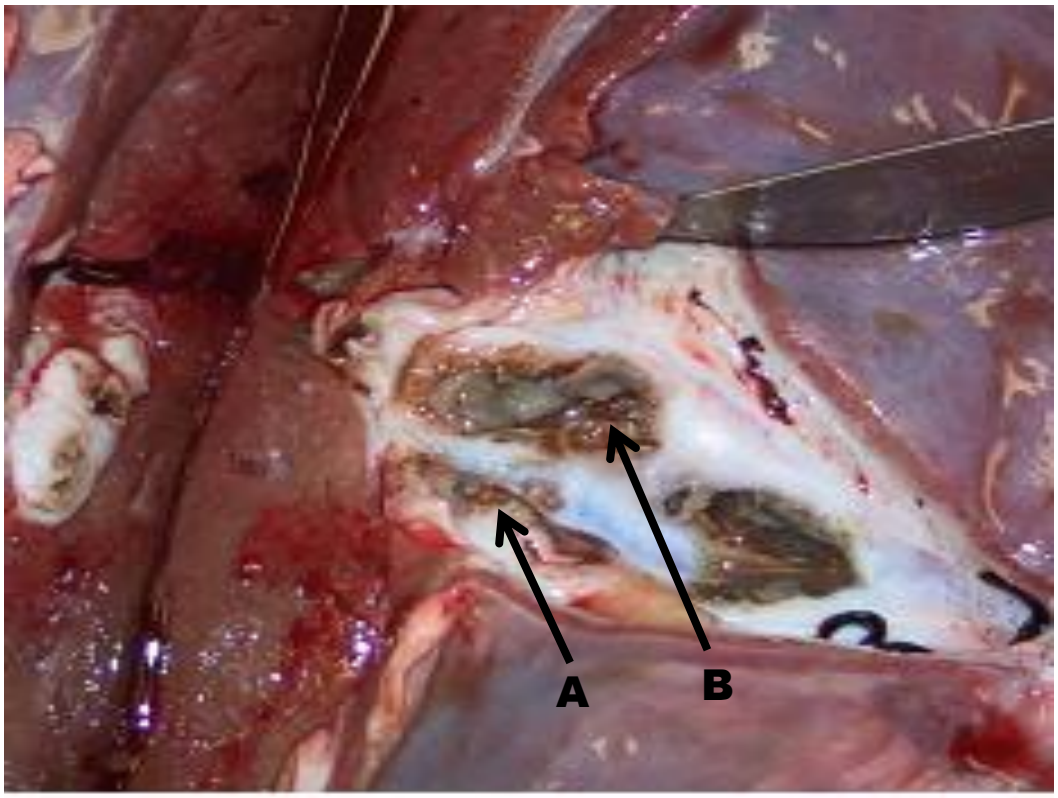


Plate III:Liver of Cattle from Nguroje slaughter slab showing enlarged bile duct (A)opened to reveal adult flukes (B).

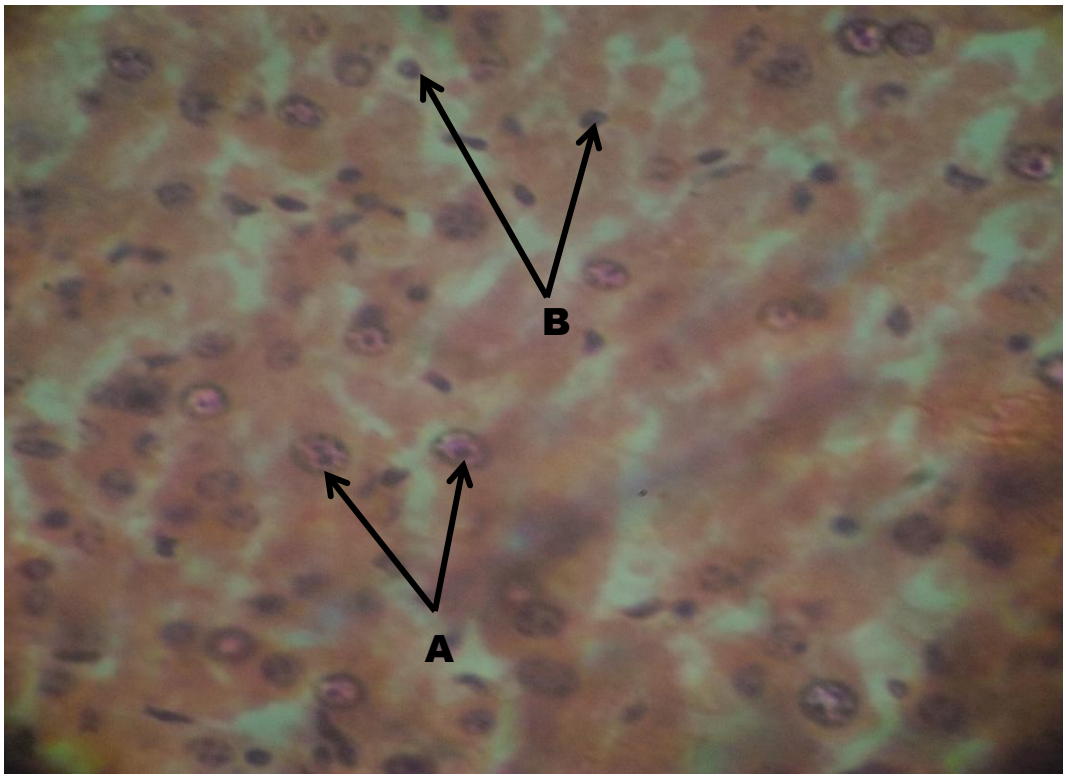


Plate IV:Histopathological section of liver tissue of Cattle slaughtered at Gembu slaughter slab showing

(A) Hepatocytes with fragmented nuclei and others devoid of nuclei

(B) Mononuclear cells infiltration (H&E X400)

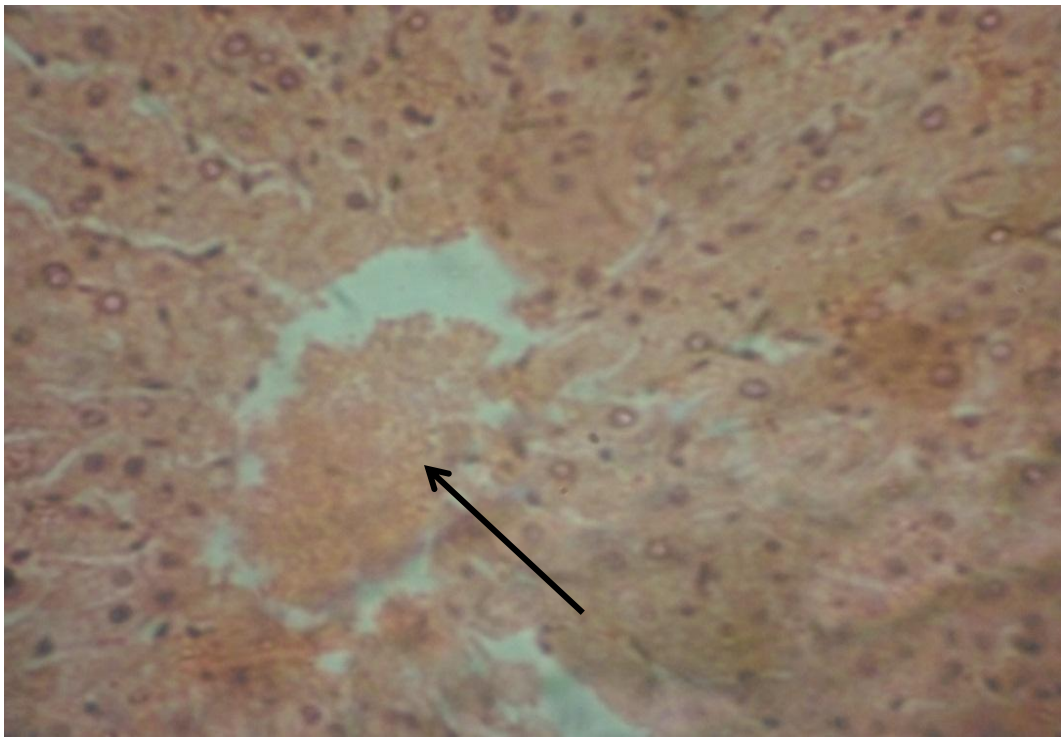


Plate V:Histopathological section of liver of Cattle slaughtered in Gembu slaughter slab showing Congestion within hepatic vein (H&E X 400)

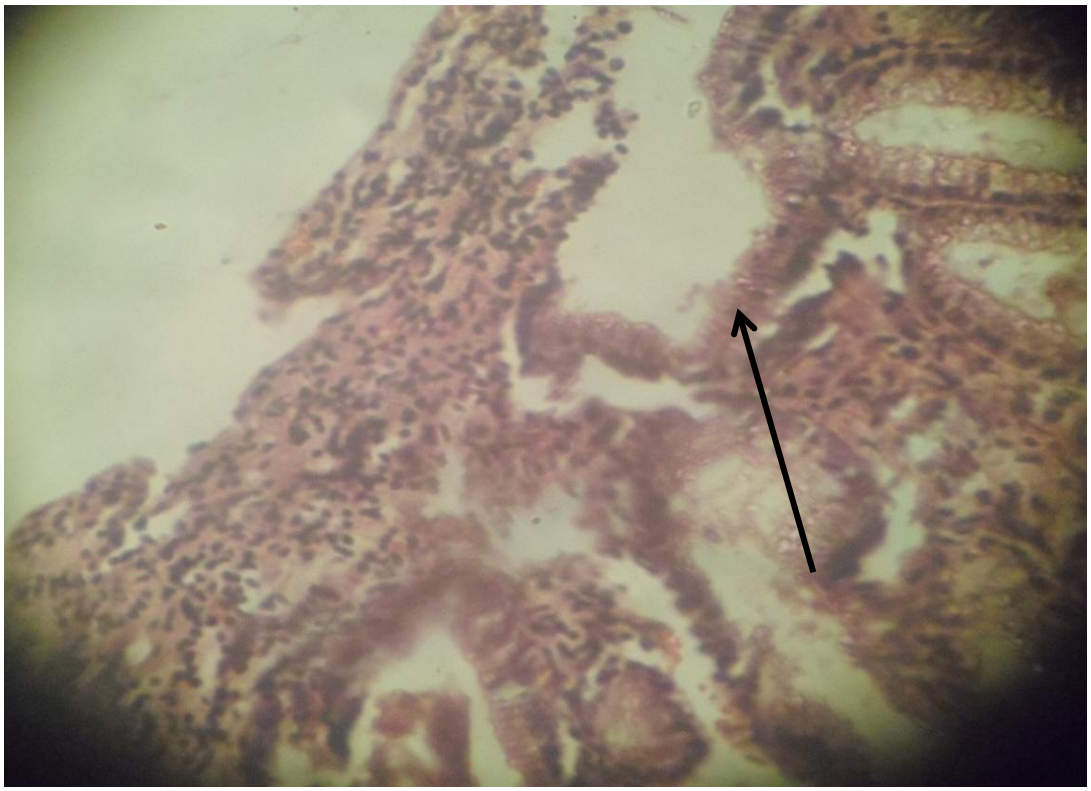


Plate VI:Histopathological section of Bile duct of Cattle slaughtered at Nguroje slaughter slab showingBile duct epithelium sloughing into the lumen and forming granules (H&E X 400)

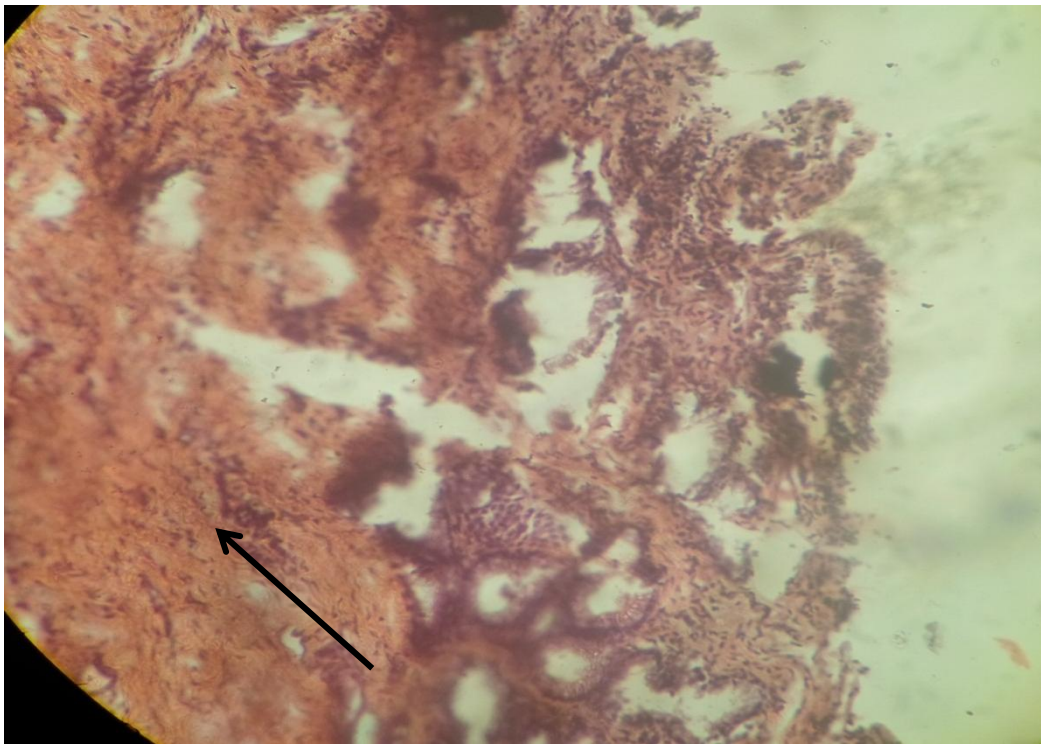


Plate VII:Histopathological section of liver of Cattle slaughtered at Gembu slaughter slab showing fibrous connective tissue infiltrating the muscularis of the bile duct (H&E X 400)

4.4Survey forSnails inAquatic Habitats on the Mambilla Plateau.

Among the 48 aquatic habitats surveyed for fresh water snails, none was found to harbor *Lymnaeid* species of snails however;*Bulinus* species of snails were identified in a dam containing clear fresh water and abundant vegetation built by the Nigerian beverages production company (NBPC)for production of Highland Tea. They were found either freely floating or attached to dead leaves and other organic debris in water. One hundred and fifty eight(158) of the snails were collected and transported to the Laboratory. When crushed and examined under the microscope at ×10 Magnification, none contained the larval stage of *Fasciola* species.It was also observed that snail density decreased as sampling progressed away from the edge of the water body. Other sites that were examined were streams, rivers and marshy areas located in the valleys which were prone to flooding, but no snail was found.

4.5Questionnaires Administered to Cattle Owners and Herdsmen on the Mambilla Plateau, to Assess their Knowledge on the Mode of Transmission of *Fasciola*Species Infection.

Questionnaires administered to cattle owners and herdsmen on the Mambilla Plateau, to assess their knowledge on the mode of transmission of *Fasciola* species infection is shown in Appendix 1.Fifty (50) questionnaires were administered to cattle owners and herdsmen on the Mambilla Plateau, Sardauna LGA of Taraba state. All respondents were males from the ages of 20 years and above and had cattle with herdsizes ranging from 25to above 50 cattle. All respondents practiced the extensive system of management and the method of increasing herd sizewas mostly natural breeding and purchase from the cattle markets. In the rainy season, all respondents depended on range browsing only for source of feed while

in the dry season, they moved their cattle down to the valleys and river banks to browse on the few green pasture remaining without provision of supplements. Considering the sources of water for the cattle, 48 (96.0%) respondentsused natural streams, rivers and ponds while 2(4.0%)used improvised dams and wells to provide water for the animals during the dry season. 27(54.0%) respondents stated that snails were encountered during grazingwhile 23(46.0%) stated otherwise.Among those that claimed to have seen snails, 22(44.0%)saw them in the rainy season, 1 (2.0%) claimed to have encountered them in the dry season while 4(8.0%) said they saw snails all year round. When asked where the snails are normally seen 14(28.0%) respondents said they saw snails on pasture on the hills, 10(20.0%) saw snails on pasture in the valleys while 3 (6.0%) saw them in the waters where cattle go to drink.All of the respondents (100.0%) did not know that snails are involved in the transmission of *Fasciola* species.

All respondents were aware of the infectioncaused by *Fasciola* species and they called it Ciwon hanta, Mbalkum or Balki in their local languages but only 1(2.0%)of the respondents claimed to be able to identify it on farm stating evidences of sub mandibular oedema and pelleted faeces as signs. When asked how they thought cattle contacted the infection,3 (6.0%) thought it was ingested with pasture,41 (88.0%) thought cattle contacted it through contaminated water,3 (6.0%) believed that the parasite falls from heaven with the rain while3 (6.0%) admitted that they didn't know how animals contacted the infection. Concerning the measures taken to prevent cattle from contacting this infection, 19(38.0%)respondents said they took no preventive measures, 18 (36.0%) said they avoided grazing near infested waters while13 (26.0%) used regular dewormers such as albendazole, levamisole and ivermec.

Table 4.8 General informations obtained from questionnaire administered to cattle owners on the Mambilla Plateau.

GENERAL INFORMATION			
System of management	Intensive	Semi intensive	Extensive
Percentage (%)	0.0	0.0	100.0
Increase herd size	Purchase only	Calving only	Purchase & calving
(%)	0.0	4.0	96.0
Feeding in rainy season	Range browsing	Feed suppliment	Other
(%)	100.0	0.0	0.0
Feeding in dry season	Valley browsing	Feed suppliment	Other
(%)	100.0	0.0	0.0
Source of drinking rainy season	Streams and rivers	Improvised source	Others
(%)	100.0	0.0	0.0
Source of drinking dry season	Streams and rivers	Improvised source	Others
(%)	96.0	4.0	0.0
What season are snails seen	Rainy	Dry	Don't know
(%)	44.0	2.0	54.0
Where do you normally see	Hills	Valleys	In water
snails (%)	28.0	20.0	6.0

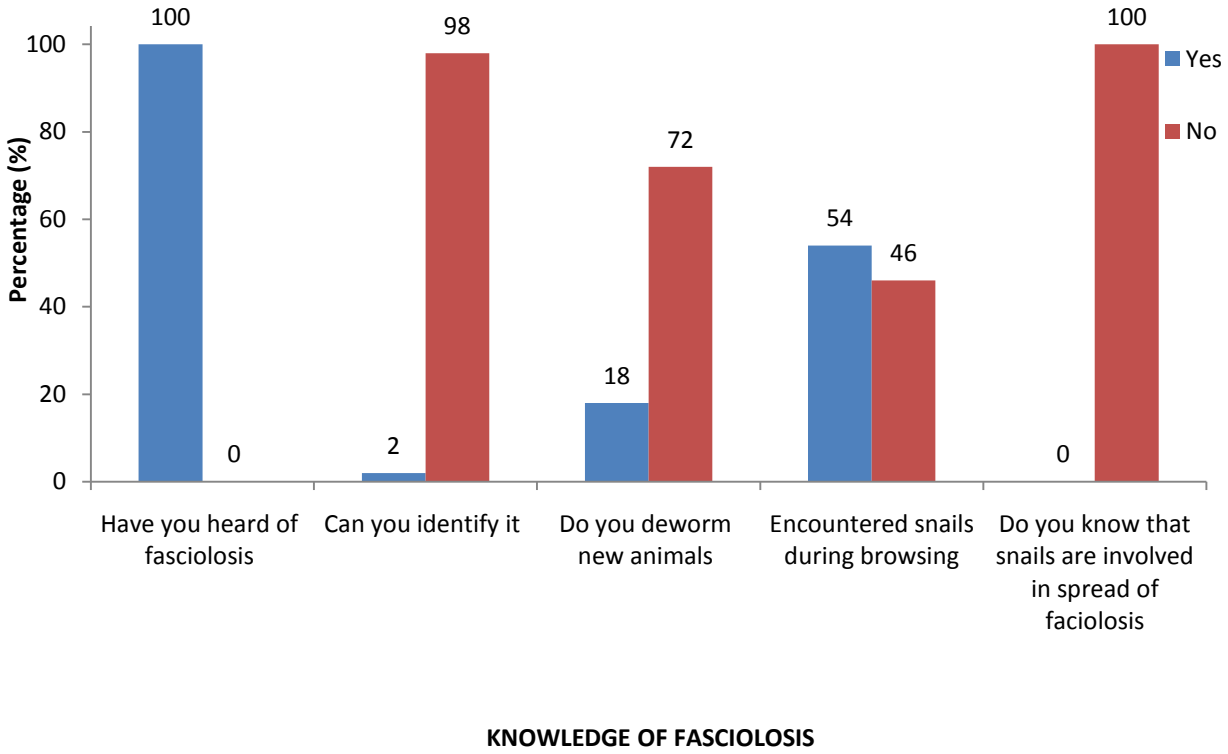


Figure 4.1: Outcome of questionnaire assessing the level of knowledge of Cattle owners on *Fasciola* species infection on the Mambilla Plateau, Sardauna Local Government Areaof Taraba State.

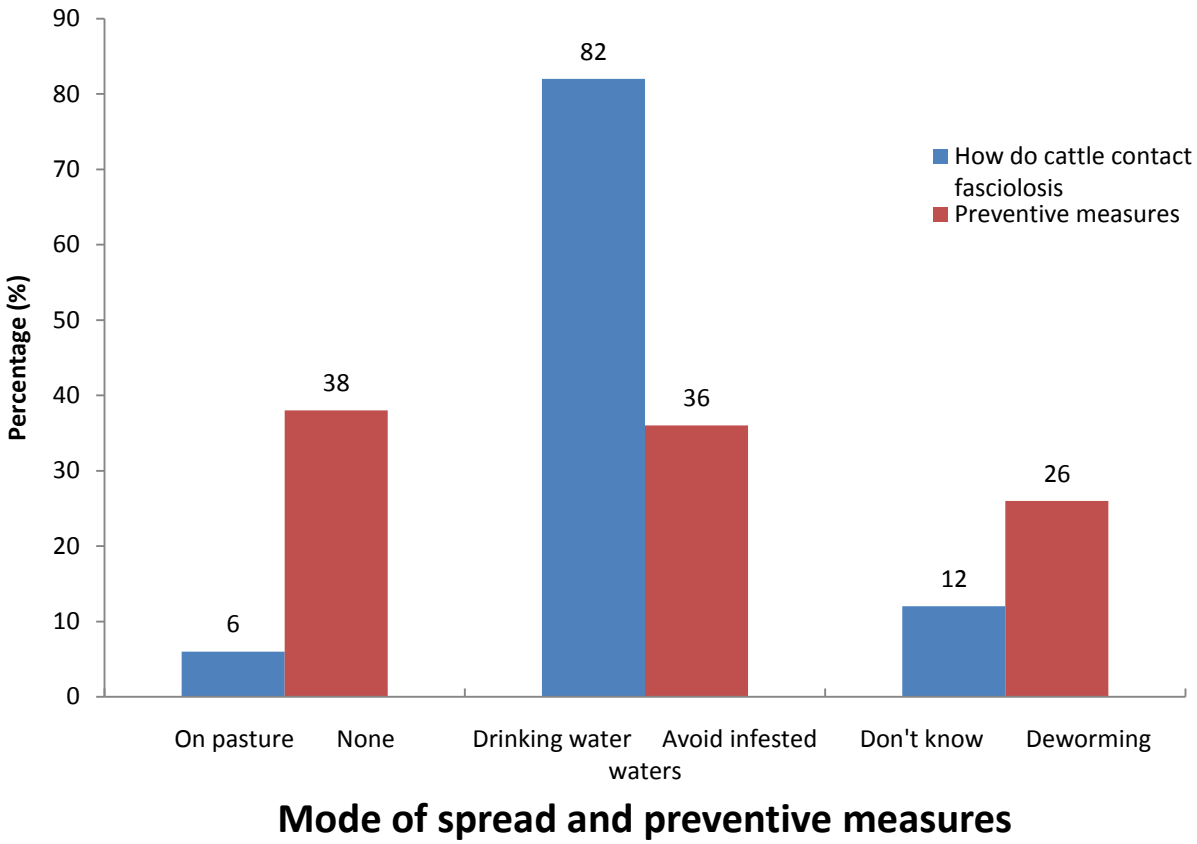


Figure 4.2: Outcome of questionnaire assessing the knowledge of Cattle owners on mode of transmission of *Fasciola* species on the Mambilla PlateauSardauna Local Government Area of Taraba State.

CHAPTER FIVE

5.0 DISCUSSION

In this study, the prevalence of *Fasciola*species infection in slaughtered animals based on post mortem liver examination was 17.9%. Thisis higher than the findings of Umar*et al*. (2009) who conducted a 4-year studyin cattle slaughtered at the Jalingo abattoir, Taraba Stateand recorded a prevalence of 14.1%.Thisslight discrepancy can be attributedmainly to variations in the ecological and climatic conditions such as altitude, rainfall and temperaturebetween the two areas studied.The result obtained in this study was however lower than the findings of Babalola and Schillhorn van Veen (1976) in Bauchi and Ardo *et al*.(2013) inAdamawa who reported prevalence of 31.7% and21.8% respectively. Differences in thesample size and number of abattoirs sampled could account for the difference in prevalence. Furthermore, Ikeme and Obioha (1973) reported a prevalence of 26.0%when they conducted post mortem liver examination on trade cattle in South Eastern Nigeria whileOzung *et al*.(2011) reported a prevalence rate of 92.65% of partially condemned livers due to infection with*Fasciola* speciesin a four year study conducted in Ikom abattoir of Cross River state. This was by far higher than the prevalence obtained in this study and could be attributed to the fact thatmost of the fluke infected cattle slaughtered in abattoirs in the South Eastern states originate from the Northern parts of the country including the Mambilla plateau as reported byUzoukwu and Ikeme (1978). The prevalence obtained in this study however agreed with the findings of Regassa *et al.* (2012) in Ethiopia who reported 17.1% andKnapp *et al*. (1992) who used meat inspection records to determine the distribution and incidence of liver flukes in cattleslaughtered in United States Deparment of agriculture USDA-inspected packing plants in Montanaand reported a prevalence rate of 17.2%.

Elsewhere in Africa, Magona *et al*.(1999) reported a prevalence of 36.5% in Uganda while Pfukenyi and Makaratirwa(2004)in Zimbabwereported 31.7%.

Examination of faecal samples collected from animals at slaughter revealed an infection rate of 27.4%. This was higher than the findings of Aliyu*et al*.(2014)who reported infection rate of 19.5% in Zaria and Rehman*et al*. (2013) who reported25.8% inPakistan.This couldbe attributed to thehigher sample sizeused in this study and the fact that this study was conducted during the rainy season which accounts for high level of *Fasciola* species infection as opposed to the one being compared to which were conducted in the dry season.The resultwas however lower than the findings of Adedokun *et al*.(2008) who reported a prevalence of 33.5%at Ibadan. Lower prevalence in coprological examination is probablydue to low sensitivity of thestandard sedimentation technique used in this study whichis attributed to long prepatent period and intermittent shedding of eggs (Abunna*et al*., 2010) given that eggs of*Fasciola*species cannot be detected by coprological examination until 8-15 weeks the time needed for *Fasciola* species tomature into adults and reach the bile duct (Hillyer,1999; Sanchez-Andrade *et al*., 2008).

Faecal sample collected from cattle on farms revealed that 28.0% harbored eggs of*Fasciola* species. This result was by far lower than the findings of Sugun*et al*. (2010) who reported a prevalence of 76.9% in a survey carried out in 5 Local Government Areas of Bauchi state over a period of 12 months. This difference can be attributed to the fact that only one local government was used in this study and also variation in ambient temperature as agreed byDinnik and Dinnik(1963) who reported that cercariae development can be inhibited in infectedsnail hostswhen mean maximum temperature gets below 20^0C as can be obtained on the Mambilla Plateau especially during the rainy seasons

when this study was undertaken.The result was however higher than the findings of Aliyu*et al*. (2014) in Zaria who sampled 186 cattle on farms and reported a prevalence of 14.5%.

All the animalsthat showed pallor of mucous membrane (9.9%)had*Fasciola*in their liver. This is in agreement with the reports of Adama *et al.*(2011) who infected 6 ewes with 1200 metacercariae of *Fasciola gigantica* and monitored them for a period of 16weeks and observed signs which included pallor of mucous membranes, depression, emaciation andsubmandibular edema. It also agrees with the reports of Okaiyeto *et al.* 2012 who observed pallor of mucous membrane in eleven (11) pregnant Holstein Friesian cattle that turned out to be positive for*Fasciola* species infection in their liver when post-mortem was conducted.

Icterus (Jaundice), which is a yellowish discoloration of the conjunctival membranes over the sclera (whites of the eyes), and other mucous membranes was also observed in 0.2% of the animals, this could have been caused by hyperbilirubinemia which occurs in chronic terminal stage fasciolosis.

Ante-mortem examination of the hair coat and skin of cattle revealed that 11 (2.1%) haddry rough starry hair coats, some with patches of alopeciaalong the back and thighs.This could signify early signs of a chronic ill-health ordue to some conditions such as heavy parasitism as agreed byWilliams and Loyacano(2001).

The 28.5% infection rate recorded from infection with*Fasciola* species in the gallbladder of slaughtered cattlewas lower than the findings of Nwosu and Strivastava (1993) who reported a 42.7% prevalence of *Fasciola gigantica* eggs found in the gallbladder of cattle

slaughtered in Maiduguri abattoir. Adedokun *et al*. (2008) also reported a higher infection rate of 47.4% by analyzing bile of slaughtered cattle in Ibadan. The lower infection rate could be attributed to theundulating landscape which favors the washing of faecal materials down the valleys which are prone to over flooding especially in the rainy season in which this study was conducted and also the cold weather which can inhibit cercarial development in infected snail intermediate hosts with consequent reduced amount of infective larval stage of *Fasciola* species to be picked up by cattle as reported by Dinnink and Dinnink (1963).

The prevalence of infection with*Fasciola*species in cattle sampled in slaughter slabs revealed a higher infection ratein the gallbladder (28.5%)than in the faeces (27.4%)and liver (17.9%). This is likely due to the factthat eggs laid by adult *Fasciola* speciesin the bile duct congregate within the gallbladder and are only found in the faeces when they are intermittently passed into the intestine with bile as agreed by the reports of Shah-fischer and Say(1989). Although the direct bile examination proved to be more sensitive with a statistically significant ($p<0.05$) difference over faecal egg examination reported in this study, its use as a diagnostic procedure is limitedonly at necropsy since locating the gallbladder in live animals is difficult.

The result based on sex of slaughtered animals showed that more females (91.3%)than males (8.7%) were slaughtered. This is a norm in the study area, the reason being that most cattle owners on the Mambilla plateau prefer to sell their bulls to cattle traders coming from the Eastern and Southern parts of this countryat higher gains than selling to butchers.

Sex prevalence of infection based on examination of the liver at slaughter showed no difference for both males (17.8%) and females (17.9%). This result agrees with the findings of Umar *et al*.(2009) in Jalingo and Rehman *et al*.(2013) in Pakistan who also did not observe asignificant differencein infection ratebetweenmales and female cattle. This could be attributed to the system of management in this study area which is predominantly the extensive system where both male and female cattle go browsing in the same location and at the same time. However,the infection rate of *Fasciola* species in the gallbladder and faeces basedon sex was higher in male cattle (35.6% and 33.3%)than in the females (27.9% and 26.8%). This is in line with the findings of Njoku-Tony (2007) and Njoku-Tony and Okoli (2011) and could be attributed to the fact that most of the males that were brought for slaughter had impediments which could have been infections with *Fasciola* species.

Sex prevalence from cattle sampled on farms showed that the females had a higher infection rate(30.3%) than the males (25.1%). The difference was however not significant. This findings agree with the reports of studies performed by Ulayi *et al*. (2007) in Zaria, Nigeria, Dhar *et al*.(1988) and Fatima *et al*.(2008)in Egypt; Phiri *et al*. (2005) in Zambia; Keyyu *et al*. (2005) in Tanzania and Vassilev (1999) in Zimbabwe who all reported that female cattle had higher infection rate than male cattle on the field. Doyle (1971); Schillhorn Van Veen (1997) Soulsby (1982) and Ibrahim *et al*.(2001) have all attributed the higher infection rate in female cattle to a hormone-controlled relaxation of immunity in female animals during pregnancy and lactation; which increases their susceptibility to infection. Another reason could be because the female animals stay on farm longer than the males (for breeding purposes) thus having a higher burden of this infection (Schillhorn

Van Veen *et al.*, 1980). However, Phiri *et al*. (2005) suggested that differences in susceptibility between opposite sexes may exist in either direction depending on predisposing factors.

Based on different ages,infection in the liver and faeceswas highest in the age group of <2 years old (50.0% and 50.0%) and lowest in the age group of >5 years old (16.3% and 24.9%). This could be attributed to the fact that cattle less than two years old are seldom brought for slaughter except it is necessary due to an impediment or some other reason. In the animals that were sampled on farm, the infection was highest in the age group of >5years (31.6%) and lowest in the age group of <2 years old (23.5%). This result agrees with the findings of earlier workers (Ogunrinade and Ogunrinade, 1980; Fabiyi and Adeleye, 1982 and Ardo *et al*., 2013). Similar observations were reported by Keyyu *et al.*(2005) who associated higher infection rate in older animals with age and consequently longer exposure time. Yilma and Mesftin (2000) in Ethiopia also reported the existence of significant difference and inverse correlation in infection prevalence among different age groups. The presence of flukes in the liver was correlated with presence of eggs in the bile and there was a significant ($p<0.05$) association.

The increase in size of the liver resulting in rounded or blunted edges could have been due to the inflammatory changes in the parenchyma resultingin massive necrosis recorded in histopathologic sections in this study. Thisis in agreement with the findings of Talukder *et al*.(2010) who performed a study in Sylhet district of Bangladesh where they investigated the pathological effect of liver fluke in Black Bengal goats of different ages, sexes and in different seasons and reportedmigratory tract with lymphocytic infiltration, atrophy, necrosis and fatty changes in the liver.However the fatty changes described by him were

not seen in this study probably due to the level of infection or the part of the liver that was sectioned or due to species variation. Badr and Nasr (2009) also reported similar lesions in a study conductedin El- warak abattoir in Egypt to evaluate the histopathological and bacteriological lesions on livers affected with *Fasciola* species in cattle. The presence of numerous mononuclear cells accompanied by haemorrhage and congestion of branches of portal and hepatic veins with some containing thrombus correlates with the findings of Dow*et al*. (1967) who recorded the same changes in livers of calves experimentally infected with*Fasciola gigantica*. They explained that the migrating flukes produced some inflammatory reactions in the host tissue which later became surrounded by macrophages, eosinophils and granular tissue. Sloughing of bile duct epithelium as reported in this study agreed with the works of Ross *et al*. (1967) who described similar pathological changes in the livers of calves and pigs infected with*Fasciola*.

When water bodies were surveyed, only one habitat was found to have snails. The inability to find snails in other habitats could be attributed to the fact that this survey was undertaken during the rainy season in which the valleys get over flooded there by washing the snails away. This is in agreement with the findings of Kendall (1949) and Gloer and Pesic, (2002)whoreport that most snails of medical and veterinary importance occur commonly in shallow, well aerated water bodies and that in case of larger water bodies, the snails occur mostly on the edge between water and land implying that the snails cannot survive in deep or fast flowing waters.

The extensive system of management was predominantly practiced by allcattle owners. This can be attributed to the long period of rainy season (9 months) which enables abundant vegetation to be made available for browsing onthe hills and valleys. Also, water

was always available in the valleys either in streams, rivers or ponds making it easier for the animals to drink from it than from alternative sources.The questionnaire also revealed thatall (100%) of the respondentsdid not know that snails are involved in the transmission of *Fasciola*species at such, they showed little or no enthusiasm as to whether snails existed or not around the areas where their animals grazed. This can be attributed to the lack ofproperinteraction between cattle owners and Veterinarians due to the activities of quacks. All respondents claimed to have heard about fasciolosis, this was to be expected since animals that have suffered chronic or acute infections and cannot withstand it anymore are being sold to butchers who immediately slaughter them either on farm or at abattoirs and report presence of flukes in the liver.Concerning the preventive measures undertaken to curtail incidences of *Fasciola*infection in their farms, some respondentsadmitted that they do not adopt any preventive measures,others claimed to depend on routine deworming of their animals while a few others said they try to avoid grazing near flukeinfested water bodies.When probed further, it was discovered that what they referred to as fluke-infested waters were actually water bodies which contained leeches. It was also discovered that all respondents introduced new animals into their herd regularly without deworming them.These further proved their ignorance on the mode of transmission of this parasite.

CHAPTER SIX

6.0 CONCLUSIONS AND RECOMMENDATIONS

6.1 CONCLUSIONS

The Findings in this study is the first to be reported from this study area. The results have shown that infection due to *Fasciola* species is prevalent in cattle on the Mambilla plateau, Sardauna Local Government Area with an overall abattoir prevalence of 17.9%, 28.5% and 27.4% for infections in the liver, gallbladder and faeces respectively and a field prevalence of 28.0% from coproscopy. The infectionwas seen to be evenly distributed throughout the MambillaPlateau given that samples collected from the eleven (11) wards yielded various levels of infection. Considering sex distribution of the infection in the gallbladder and faeces of slaughtered cattle, themales had a higher prevalence (35.6% and 33.3%) than the females (27.9% and 26.8%) while no difference was seen between the males (17.8%) and females (17.9%) when infection in the liver was considered. However,Sex distribution of the infection in cattle sampled on farms revealed that the prevalence was higher in females (30.3%)than males (25.1%).

Considering distribution of the infection inslaughtered cattle based on the different age groups, the prevalence was highest inthose within the age group of <2 years old(50.0%) and (50.0%) while it was lowest in those within the age group of >5 years old (16.3%) and 2-5 years old (24.0%)for liver and gallbladder respectively. However the distribution of the infection in cattle that were sampled on field revealed the highest prevalence in the age group of >5 years old (31.6%) while the lowest prevalence wasrecorded in the age group

of <2 years old (23.5%). These differences in infection rate were not significantly ($p>0.05$).

This present study further revealed that most livestock farmers are ignorant of the mode of transmission of *Fasciola.*Furthermore, the farming practice going on in this study area is such that puts domestic animals and the general public at risk of infection with *Fasciola*species hence the need for an effective massive campaign to educate the populace on the epidemiology of this snail-borne infection, and to device effective control measures against the snail intermediate hosts.

6.2 RECOMMENDATIONS

The launching of a massive awareness campaign for the benefit of all cattle owners and the entire populace on the epidemiology of this infectionand its pre-disposing factors is highly recommended. It is also recommended that cattle owners should be well educated on pasture and range management and how to eliminate the intermediate hosts.

Meat inspection should be intensified in all the slaughter slabs, slaughter houses and abattoirs and proper records kept so that infected animals can be traced back to the herd from which they were brought and prompt treatment instituted at herd level.

Government and all stakeholders should step in and provide funding and logistics that will enable Veterinarians perform their duties effectively in terms of creating diagnostic laboratories and equipping them with prompt diagnosing kits which are affordable. Existing legislations should also be reviewed to make it stiffer for quacks that have infiltrated everywhere and are doing more damage than *Fasciola*species itself.

The diagnostic methods used in this study had so many limitations in terms of sensitivity and ability to diagnose infections due to*Fasciola*species early enough in live animals. This has necessitated the quest for a better and less expensive diagnostic procedure that can detect the infection early and can be used on the field.

Lastly, a more in-depth study should be undertaken in this study area with the use of faster diagnostic facilities that can pick recent and acute infections and a molecular dimension introduced to ascertain the specific species of *Fasciola* and their associated snail intermediate host involved in the epidemiology of the infection up in the Mambilla Plateau.

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APPENDIX 1

QUESTIONAIRE ASSESSING THE KNOWLEDGE OF CATTLE OWNERS ON MODE OF SPREAD OF FASCIOLOSIS ON THE MAMBILLA PLATEAU OF TARABA STATE, NIGERIA.

INSTRUCTION: PLEASE ANSWER THE FOLLOWING SET OF QUESTIONS BY FILLING THE BLANK SPACES OR BY TICKING THE APPROPRIATE OPTION. YOUR PARTICIPATION WILL BE HIGHLY APPRECIATED AND ALL INFORMATION WILL BE TREATED AS CONFIDENTIAL

FARMER BIODATA

1. Farm Location (Ward) ……

2. Sex Male[ ] Female [ ]

3. Age Less than 20 [ ] Between 20 and 40 [ ] Above 40 years [ ]

FLOCK DATA

4. What is the size of your herd; less than 25 [ ] 25-50 [ ] 50 and above.

5. What system of management do you use; intensive [ ] semi intensive [ ] extensive [ ]

6. How do you increase the number of animals in your herd? Purchase from cattle market only [ ] From calving only [ ] From both market and calving [ ]anyother (specify) [ ]

GENERAL INFORMATION

7. How do the animals feed in the rainy season? Range browsing [ ] Feed supplement provided [ ] Others (specify) [ ]

8. How do the animals feed in the dry season? Migrate and Browse in valleys around river banks [ ] provide husks and chaff [ ] Other (SPECIFY) …….

9. What is the source of drinking water for the animals in the rainy season? Natural streams and rivers [ ] Improvised dams [ ] Any other (specify) [ ]

10. What is the source of drinking water for the animals in the dry season? Natural streams and rivers [ ] Improvised dams [ ] pipes and drinking troughs [ ] Any other (specify) ……

11. Have you ever come across snails during grazing? Yes [ ] no [ ]

12. If yes, at what season of the year do you usually see these snails? Rainy season [ ] dry season [ ] all year round [ ] none [ ]

13. Where do you normally see them? On the hills [ ] in the valleys [ ] in the water [ ] others ……

14. Do you know that snails are involved in the spread of this disease? Yes[ ] No [ ]

KNOWLEDGE OF FASCIOLOSIS

15. Do you know a disease called fasciolosis? Yes[ ] No[ ]

16. What is the local name of this disease?

17. Can you identify it in your farm? Yes [ ] no [ ]

17. How do you think animals contact this disease? On pasture [ ] in drinking water [ ] fall with rain [ ] others (specify)…….

18. What measures do you take to prevent your animals from contacting fasciolosis? ……..

19. Do you deworm new animals before introducing them into the farm? Yes [ ] No [ ]

20. Do you de-worm your animals? Yes [ ] No [ ], if yes, how often?