EPIDEMIOLOGY AND CONTROL OF FASCIOLOSIS IN CATTLE IN IMBO REGION, BURUNDI

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DECLARATION AND RECOMMENDATION

Declaration

This thesis is my original work and has not been presented in this university or any other for the award of a degree.

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Recommendation

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DEDICATION

This work is dedicated to the Almighty God for his glory and mercy that gave me strength to complete my study, to my loving wife, Yvette Nshimirimana, and lovely children Kyle-Alain Nkurunziza, Kelcy-Alaine Nkurunziza and Kydy-Toussaint Nkurunziza who gave me every motivation and encouragement needed to get me in the position I am today; without forgotten my sincerely parents Gérard Ntuyahaga and Suzanne Niyonkuru, brothers and sisters family for their contributions of blessings and prayers, and others supporting to achieve on this current study.

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ABSTRACT

Fasciolosis is a significant zoonotic parasitic disease which affecting animals and humans globally. It reduces the productivity in the ruminants, however, there is a scarcity of information on the epidemiology of fasciolosis and associated risk factors in animals in Burundi. Therefore, the aim of this current research work was to dress the knowledge gap regarding the Imbo region of Burundi through a two-stage approaches: first, by determining the level of knowledge, practices, and attitudes regarding fasciolosis infection among farmers, and perception and practices among veterinary professionals. Second, by investigating the occurrence and risk elements associated with the fasciolosis infection. Two structured questionnaires were used to survey 168 farmers and 26 veterinary professionals who were randomly selected. Fresh stool from the rectum of 426 cattle was collected for microscopic examination, and a questionnaire focused on an individual animal was utilized to collect supplementary information regarding cattle identification and risk elements associated with the disease. Liver fluke examinations were conducted on 467 cattle in abattoirs. Results among surveyed farmers showed that 57.7% knew fasciolosis or "UMURAGU" in the local language, but only 28.6 % knew its causes. Among farmers, 40.5% indicated that they knew cattle could be infected by fasciolosis, and only 0.6% indicated that humans could get the infection. Among surveyed veterinary professionals, only 39% indicated that they had a record-keeping book of parasitic infections, and, 65.4 % indicated that fasciolosis is classified among the top two diseases caused by parasites based on frequency of occurrence and magnitude of economic losses in cattle. The prevalence rate of fasciolosis in cattle was 47.7% (42.9 - 52.4, 95% CI) through microscopic examination tools and 33.2% (28.9 - 37.5, 95 CI) through postmortem examinations. Most of the infected cattle (60.6%) had light intensity infections as measured by eggs per gram of feces (epg). In addition, postmortem examinations confirmed this finding by showing that most of the infected cattle (80%) had light intensity infections. Chi-square analysis revealed a significant association ($P<0.05$) between bovine fasciolosis and cattle-related factors such as age, sex, and origin, as well as the management practices of cattle owners. This current study further indicated that farmers demonstrated low to moderate levels of knowledge, attitudes, and practices concerning fasciolosis infection. Moreover, the findings confirmed that fasciolosis is highly endemic in the Imbo region, which could continue to cause a potential public health risk given its zoonotic character.

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LIST OF ABBREVIATIONS AND ACRONYMS

CDC	Centers for Disease Control and Prevention
DNA	Deoxyribonucleic acid
ELISA	Enzyme-linked immunosorbent assay
EPG	Egg present per gram
FAO	Food and Agriculture Organization
GDP	Gross Domestic Product
GGT	Gamma-Glutamyl Transferase
GLDH	Glutamate Dehydrogenase
IEA	Indirect Enzyme Immunoassay
IFAD	International Fund for Agricultural Development
ILRI	International Livestock Research Institute
KAP	Knowledge, Attitudes and Practices
LAMP	Loop-mediated isothermal amplification
LDH	Lactate Dehydrogenase
NEJ	Newly Excysted Infectious Juveniles
NTD	Neglected Tropical Disease
PCR	Polymerase Chain Reaction
PM	Post-mortem
PRDAIGL	Projet régional de développement agricole intégré dans les Grands Lacs
SD	Standard Deviation
SSA	Sub-Saharan Africa
UGX	Ugandan Shilling
USD	United States Dollar
WHO	World Health Organization

CHAPTER ONE

INTRODUCTION

1.1 Background information

Livestock provides important nutrients and is often used for traction in smallholder mixed systems, where it contributes to resource use efficiency through nutrient cycling. The livestock sector sustains the livelihoods of nearly 600 million poor smallholder farmers in developing countries (Thornton, 2010). Fasciolosis is a helminthic disease affecting cattle, buffaloes, sheep, goats, horses, and humans of all ages, caused by liver flukes of the genus *Fasciola* (Ardo *et al*., 2014). The infection occurs following ingestion of the encysted metacercaria which is the infective stage of the parasite (Figtree *et al.*, 2015; Najib *et al*., 2020). In livestock, transmission of fasciolosis infection typically happens through consumption of forage contaminated with metacercaria or by drinking water carrying encysted metacercaria attached to soil particles or plant debris (Irsik *et al*., 2008). Humans, on the other hand, become infected with fasciolosis infection not directly from animals but by ingesting metacercaria present on raw vegetables or aquatic plants, through drinking contaminated water, consuming food washed with such water (Ibrahim, 2017), or, in some cases, via exposure to contaminated human sewage (Aleixo *et al*., 2015).

The World Health Organization (WHO) recognizes human fasciolosis as one of the neglected tropical diseases (NTDs) (Abah *et al*., 2019; Ai *et al*., 2017). This parasitic infection represents a significant health burden in many regions of the world, with an increasing number of human cases reported across Europe, the Americas, Oceania, India, and Africa (Aksoy *et al*., 2005; Soliman, 2008). In Certain countries, such as Bolivia, Peru, and Egypt, fasciolosis in humans is considered hyper-endemic, with the prevalence rates exceeding 70% when detected through stool examination and reaching almost 100% when diagnosed by serological methods (Aleixo *et al*., 2015). In Bolivia and Egypt, children are among the most affected groups, often acquiring the infection by consuming wild of cultivated plants during herding and other livestock-related activities (Aleixo *et al*., 2015; Mas-Coma, 2014). Both human and animal infections can cause severe pathological effects on the liver, largely due to the migration of immature flukes through hepatic tissues or their presence in other organs (Najib *et al*., 2020). From a clinical perspective, fasciolosis infection is commonly associated with inflammation of the bile ducts and obstruction of the biliary system in both animals and humans (Sah *et al*., 2017).

The identification of fasciolosis infection in both human and animals relies on several diagnostic methods such as stool examination under the microscope, serological tests such as the enzyme-linked immunosorbent assay (ELISA) that identifies antibodies produced in response to Fasciola infection, and the copro-antigen ELISA, which detects parasite antigens directly in fecal matter. Molecular methods, such as Polymerase chain reaction (PCR) are also employed to identify fragments of Fasciola DNA in stool samples (Caravedo & Cabada, 2020). In addition, milk samples from infected animals can be also be tested to confirm the presence of the parasite (Rana *et al*., 2014). Treatment of fasciolosis infection differs between humans and animals, where for human, treatment is manly the use of antiparasitc such as triclabendazole, nitazoxanide, and albendazole (Cwiklinski *et al*., 2016). For fasciolosis treatment in animals, drugs commonly used are including nitroxynil, oxyclozanide, albendazole, clorsulon (Isabirye *et al*., 2012).

The spread of fasciolosis infection is closely linked to the presence of freshwater snails, particularly *Lymnaea truncuta* and *Lymnaea natalensis*, which act as the essential intermediate hosts in the parasite' life cycle (Boray & Love, 2007; Ibrahim, 2017). This parasitic infection is considered widespread across the globe and has been documented in multiple regions, including Europe (Isabirye *et al*., 2012), the Americas (Kleiman *et al*., 2007), Asia (Nguyen *et al*., 2011), the Middle East, Oceania (Isabirye *et al*., 2012), and Africa (Abunna *et al*., 2010). Within sub-Saharan Africa, fasciolosis infection has been recorded in several sub-regions such as East Africa (Dida *et al*., 2014), West Africa (Elelu *et al*., 2016), Southern Africa (Nyirenda *et al*., 2019), and North Africa (Elshraway & Mahmoud, 2017). In East Africa, for example, research on ruminant populations has demonstrated its presence in Tanzania (Mellau *et al*., 2010) and Kenya (Mungube *et al*., 2006). Epidemiological investigations in livestock populations highlight that several factors such as the animal's age, sex, breed, as well as the type of husbandry and management practices adopted by farmers strongly influence how frequently the disease occurs and all these factors play an important role in determining levels of infection (Abunna *et al*., 2010; Keyyu *et al*., 2006; Phiri *et al*., 2005). Reports from veterinary services of Burundi show cases of morbidity in cattle and in small ruminants, and liver flukes burden during abattoir meat inspections (Report from General Directorate of Livestock in Burundi, 2021).

Fasciolosis is widely recognized as a significant disease affecting livestock, particularly sheep, goats, and cattle, where it represents a major health and management challenge (Soliman, 2008). The infection leads to considerable financial losses for farmers and the livestock industry.

These losses are associated with several factors such as deaths of infected animals, reduced health and vitality (morbidity), slower weight gain, condemnation of livers during meat inspection, and a higher vulnerability of infected animals to other diseases. In addition, the costs of preventive measures and medical treatment further increase the financial losses. Beyond these, fasciolosis also lowers the overall productivity of herds by reducing fertility rates and decreasing milk yields (Bernardo *et al*., 2011; Elshraway & Mahmoud, 2017). Globally, the economic impact of this parasitic disease has been estimated at around three million dollars annually (Ojeda-Robertos *et al*., 2020).

In Burundi, information concerning the spread, management, and prevention of fasciolosis infection remains very limited. The present study was therefore designed to address this gap by assessing farmers' knowledge, practices, and attitudes related to bovine fasciolosis, as well as the practices and perceptions among veterinary professionals in the Imbo region. In addition, the study was to determine the occurrence of fasciolosis infection in cattle in this area and to identify the main factors that contribute to its occurrence.

1.2 Statement of the problem

Fasciolosis, a parasitic disease caused by *Fasciola hepatica* and *Fasciola gigantica*, is among the most important helminth infections affecting livestock. It represents a serious health challenge for ruminants in many tropical and subtropical regions, including Burundi, where it is responsible for considerable economic losses in animal production. Reports from the veterinary services of Burundi show huge cases of morbidity due to fasciolosis in cattle herds and other small animals in the Imbo region more than in other regions of Burundi (Report from General Directorate of Livestock in Burundi, 2021). Once, by misfortune, the cattle suspected of fasciolosis die on the farm, at the time of the autopsy the liver fluke burden is found in the liver. At the time of the inspection of the meat in the slaughterhouses, liver fluke burden in the cattle liver is reported. Therefore, in the year 2021, the veterinary services of the Imbo region reported a prevalence of 12.23 % for bovine fasciolosis. A recent investigation carried out by Minani *et al*. (2023) at the slaughterhouse in Muyinga Province, Burundi, revealed that fasciolosis was present in 13.04% of cattle, 3.16% of goats, while no cases were detected in sheep. Despite these findings, information on the occurrence of fasciolosis remains scarce, and knowledge about its epidemiology and control in cattle within Burundi is still very limited.

The present research was therefore designed to determine both the prevalence and the main risk factors of bovine fasciolosis, while also assessing farmers' knowledge, attitudes, and practices, as well as the practices and perceptions of veterinary professionals working in the Imbo region of Burundi.

1.3 Objectives

1.3.1 General objective

To evaluate the epidemiology of bovine fasciolosis to contribute to the control of such disease in the Imbo region in Burundi.

1.3.2 Specific objectives

i. To determine the level of knowledge, practices, and attitudes regarding bovine fasciolosis among farmers and practices and perceptions among veterinary professionals in the Imbo region of Burundi.
ii. To determine the prevalence of bovine fasciolosis in the Imbo region of Burundi
iii. To determine the risk factors associated with bovine fasciolosis in the Imbo region of Burundi.

1.4 Null hypothesis

i. There is no significant difference in the level of knowledge, practices, and attitudes regarding bovine fasciolosis among farmers and practices and perception among veterinary professionals in the Imbo region of Burundi.
ii. There is no significant difference in the prevalence of bovine fasciolosis in the Imbo region of Burundi.
iii. There is no significant difference in the risk factors associated with bovine fasciolosis in the Imbo region of Burundi.

1.5 Justification

Burundi is a predominantly agricultural country, with over 90% of its inhabitants practicing agriculture and farming as their main source of livelihood. Livestock production is practiced extensively across the country and plays an important role in the national economy, contributing around 4.6% of the Gross Domestic Product (GDP) (Hatungumukama *et al.*, 2007). To support Burundi's economic growth by increasing animal production, animal health is the main parameter enabling animal productivity by reducing the rate of morbidity and mortality, increasing growth rate and fertility, and reducing the seizure of liver of livestock through the evaluation of epidemiology and control of animal diseases including fasciolosis in cattle to establish the control measures.

Information on the epidemiology and management of bovine fasciolosis in Burundi is limited. This study was therefore designed to create a framework for investigating both the spread and control of the disease in cattle. It also examined the roles of farmers and veterinary professionals in the persistence, transmission, and challenges in controlling *Fasciola* species in the Imbo region. This was achieved by assessing farmers' knowledge, practices, and attitudes toward bovine fasciolosis, alongside the practices and perceptions of veterinary professional in the same area. Additionally, the study collected data on the prevalence of the disease and the factors that increase the risk of fasciolosis infection. Findings from the assessment of knowledge, attitudes, and practices, together with prevalence and risk factor data, provided valuable insights into the current situation of bovine fasciolosis in the Imbo region. Such information can assist veterinary authorities in developing and implementing effective control strategies for the disease in ruminants across Burundi

1.6 Definition of terms

Liver fluke: This term refers to a diverse group of parasitic flatworms belonging to the phylum Platyhelminthes which are able to infect the liver of a variety of mammals, including humans. They are also able to travel through the bloodstream and can also be found in the bile ducts, gallbladder, and liver tissue.

Parasite: An organism that lives in, on, or together with another organism, often deriving nutrients at the host's expense.

Prevalence: This is a statistic of disease occurrence. It is a proportion of the population that is diseased at a given point in time (alternatively referred to as point prevalence rate).

Trematodes: These are unsegmented, flat (flatworms), leaf-shaped helminth parasites. Their life cycles are distinctive, featuring sexual reproduction within mammalian and other vertebrate definitive hosts, while asexual reproduction occurs in intermediate hosts such as snails, mollusks, and fish.

Zoonotic parasites: They are animal parasites (helminths, protozoans, and one arthropod such as Sarcoptes scabies) that can infect humans.

CHAPTER TWO
LITERATURE REVIEW

2.1 Etiology and classification

Fasciolosis is caused by several liver fluke species classified under the Phylum Platyhelminthes, Sub-phylum Flatworm, Class Trematoda, Subclass Digenea, Order Distome, and Family Fasciolidae, including the genera *Fasciola* and *Fascioloides* (Ibrahim, 2017; Houda, 2011; Lotfy, 2014). Within these genera, the most significant species are *Fasciola hepatica* and *Fasciola gigantica* (Zimmerman, 2013). Other less common species include *F. nyanzae*, found in hippopotamuses in Uganda; *F. tragelaphi*, identified in Sitatunga antelopes; and *F. jacksoni*, present in elephants. *F. nyanzae* is restricted to certain regions of East Africa, while *F. jacksoni* is native to India and Pakistan. Additionally, *Fascioloides magna*, a liver fluke of deer in North America, can also infect domestic ruminants (Lotfy, 2014).

2.2 Biology and life cycle of *Fasciola* species

Similar to other trematodes, *Fasciola hepatica* and *Fasciola gigantica* exhibit a complex life cycle (Mas-Coma *et al*., 2009), involving both a vertebrate primary host, where sexual reproduction takes place, and freshwater snails from the family *Lymnaeidae*, which serve as intermediate hosts supporting asexual reproduction (Rondelaud *et al*., 2009). In the definitive host, these liver flukes inhabit the bile ducts and gallbladder, where they carry out their reproductive processes.

Fasciola species are hermaphrodites, meaning each individual has both male and female reproductive organs and can fertilize itself. Despite this, reproduction typically occurs through cross-fertilization between two adult flukes, which helps generate genetic diversity within the population (Cwiklinski *et al*., 2015). Following cross-fertilization, a single fluke can produce up to 25,000 eggs per day. These eggs are expelled through the host's intestine and enter the external environment via feces. Initially immature, the eggs undergo a period of development called embryonation, after which they hatch into a ciliated larva known as a miracidium. The miracidium exits the egg through a small opening called the operculum and actively searches for a suitable snail to serve as an intermediate host. Its lifespan ranges from 8 to 24 hours, largely constrained by the limited glycogen stored in its body, which serves as its primary energy source (Zhang *et al*., 2019). To improve the likelihood of finding and invading a snail during this brief window, the larva has evolved specialized sensory mechanisms, including attraction to light and the production of pheromones and tissue-degrading metallopeptidases that facilitate host entry (Lalor *et al*., 2021).

Within the snail, the parasite develops asexually, progressing through several stages such as sporocyst, rediae, and ultimately cercariae. This process, called clonal multiplication, allows a single miracidium to generate between 10 and 700 cercariae (Lalor *et al*., 2021). The release of numerous cercariae from each snail ensures that the parasite's life cycle continues (Husch *et al*., 2020). Favorable temperature and light conditions trigger the snails to release the cercariae. These relatively large (~250 µm), motile larvae swim in the water and eventually attach to leafy plants or the water surface, forming durable cysts known as metacercariae, which are the infectious stage for the mammalian host. The formation of these cysts is stimulated by environmental changes, such as oxidative stress, UV exposure, salinity fluctuations, and CO_2 levels, which are detected by the cercariae (Cwiklinski *et al*., 2015).

Infection with *Fasciola* species occurs only when a mammal consumes plants or water contaminated with metacercariae (Rondelaud *et al*., 2009). In the small intestine, the metacercariae excyst, releasing newly excysted juveniles (NEJs). These tiny (~0.1 mm), active parasites penetrate the intestinal wall and enter the abdominal cavity within 6–72 hours after ingestion, depending on the host species. This early stage is crucial for establishing infection and initiating the disease process. Within a week, the juveniles traverse the peritoneum and invade the liver by penetrating Glisson's capsule. As they migrate through the hepatic tissue, the flukes grow substantially by consuming host cells and eventually blood (Han *et al*., 1993), which is largely responsible for the clinical signs seen in acute fasciolosis. At this stage, the parasites reach about 5 mm in size, causing mechanical injury through tegument abrasion, tissue digestion, and the action of their suckers. These movements create hemorrhagic migratory tracks in the liver tissue (Mas-Coma, 2014). Contact between the parasite's tegument and liver cells generates enough trauma to destroy host cells (Zafra *et al*., 2013). The spines on the tegument also aid feeding by piercing small blood vessels. *Fasciola* species possess two suckers such as an oral sucker at the front surrounding the mouth and a ventral sucker. Both contribute to significant tissue damage as the flukes use them to feed, attach to the bile duct walls, and migrate through the liver (Lalor *et al*., 2021).

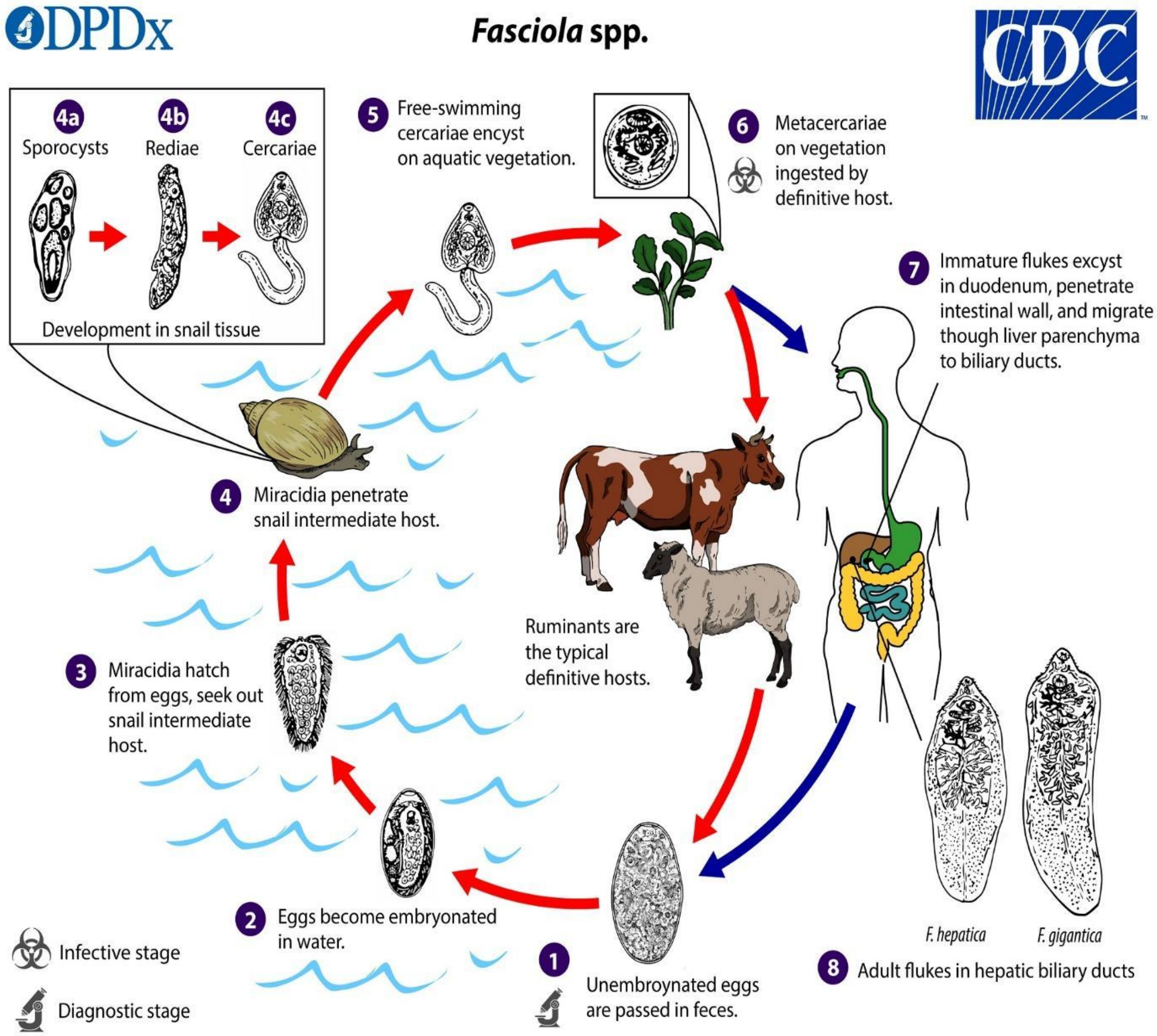

Figure 1: Life cycle of *Fasciola* species

Source: https://www.cdc.gov/parasites/fasciola/biology.html, Accessed 24 April 2022

2.3 Intermediate hosts of *Fasciola* species and their geographical distribution

The intermediate hosts of *Fasciola* species belong to the phylum Mollusca, class Gastropoda, subclass Euthyneura and Pulmonata, within the family *Lymnaeidae* (Ernest, 2014). Not all Lymnaea species are capable of transmitting *Fasciola*; some specifically transmit *F. hepatica,* others *F. gigantica*, and a few can carry both species (Bargues *et al*., 2001). Molecular research indicates that *Fasciola hepatica* is mainly spread by snails in the Galba (Bargues *et al*., 2007) or Fossaria groups (Bargues *et al*., 2011), with Galba truncatula being the primary intermediate host. This species is widely distributed across Europe and also occurs in Africa, Asia, and South America. Other relevant species include *Lymnaea humilis*, *L. bulimoides*, and *L. cubensis* in North America; *L. cubensis* in the Caribbean; *L. neotropica*, *L. cousini*, and *L. viator* in South America; and *L. tomentosa* in Australia. A recently identified species, *Lymnaea schirazensis*, appears to have been historically misidentified as G. truncatula or other similar vector species and has been found in Asia, Europe, Africa, the Caribbean, North America, and South America (Bargues *et al*., 2011).

Fasciola gigantica is primarily transmitted by snails of the genus *Radix*, particularly *R. natalensis* in Africa and various forms of *R. auricularia* and *R. viridis* in Asia (Bargues *et al*., 2001). The genus *Pseudosuccinea*, which includes only *P. columella*, has spread across all continents and appears capable of carrying both *Fasciola* species. Some lymnaeid snails within the stagnicoline group have shown the ability to transmit *F. hepatica* under specific or localized natural conditions, such as *Lymnaea* (*Stagnicola*) *palustris*, *L.* (*Stagnicola*) *fuscus*, and related species like *Omphiscola glabra* (Bargues *et al*., 2003).

The presence and distribution of lymnaeid snails not only determine where fasciolosis occurs but also help explain the patterns of human infection within a country. This has been observed in places like Venezuela (Bargues *et al*., 2011) and Chile (Artigas *et al*., 2011), where the snails' presence influences both seasonal and continuous transmission in endemic regions (Mas-Coma *et al*., 1999).

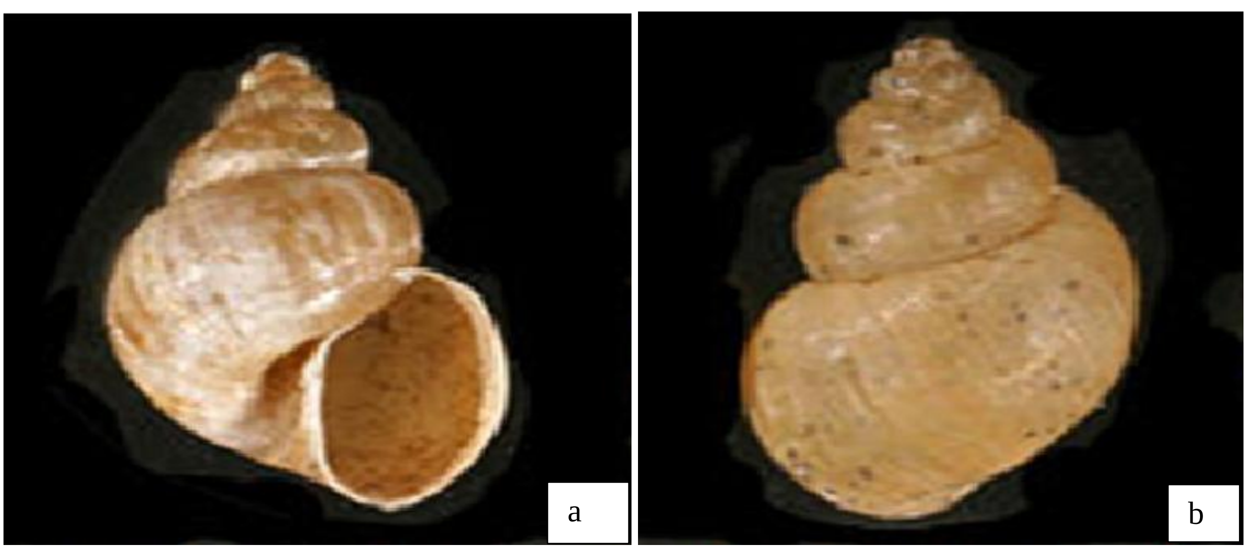

Figure 2: Shell layers showing ventral (a) and dorsal (b) views of Lymnaeid snails (Galba truncatula), intermediate host of Fasciola hepatica (Ayele & Hiko, 2016)

2.4 Ecology and factors influencing Lymnaeid snail

In general, snails thrive and reproduce quickly in areas with high moisture. Lymnaeid snails favor poorly drained soils, channels, and areas where water seeps from springs or damaged drains. They also do well in low-lying, neutral or slightly alkaline marshlands with slow-flowing water. Small streams, springs, spillages, leaks from water troughs, or irrigated land can also support their survival. Human activities such as altering land drainage or applying lime to improve pastures may unintentionally create conditions favorable for snail populations, thereby promoting the transmission of Fasciola species (Ernest, 2014).

Different snail species have varying water requirements where some are more aquatic, while others are mainly amphibious (Boray, 1964). Their habitats may be permanent or temporary and can expand or shrink depending on water availability. Temporary habitats often appear after heavy rainfall or flooding and may include muddy paths, vehicle tracks, hoof prints, springs, riverbank mud along slow-flowing streams, and areas near water troughs. Permanent habitats include irrigation ditch banks, deep streams, large dams, lakes, swamps, and pond or dam edges (Boray, 1964). Under optimal conditions of moderate temperature and moist pastures, snail populations can reach densities of up to 3,300 per square meter.

Research indicates that snails require a minimum temperature of around 10˚C to begin reproducing, while temperatures exceeding 35˚C can be lethal to their larvae. Reproduction is generally effective only when the average temperature remains above 10˚C, and significant population growth of both snails and larval flukes occurs once temperatures reach 15˚C or higher (Amato, 1986). The optimal conditions for the survival and spread of metacercariae are around 20˚C with relative humidity close to 95%. Other environmental factors influencing their distribution include water flow and slope, turbidity, light exposure, moisture levels, soil characteristics, oxygen availability, salinity, presence of aquatic plants, food sources, and pollution (Hourdin *et al*., 2006).

2.5 Pathology of infection

2.5.1 Pathogenesis

The development of fasciolosis-related disease is linked to both the migratory juvenile stages in the liver and the adult flukes feeding on blood within the bile ducts. The severity and characteristics of the pathology depend on the quantity of metacercariae ingested, the stage of parasite development within the liver, and the specific *Fasciola* species involved. There are two phases of pathogenesis among which the first is the parenchymal (migratory) phase or acute phase caused by the migration of larvae through the liver parenchyma resulting in hepatic hemorrhagic tracts of blood (causing hypoalbuminemia and eventually causing anemia), fibrin, cell debris, and necrosis in the parenchyma causing infectious hepatic necrotic hepatitis (black disease). The second stage, known as the biliary or chronic phase, arises from the blood-feeding activity of adult flukes and the injury they cause to the bile duct lining with their cuticular spines. This leads to inflammation of the bile ducts, blockage, fibrosis, and leakage of plasma proteins through the epithelial layer (Ernest, 2014).

The disease manifestations caused by *Fasciola hepatica* and *Fasciola gigantica* are largely similar, resulting in comparable clinical outcomes. Additionally, fasciolosis has been reported to increase cattle's vulnerability to *Salmonella Dublin*, promoting longer-lasting infections and extended fecal shedding (Ibrahim, 2017).

2.5.2 Macroscopic and histopathology during post-mortem

During inspection, the liver may appear irregularly contoured and pale and firm**.** The tunnels left by larval passage become filled with defense cells such as neutrophils, eosinophils, and lymphocytes, and expand as young flukes develop, making them visible to the naked eye as dark red streaks during the early stage of infection (Ernest, 2014).

At this acute stage, the liver is noticeably swollen, fragile, blood-stained, and riddled with winding passages of the parasites. The ventral lobe commonly displays a coating of fibrin deposits. In the subacute stage, the organ remains enlarged, with numerous zones of tissue death and bleeding both on the surface and deeper inside. When the disease advances to its chronic form, the liver becomes misshapen, often pale and rigid, with the ventral lobe typically shrunken. Long-standing or severe infections lead to scarring and excessive tissue growth due to inflammation of the bile ducts (Yadav *et al*., 1999). The liver tissue becomes heavily fibrous, while the lymph nodes of the organ are moderately swollen, showing a cut surface of dark brown or greenish color (Ernest, 2014).

2.5.3 Clinical signs

The way fasciolosis presents itself in animals depends mainly on how many infective larvae (metacercariae) are swallowed, since the size of the infectious dose is closely tied to the number of parasites entering the host. In sheep, the disease can appear in three distinct patterns: sudden (acute), intermediate (subacute), or long-standing (chronic). In cattle, however, the persistent or chronic form is the most frequently observed, although younger animals, especially calves, may also suffer from sudden or intermediate forms when they are exposed to a very high number of larvae in a short time (Ernest, 2014; Houda, 2011). When animals ingest a large quantity of infective stages all at once, the disease develops rapidly and manifests as acute illness. If the parasites are consumed in smaller amounts but over many weeks or months, the outcome is a chronic condition. Subacute fasciolosis, on the other hand, arises when the host takes in a moderate number of larvae repeatedly over a prolonged period, leading to an illness that falls between the acute and the chronic patterns (Houda, 2011).

The signs of fasciolosis depend on how severe the infestation is and at what stage the illness has reached. In the acute stage, when young parasites are actively invading the liver, animals may suddenly collapse and die. Other signs at this stage include extreme weakness, pale gums and inner eyelids, difficulty in breathing, yellow discoloration of tissues, frothy blood-tinged fluid from the nostrils, diarrhea, bleeding from the rectum, abdominal discomfort, and accumulation of fluid in the belly. In the subacute stage, animals tend to lose their appetite, appear dull and inactive, grow progressively thinner, show pale mucous membranes, and develop jaundice; in some cases, death may also occur (Houda, 2011). The long-term or chronic stage, usually linked to the presence of adult flukes, may sometimes pass unnoticed when mild, but more often it is a slow wasting condition. This form is marked by progressive emaciation, paleness of mucous tissues, reluctance to move, swelling under the jaw due to loss of blood proteins, buildup of fluid in the abdominal cavity, and eventually death if the disease is left unchecked (Ernest, 2014, Yadav *et al*., 1999)**.**

In humans, fasciolosis evolves through four main stages. The incubation period begins after swallowing the infective form of the parasite and lasts until the first signs appear. Its exact duration is uncertain and may vary from a few days to several weeks or even a few months (Mas-Coma, 2014). The acute or invasive stage corresponds to the movement of the young flukes toward the bile ducts. During this time, the larvae damage the liver and surrounding membranes, leading to tissue injury and provoking both toxic and allergic reactions that can persist for two to four months. Common complaints in this stage are fever, pain in the right upper abdomen or beneath the sternum, digestive upset such as reduced appetite, bloating, nausea, or diarrhea, as well as respiratory troubles including cough, shortness of breath, coughing up blood, and chest pain. Skin reactions like hives may also occur. The latent stage follows, marked by the maturation of the worms and the beginning of egg production. This phase may last for many months or even years, often going unnoticed. In many cases, infection is only suspected during family investigations after another member is diagnosed (Arjona, 1995). Some patients show high eosinophil counts, recurring digestive troubles, or occasional flare-ups resembling the acute stage. Finally, the biliary or chronic stage develops after prolonged infection, when the parasites cause persistent blockage and long-term damage within the bile ducts.

When the adult worms settle in the bile ducts, they trigger irritation, overgrowth of the lining, and thickening with enlargement of both the ducts and the gallbladder walls. These changes, together with the bulk of the parasites, are enough to block the flow of bile. At this stage, patients may suffer from inflammation of the bile ducts and gallbladder, colicky pain, discomfort in the upper abdomen, poor tolerance to fatty meals, nausea, yellowing of the skin and eyes, itching, and tenderness in the right upper quadrant. Gallstones often develop in the bile ducts or gallbladder, though liver cirrhosis is not typically observed. Sometimes the common bile duct or gallbladder contains blood mixed with bile, clots, or plugs of fibrin. Among children living in endemic regions, pain centered in the upper middle abdomen is frequent, often accompanied by Murphy's sign and jaundice, while other symptoms are less specific. Out of the four stages of the disease, the second and the fourth are the most clinically significant, since patients are usually diagnosed during one of these two (Mas-Coma, 2014).

2.6. Diagnosis

In animals, the diagnosis of fasciolosis is based predominantly on the detection of eggs by feces examinations, immunological and molecular methods (Rana *et al.*, 2014). In addition, clinical signs, seasons, climate conditions, and examinations of snails must be considered. Based on this information, several techniques are used to diagnose the disease like fluke egg count, liver enzyme detection, postmortem examination (Ayele & Hiko, 2016).

Techniques ranging from a simple direct smear to different concentration methods can be used during coproscopy and *Fasciola* eggs become visible after 8-10 weeks post-infection. Egg concentration was obtained by flotation and sedimentation techniques (Mas-Coma *et al*., 2009). Sedimentation techniques seem to be more precise and sensitive than flotation techniques (Mas-Coma *et al*., 2009). The Fluke Eggs Count method (Macmaster method) used in the diagnosis of *Fasciola* is confirmed by finding the eggs in the feces. These eggs must be distinguished from the eggs of other flukes, especially the large eggs of *Paramphistomes*. The *Fasciola* eggs are oval, yellow-brown, and measure (130 to 150µm by 60 to 90 µm). Each egg will possess a distinct operculum (Ayele & Hiko, 2016). Even though it is impossible to detect *Fasciola* species in live animals, examination of the liver at slaughter or autopsy has proven to be the most direct, reliable, and cost-effective technique.

Various serological techniques on blood samples can be used to detect *Fasciola hepatica* antibodies with a high level of specificity**.** Antibodies to *Fasciola hepatica* can be detected for the first time by indirect ELISA between 3 and 6 weeks after infection during the hepatic migration phase of immature worms (Rana *et al.*, 2014). Antibodies to fluke components are detected in serum or milk samples (Village *et al*., 2010)**,** with ELISA and passive hemagglutination being the most reliable (Mars *et al*., 2010)**.** Studies show that coproantigens detection with a commercial Kit (antigens in faecal samples) is preferred to antigenemia (presence of antigens in the blood) because in the latter, circulating antigens rapidly disappear in the serum (Sarkari & Khabisi, 2017). The detection of antigens rather than antibodies is considered the best procedure for evaluating the status of infection. Sandwich-ELISA is a rapid, easy, and sensitive test to detect coproantigens earlier than the liver fluke eggs in feces (Estuningsih *et al.*, 2004). An indirect enzyme immunoassay IEA allows an early diagnosis of fasciolosis. Elevation of liver enzyme activities, such as 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 (Ayele & Hiko, 2016).

Less than a decade ago, nested PCR was developed to increase the sensitivity and specificity of current diagnostic techniques with the view that fasciolosis could be detected in the feces of ruminant weeks after infection. Since molecular diagnostics using PCR is not readily available everywhere and loop-mediated isothermal amplification (LAMP) has been introduced as an alternative (Martínez-Pérez *et al*., 2012)**.** Loop-mediated Isothermal Amplification test (LAMP test) has been developed for the sensitive and rapid detection and discrimination of *Fasciola hepatica* and *Fasciola gigantica*. This LAMP test was about 10^5 times more sensitive than conventional specific PCR tests and may find applicability in the field or poorly equipped laboratories in endemic countries (Ai *et al*., 2010). Post-mortem liver inspection at the slaughterhouse and individual faecal diagnosis need to be improved to reach an acceptable sensitivity (Beesley *et al*., 2018). For this, a complementary tool such as a serological test and molecular test, especially in critical pathological situations make it possible to effectively identify cattle herds affected by clinical fasciolosis in the event of failure of microscopy and are very specific and sensitive, providing useful and practical tools for the epidemiological investigation of *Fasciola* (Houda, 2011; Schweizer *et al*., 2007).

2.7 Treatment

For most intestinal helminth infections, safe and effective drugs against fasciolosis are available (Panic *et al*., 2014). Treatment is essential to control the spread of fasciolosis when infected humans and animals pass the eggs in the stool. The treatment is also essential with efficiency to kill the different stages of development of the parasites (immature and mature stages) (Rana *et al*., 2014).

Many fasciolicides recommended for livestock are available and used for the treatment of fasciolosis, including benzimidazoles (triclabendazole, albendazole) (Fairweather, 2011), salicylanides (oxyclozanide, closantel and rafoxanide), sulfonamides (clorsulon) (Villegas *et al*., 2012), halogenated phenols (nitroxynil, niclofolan, bithionol, hexachlorophene), phenoxyalkanes (diamphenethides) (Elelu & Eisler, 2018). For fasciolosis in humans, triclabendazole is currently the recommended treatment. Metronidazole and albendazole are imidazole derivatives which have also been applied for the treatment of human fasciolosis with varying degrees of success (Mas-Coma, 2014)**.** The presence of emerging drug-resistant liver fluke strains has led to the need for vaccine development. Despite the immense effort of researchers in this regard, no commercial vaccine is yet available (Molina-Hernández *et al*., 2015).

2.8 Prevention and control

The prevention and control of fasciolosis is important for two reasons which on the one hand to minimize the economic losses linked to the reduction of performance of animals infested with *Fasciola* species and to the seizure of livers at the slaughterhouse, and on the other hand to reduce the pressure of parasitic infestation of the herd by limiting the course of the cycle (Ibrahim, 2017a). The measures to be taken are either sanitary (sanitary prophylaxis) or medical (medical prophylaxis) (Houda, 2011).

The sanitary method requires the drainage and the rational use of grasslands and other places of pasture (Ibrahim, 2017). If the water points are reduced, the grass must be mowed and treated with molluscicides. In areas where the means are available, the use of predatory mollusks proves to be an effective method (Rana *et al*., 2014). The use of hygienic measures and grass harvested in risk areas is essential. This boils down to the consumption of hay and silage at least six months after harvest because of the death of the metacercariae beyond this period. The drainage of grasslands and other places of pasture is generally used to avoid flooding which occurs following heavy rainfall, it is also a technique to fight against fasciolosis by destroying the environment in which the snails live (Ibrahim, 2017). Controlling pasture contamination by fluke eggs is the key to rapid and effective fluke control (Houda, 2011).

Medical prophylaxis is based on the chemotherapeutic approach (Fairweather, 2011). This preventive chemotherapy has been the main control strategy for major helminthiasis (Greter *et al*., 2017). Intervals of 12 to 13 weeks of periodic use of anthelmintics are effective against mature and immature flukes. Some author recommend that cattle be dewormed regularly (Aliyu *et al.*, 2014), while others recommend treatment as soon as clinical fasciolosis appears. At the same time, two or three annual treatments have been proposed: at the start of the rainy season, in the middle of the rainy season, and the start of the dry season (Houda, 2011). Vaccines against fasciolosis are not yet commercially available, but some are under development (Ibrahim, 2017).

2.9 Epidemiology of fasciolosis

2.9.1 Prevalence, distribution, and risk factors

Fasciolosis is endemic on all continents of the world including Europe (Isabiryе *et al*., 2012), the Americas (Kleiman *et al*., 2007)**,** Asia (Nguyen *et al*., 2011); Middle East, Oceania (Isabirye *et al*., 2012) and Africa (Abunna *et al*., 2010)**.** The spread of fasciolosis mainly depends on the availability of specific freshwater snails that act as transitional hosts. Beyond this, its occurrence in a given region is shaped by a combination of influences related to the animal host, the parasite itself, and surrounding environmental conditions. For this reason, it is essential to clearly grasp how these three elements interact in order to manage and reduce the disease effectively (Maqbool *et al*., 2002). In sub-Saharan Africa (SSA), fasciolosis has been reported in West Africa (Elelu *et al*., 2016), East Africa (Dida *et al*., 2014), South Africa (Nyirenda *et al*., 2019)**,** and North Africa (Elshraway & Mahmoud, 2017)**.**

Fasciolosis infection is most prevalent and a serious problem in areas with sheep, goats, and cattle production (Soliman, 2008), with prevalence estimates as 5-45% of *Fasciola hepatica* in Europe, 1-up to 94% of *Fasciola hepatica* in America, 5-30% of *Fasciola hepatica* in Oceania, 0-85% of *Fasciola gigantica* in Asia, 2-65 % of *Fasciola gigantica* in Middle East, 14-24% of *Fasciola hepatica* and 7-up to 97% of *Fasciola gigantica* in Africa (Ernest, 2014). Studies conducted in Malang District- East Java in Indonesia reported 30% (, in Mardan District in Pakistan reported 42.8% (Khan *et al*. 2020), in Southern Espirito Santo reported 24.9% (Bernardo *et al*., 2011). in Kware State north-central of Nigeria reported 74.9% (Elelu *et al*., 2016), in Western Ethiopia reported 39.1% (Tulu, 2018), in Southwestern of Ethiopia reported 21.4% (Abebe *et al*., 2018), in Central, Southern and Western provinces of Zambia reported 48 % (Phiri *et al*., 2005), in abattoir of El-Kharga of Egypt reported 30.9% (Elshraway & Mahmoud, 2017), in Western of Ethiopia reported 39.1% (Tulu, 2018), in Kombolcha ELFORA abattoir located in North Eastern part of Ethiopia reported 35% (Mequaninit, 2021), in Saddo municipal abattoir - Southern of Ethiopia reported 14.0% (Abunna *et al.*, 2010). Other studies conducted regarding fasciolosis prevalence determination showed in Lira Municipality abattoir in Northern Uganda a prevalence of 65.7% (Opio *et al*., 2021), in semi-arid coastal Kenya a prevalence of 26% (Mungube *et al*. 2006), in Iringa District of Tanzania a prevalence of 42.1% (Keyyu *et al*., 2006). A prevalence of 9-31% of *Fasciola gigantica* was reported in Tanzania (Mellau *et al*., 2010), and 7-26% of *Fasciola gigantica* in Kenya (Mungube *et al*., 2006). Studies conducted on the epidemiology of fasciolosis among animals have shown that risk factors, including age, sex, breed, and livestock management, have a significant influence on the prevalence of fasciolosis (Abunna *et al*., 2010; Keyyu *et al*., 2006; Phiri *et al*., 2005)**.**

2.10 Economic importance

Fasciolosis in ruminants poses a major health challenge that leads to heavy financial setbacks in animal farming (Abunna *et al*., 2010). These losses arise from deaths, illness, slower weight gain, rejection of damaged livers at slaughter, higher risk of other infections, expenses linked to prevention and treatment, loss of body condition, decline in overall output, reduced resistance during dry periods, poor fertility, and lower milk yield (Bernardo *et al*., 2011).

On a global scale, the reduction in animal productivity caused by this parasite has been valued at more than 3 billion US dollars each year (Ernest, 2014). In the United States, for example, the cattle sector in Florida alone is estimated to lose around 10 million US dollars annually due to liver fluke (Irsik *et al*., 2008). In Africa, research from Uganda reported yearly financial damages of about 37.88 million Ugandan shillings, equivalent to roughly 10,300 dollars (Opio *et al*., 2021).

2.11 Zoonotic importance

Fasciolosis is a parasitic disease that affects not only farm ruminants but also humans (Mas-Coma *et al*., 2005). In humans, as in animals, the disease has a worldwide distribution. While animal infections are commonly found in regions with large populations of cattle, sheep, and goats, human cases tend to occur more often in developing nations. This parasite is a notable zoonotic agent. People generally become infected by eating aquatic plants carrying the infective stage, consuming vegetables contaminated during cultivation, or by drinking untreated water containing the parasite (Mas-Coma & Bargues, 2007).

The frequency of human disease is greatest in areas where eating habits include raw aquatic plants or undercooked animal liver. Modern factors such as expanding cities, increased human movement, and the building of dams and irrigation networks have enlarged the number of people exposed, contributing to a sharp rise in human cases. It is estimated that about 2.4 million individuals are currently infected worldwide, while nearly 180 million more face the risk of exposure. These figures demonstrate that fasciolosis represents a major health threat, justifying its recognition as a zoonotic disease of both international and regional concern (Soliman, 2008).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Description of study area

Burundi is a country which made up of 11 natural regions. Among those regions, the current study was conducted in 5 communes namely Nyanza-lac, Rumonge, Rugombo, Rugazi, and Mutimbuzi in 5 provinces respectively namely Makamba, Rumonge, Cibitoke, Bubanza, and Bujumbura Rural in Imbo region of Burundi. The Imbo region is located between 2°48'30" and 4°20'43" latitude South and 29°36'3" longitude East. It is the westernmost region and the lowest in altitude of Burundi and, also occupies the Northern sector. It extends unequally over six provinces, namely Cibitoke, Bubanza, Bujumbura Rural, Bujumbura Mairie, Rumonge, and Makamba, composed of 11 rural communes and 3 urban communes of Bujumbura town.

The Imbo region is located between Lake Tanganyika to the West and South and the foothills of Mumirwa to the East and Northeast and extends North from Lake Tanganyika towards the Democratic Republic of Congo (Figure 1). The Imbo Region is made up of vast expanses drained by the Rusizi to the North and the thin coastal plain along Lake Tanganyika to the South. The limits of the Imbo region are located between the altitude of 774 m (the mean level of the lake) and the 1000 m isohypse (MEEATU, 2013).

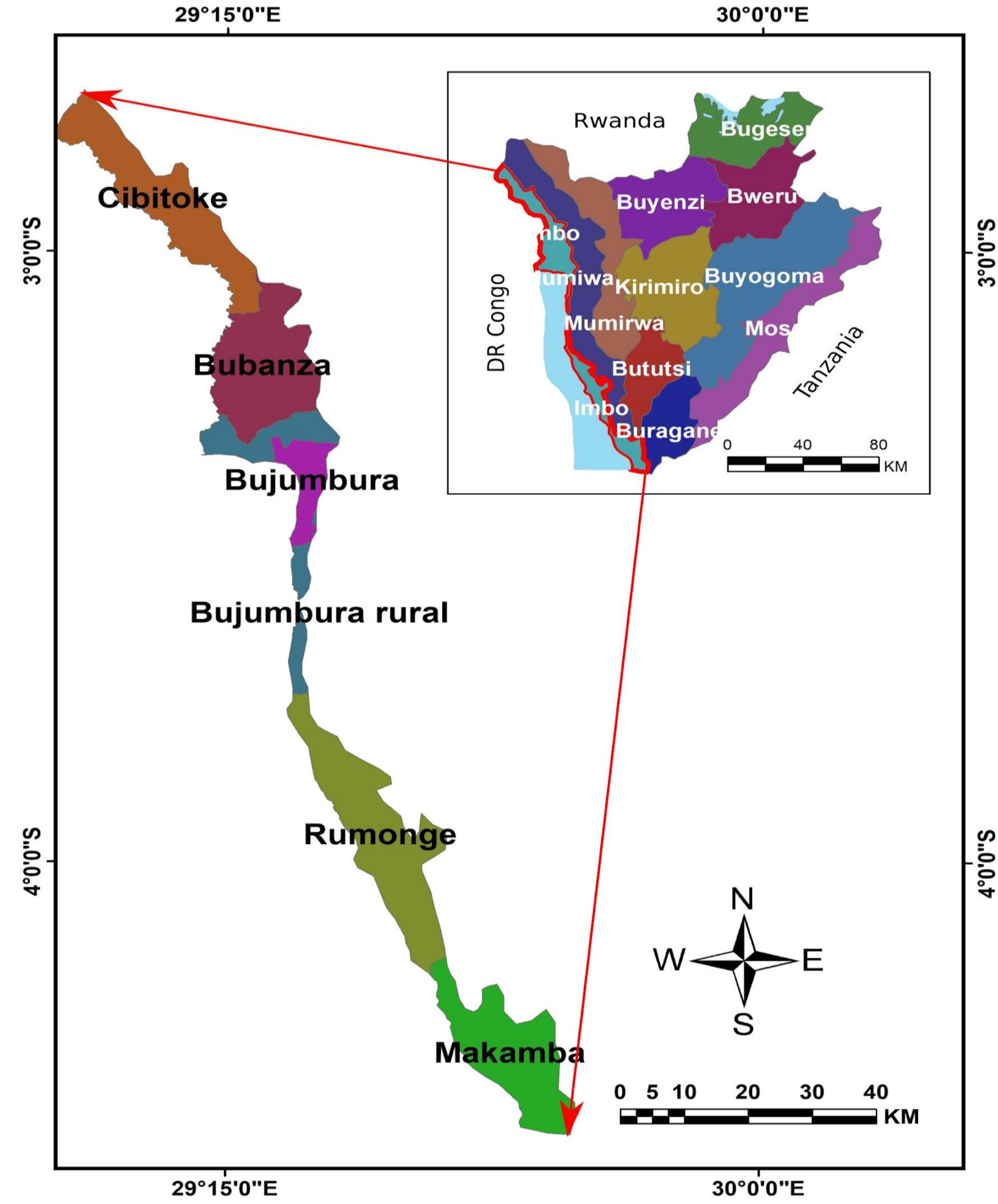

Figure 3: A Map illustrating the subdivisions of regions of Burundi including the Imbo region (Drawn by Nkurunziza S)

3.1.1 Climate and hydrography

From a climatic point of view, the ecological region of the Imbo region is characterized by a rainfall of 800 to 1,100 mm of rain spread over 7 to 8 months such as January, February, March, April, May, October, November, and December, but certain parts, especially in the north, are chronically arid. The average annual temperature is 25°C (range 15^o - 30^o C). The relative humidity is estimated at 70% (MEEATU, 2013).

The hydrography of the Imbo region is placed in the context of that of the Congo basin and precisely in the sub-basin located west of the Congo-Nile ridge. The latter is formed by the Rusizi River and its tributaries and other major tributaries of Lake Tanganyika such as Mutimbuzi, Ntahangwa, Muha, Kanyosha, Mugere, Ruzibazi, Karonge, Kirasa, Dama, Nyengwe and Rwaba (MEEATU, 2013).

3.1.2. Socio-economic conditions

With an area equal to 10% of that of Burundi, for a population density of 300 inhabitants per km², the Imbo plain is one of the most densely populated regions of the country. Its economy is largely dependent on livestock and agriculture. Cattle keeping is common, especially in the central and northern parts of the region. The extra-agricultural economy is made dynamic by the presence of the capital Bujumbura and the Towns of Rumonge and Cibitoke (MEEATU, 2013).

3.2. Ethical clearance

Permission to conduct research in the study area was obtained from the Ministry of Environment, Agriculture and Livestock of Burundi through Directorate Veterinary Services (Ref N^0: 7132/39/NT.D/n.p/2023) (Appendix 1). The inclusion of the cattle owners in the study was done upon receiving the information through the local veterinary services of their consent to be participants.

3.3. Sample size determination

Since there was no previous study in Burundi to establish the prevalence of bovine fasciolosis, the sample size was determined by taking the prevalence of 50%, fasciolosis with absolute precision of 5%, and with a confidence level of 95% using the formula as reported by Abah *et al*.(2019): n = Required sample size, t = Confidence level of 95% (standard value of 1.96), P = Estimated prevalence = 50% (0.5), M = Margin of error at 5% (standard value of 0.05).

i. Sample size of cattle from farms for coprological studies: n$= \frac{t^2 * P(1-P)}{M^2}$ =

$$\frac{1.96^2 * 0.5(1-0.5)}{0.05^2} = 384.$$

A total of 384 cattle was estimated, but 426 cattle were sampled to maximize the precision of prevalence.

ii. Sample size of cattle from abattoir for postmortem liver inspection: n$= \frac{t^2 * P(1-P)}{M^2} =$

$$\frac{1.96^2 * 0.5(1-0.5)}{0.05^2} = 384.$$

A total of 384 cattle were estimated, but 467 were sampled to maximize the precision of prevalence.

The cattle from farms for coprological studies were different from the cattle from the abattoir for postmortem liver inspection. The farmers surveyed were the cattle owners or household heads. In their absence, another adult member of the family, a minimum of 18 years old, was interviewed. For every cattle owner, one or more cattle heads were sampled depending on the herd size and the inclusion criteria, such as clinical signs including diarrhea, dullness, rough body hair, emaciation, bottle jaw, and weight loss. A total of 168 farmers were surveyed. Veterinary professionals to be surveyed were public veterinary services practicing in the study areas including provincial livestock managers, and communal and zonal veterinary technicians. A total of 26 veterinary professionals were surveyed.

3.4. Inclusion and exclusion criteria

In the farms, the study involved all cattle presenting with diarrhea, rough body hair coat, bottle jaw, emaciation, and weight loss. The cattle that were already treated or dewormed for fasciolosis within two weeks of previous sample collection, these cattle were not considered.

In the abattoirs, in addition to the criteria stated above, the study involved all cattle that came from only in Imbo region. Farmers to be surveyed were those who were at least 18 age minimum. Veterinary technicians to be surveyed were the public veterinary services practicing in the Imbo region.

3.5. Study animals and sampling technique

The cattle population sampled in the farms and abattoirs was identified based on the animal level questionnaire. The animal level questionnaire (Appendix 2) was composed of the breed of animals, age, sex, body condition, origin, and clinical signs. The age of cattle was estimated by observing the eruption of the front teeth and the permanent incisors (Ardo *et al.*, 2014) and was classified into three groups such as young (< 2 years), adult (2-4 years), and old (> 4 years). The body condition of each cattle was assessed based on a 5-point scoring system ranging from score 1 (poor) to score 5 (good) (Tulu, 2018).

A multistage random sampling strategy was used for selecting cattle and owners for both studies. Before beginning, the local veterinary services were consulted to obtain a list of provinces, communes, and villages of the Imbo region. Local veterinary services of the villages, communes, and provinces selected were consulted to obtain a list of cattle owners in each village and abattoir in each commune. Based on the lists, the names of provinces, communes, villages, cattle owners, and abattoirs visited were randomly generated using the RAND function in Microsoft Excel. Therefore, the sampled cattle were randomly selected in the households located in 20 villages of 5 communes in 5 provinces of Imbo region in Burundi.

The farmers surveyed were the cattle owners or household heads. In their absence, another adult member of the family, a minimum of 18 years old, was interviewed. For every cattle owner, one or more cattle heads were sampled depending on the herd size and the inclusion criteria, such as clinical signs including diarrhea, rough body hair, emaciation, bottle jaw, and weight loss. A total of 168 farmers were surveyed, 426 cattle were sampled from farms for coprological studies, and 467 cattle were selected from abattoirs for postmortem liver inspection.

3.6. Study design

This was a cross-sectional study to determine the level of knowledge, practices, and attitudes regarding bovine fasciolosis among farmers and practices and perceptions regarding fasciolosis among veterinary professionals through a survey. The determination of the prevalence of bovine fasciolosis was also carried out based on coprological examination of feces collected in the farms to identify the presence of *Fasciola* species eggs and the visit of a slaughterhouse for identification of the liver flukes using post-mortem liver examination of each slaughtered cattle selected. During fecal samples collection, animal level information including sex, breed, body condition scores, origin (communes and provinces) and survey data on cattle husbandry including the source of fodder, source of water, feeding system and anthelmintic commonly used to treat bovine fasciolosis were collected from cattle owners to determine the risk factors associated with bovine fasciolosis. This study was carried out between the period of September 2022 and December 2023.

3.7. Sampling collection and data management

3.7.1 Knowledge, attitudes, and practices data collection among farmers, and perceptions and practices among veterinary professionals

The determination of the level of knowledge, practices, and attitudes regarding bovine fasciolosis among farmers and practices and perceptions among veterinary professionals in the Imbo region of Burundi was conducted through a survey using a web-based mobile phone application, Kobocollect (Namgyal *et al*., 2021) (Version May 20, 2018 Copyright Act of 1998, Section 512 of the U.S. Copyright Act). The questionnaire (Appendix 3) regarding farmers included four sections (1) items concerning the respondent's information (name, age, sex, level of education, profession, status of animal owner, location, etc.); (2) questions related to knowledge and perception of fasciolosis in cattle and its intermediate host; (3) questions related to attitudes and perception of fasciolosis; and (4) questions related to practices on cattle raising (watering, feeding, husbundry and fasciolosis control). The questionnaire (Appendix 4) regarding veterinary professionals included two segments (1) items concerning the respondent's information (age, sex, level of education, experience of profession, location, etc.); (2) perceptions and practices about bovine fasciolosis management.

The farmers surveyed were the cattle owners who aged not less than 18 years. The veterinary professionals surveyed were those public veterinary services exercising their profession in the Imbo region. The questionnaire concerns the farmers was pre-tested to the 10 cattle owners who were not among the supposed to be surveyed but living in Imbo region. For the questionnaire on the veterinary professionals, a pre-test was also conducted on the 2 veterinary technicians who were not among the supposed to be surveyed but exercising their profession in the Imbo region. The participants surveyed were randomly selected using the RAND function in Microsoft Excel. The lists of farmers and veterinary professionals were obtained from the Provincial Office of Environment, Agriculture, and Livestock in each province of 5 provinces in Imbo region. Therefore, 168 farmers and 26 veterinary professionals were surveyed. After pre-testing of the survey questionnaire, there were no changes to adjust. The survey questionnaires were built in English but in the field, the questions were asked in the local language.

Plate 4: Survey on farmers and veterinary professionals at the field in the Imbo region of Burundi (Taken by Nkurunziza S)

3.7.2 Feces collection for prevalence assessment in farms

Fecal samples were taken directly from the rectum of each cattle using disposable gloves and placed in a clean screw-cap universal sample bottle and clearly labeled with the date and the code related to the animal's identification. Fecal samples preserved in 10% formalin were transported to the National Veterinary Laboratory of Bujumbura, and stored in a refrigerator at $4^{o}C$ (Mequaninit, 2021) until their use.

Plate 5: (1) Fecal samples taken directly from the rectum of each cattle, (2) Fecal samples placed in a clean screw-cap universal sample bottle preserved in 10% formalin, (3) screw-cap well labeled and placed into cooler for transport to Laboratory and (4) Fecal samples placed in screw-cap and stored in a refrigerator at $4^{o}C$ until their use (Taken by Nkurunziza S)

3.7. 3 Feces examination using a microscope for prevalence assessment in farms

In the laboratory, a sedimentation technique was used to detect the presence or absence of liver fluke eggs in the fecal samples under an optical microscope (Tulu, 2018). Five grams of fecal samples were added into 50 ml of water into container 1. The contents were then mixed using a tongue blade and filtered through a tea strainer to remove any large debris into container 2. The filtrate was left to sediment for 5 minutes. The supernatant was then very carefully discarded. The sediment was then resuspended in 40 ml of water and was left to sediment for 5 minutes. The supernatant was again very carefully discarded. The sediment was then stained with two drops of methylene blue solution to differentiate the eggs of *Fasciola* species and *Paramphistomum* species or other trematodes where the eggs of *Fasciola* species show a yellowish color while the eggs of *Paramphistomum* species stain blue methylene. The sediment was placed on a slide with a coverslip and viewed under the microscope. Eggs of *Fasciola* species were identified by their morphologic characteristics (operculated with a thin shell, broadly ellipsoidal with the estimated measurements of 130-150 Mm in length and 63-90 Mm in breadth, and yellow color)(Hussein *et al*., 2010; Miller, 1997; Roepstortf & Nansen, 1998).

The MacMaster counting technique was used to determine the egg burden of *Fasciola* species as indicated by (Miller, 1997). Five grams of fecal samples were added into container 1 of

56 ml of flotation liquid (Zinc sulfate) and mixed using a tongue blade. The solution was then passed through a sieve (mesh size 210 mm) into container 2. While swirling the filtrate in container 2, a subsample was taken with a Pasteur pipette. Both sides of the Macmaster counting chamber were filled with the subsample. The counting chambers were let to sit for 5 minutes. The counting chambers were examined under a microscope and all the eggs in the etched area were counted. The number of eggs per gram of feces (epg) was calculated as follows, the number of eggs from both chambers was added and the total was multiplied by 50.

The level of infection intensity was classified according to the number of eggs per gram of feces (epg), such as light infection (epg from 1-600), moderate infection (epg between 600-1000 eggs), and heavy infection (epg > 1000 eggs) (Tulu, 2018).

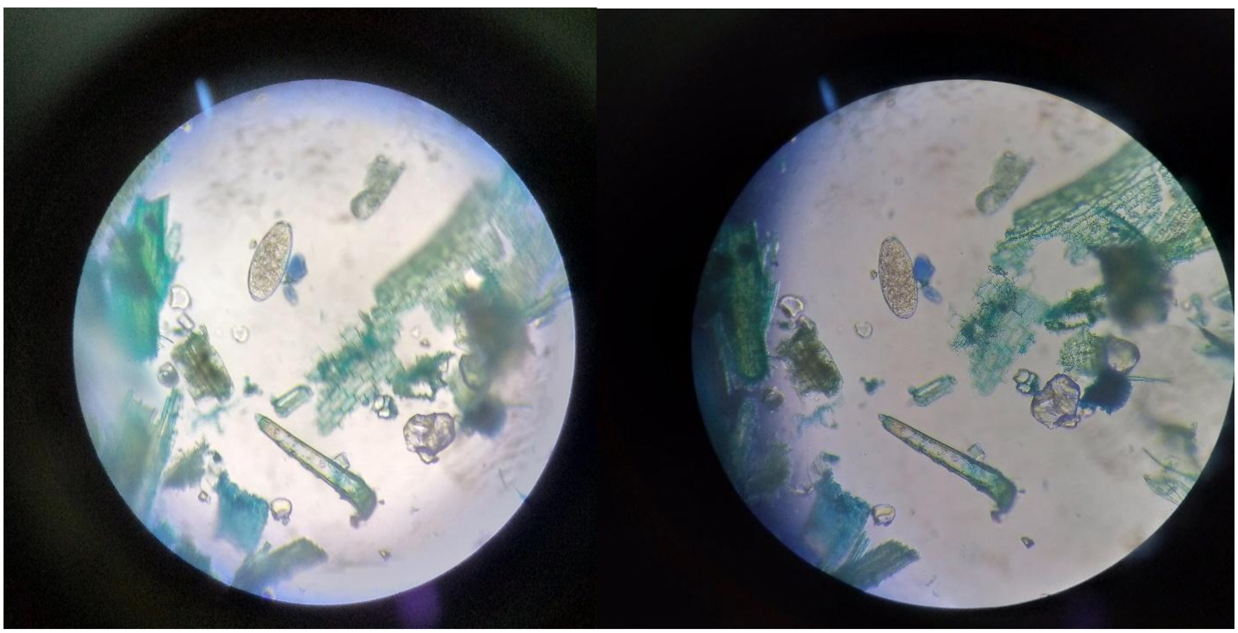

Plate 6: *Fasciola* species eggs found in microscopic field stained with methylene bleu. Photos taken using a mobile phone on microscopic ocular during this study from Burundi (Taken by Nkurunziza S)

3.7. 4 Postmortem liver inspection for prevalence assessment in abattoirs

At each abattoir, before they are slaughtered, the cattle are selected based on whether they came from the province where the slaughterhouse is located. The visit to each abattoir took a period of 10 days (2 weeks) from Monday to Friday per week at the time from 6:00 to 9:00 AM (3 hours per day). A total of 467 cattle were randomly selected during ante mortem and individual information including date, cattle owner, origin, sex, age, breed, body condition score, and clinical signs were recorded in notebooks. During post-mortem liver inspection, the liver and associated bile ducts and gallbladders were carefully examined for the presence or absence of liver fluke using visualization, palpation, and incision with a knife (Kusumarini *et al*., 2020). Liver lesions were then grouped based on the liver fluke burden into a mild infection (1-29 flukes), moderate infection (30-50 flukes), and heavy infection (>50 flukes) (Mpisana *et al*., 2022).

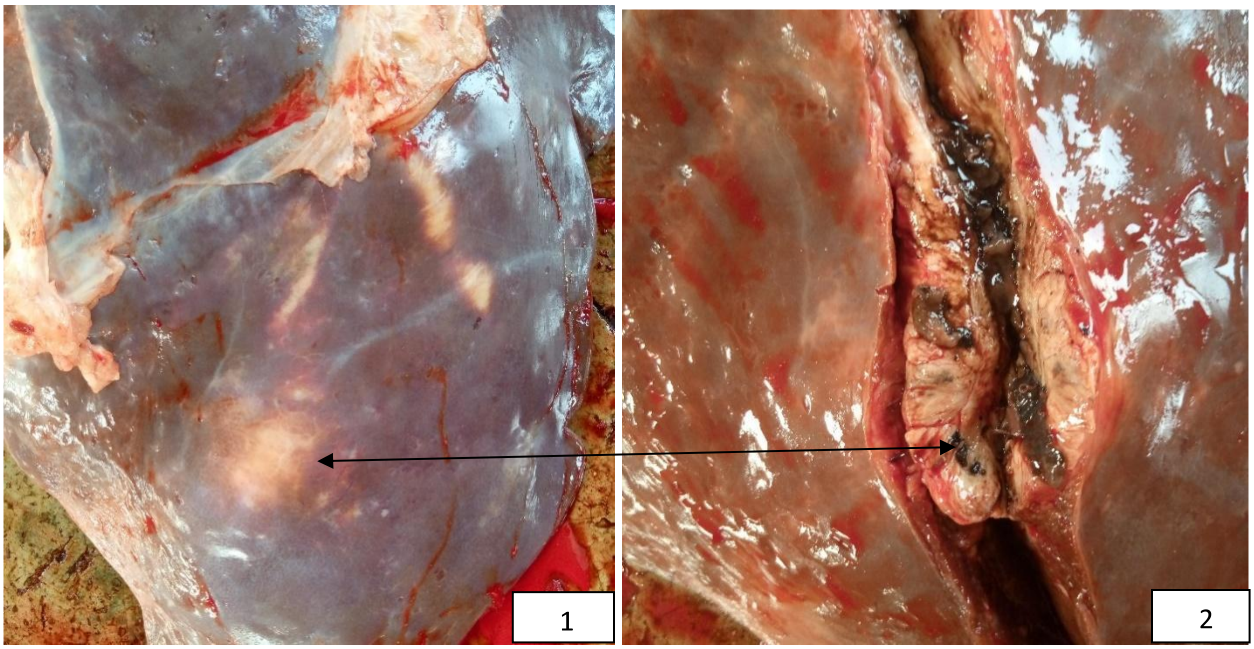

Plate 7: Liver of cattle from at abattoir of Nyanza-Lac commune of Makamba province in Imbo region of Burundi: (1) liver changed color due to liver fluke burden and (2) the site of changed color, the bile duct and associated tissues of the liver were destructed due to liver fluke burden (Taken by Nkurunziza S)

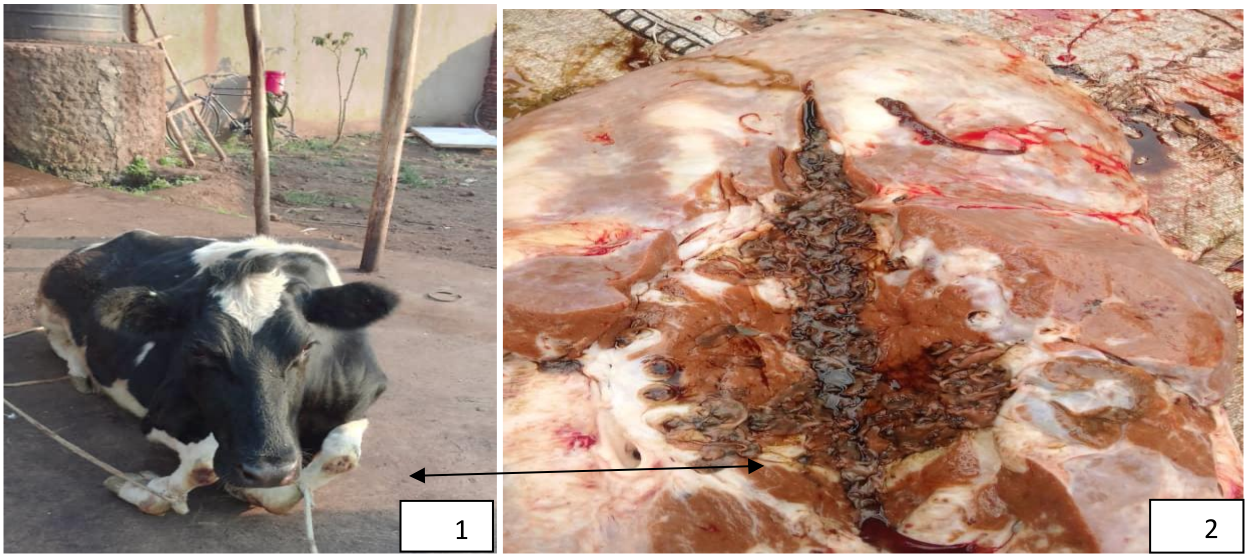

Plate 8: Cattle from the abattoir of Rugombo commune of Cibitoke province in the Imbo region of Burundi: (1) Clinically at the abattoir, cattle presented reluctance to move, weakness, and dyspnea, (2) liver fluke burden in the liver of this cattle (Taken by Nkurunziza S)

Plate 9: Discussion with butchers regarding the importance, requirements, and procedures of this study of epidemiology and control of fasciolosis in cattle in the Imbo region of Burundi (Taken by Nkurunziza S)

3.7. 5 Data collection for risk factors associated with bovine fasciolosis assessment

During feces collection in the farms, the identification of sampled cattle was collected using a web-based mobile phone application, Kobocollect (Version May 20, 2018, Copyright Act of 1998, Section 512 of the U.S. Copyright Act) through animal level questionnaire.

The animal level questionnaire included the code of cattle sampled followed by the date of collection, age, breed, sex, origin, body condition score, and clinical signs. The data from the animal level questionnaire was used to assess the risk factors associated with bovine fasciolosis based on cattle identification. In addition, the data from the survey among farmers regarding their practices towards cattle raising management was also used to assess the risk factors associated with bovine fasciolosis in the Imbo region of Burundi.

3.7. 6 Data analysis

Data collected from the survey through the Kobocollect application was imported into Microsoft Excel for cleaning and finally to R software for further analysis (Namgyal *et al*., 2021). Numerical and categorical data were presented as counts and percentages, respectively (Sazmand *et al*., 2020).

The recorded data from the feces examination study was entered into the Microsoft Excel database system and carefully cleaned. The cleaned data was imported into R statistical software for analysis where prevalence, mean, and Standard deviation were generated (Tulu, 2018). Chi-square analysis and univariate and multivariate ordinal logistic regression analysis were used to assess the association between fasciolosis with sex, age, body condition score, breed, origin, and practices regarding cattle raising among farmers.

The recorded data from postmortem liver inspection was entered into the Microsoft Excel database system and carefully cleaned. The cleaned data was imported into R statistical software for analysis where prevalence, mean, and Standard deviation were generated (Tulu, 2018). Chi-square analysis was used to assess the association between fasciolosis with origin. A 95% confidence interval and a P-value of less than 0.05 (at the 5% level of significance) was considered in all analyses (Opio *et al*., 2021).

CHAPTER FOUR

RESULTS

4.1 Knowledge, attitudes, and practices of farmers, and perceptions and practices of veterinary professionals on bovine fasciolosis

4.1.1 Socio-demographic characteristics of farmers, their knowledge, attitudes, and practices information on fasciolosis

A total of 168 respondents were interviewed and provided complete information during an interview conducted in 5 communes including Nyanza-lac, Rumonge, Rugombo, Rugazi, and Mutimbuzi in 5 provinces respectively namely Makamba, Rumonge, Cibitoke, Bubanza, and Bujumbura Rural (Figure 10).

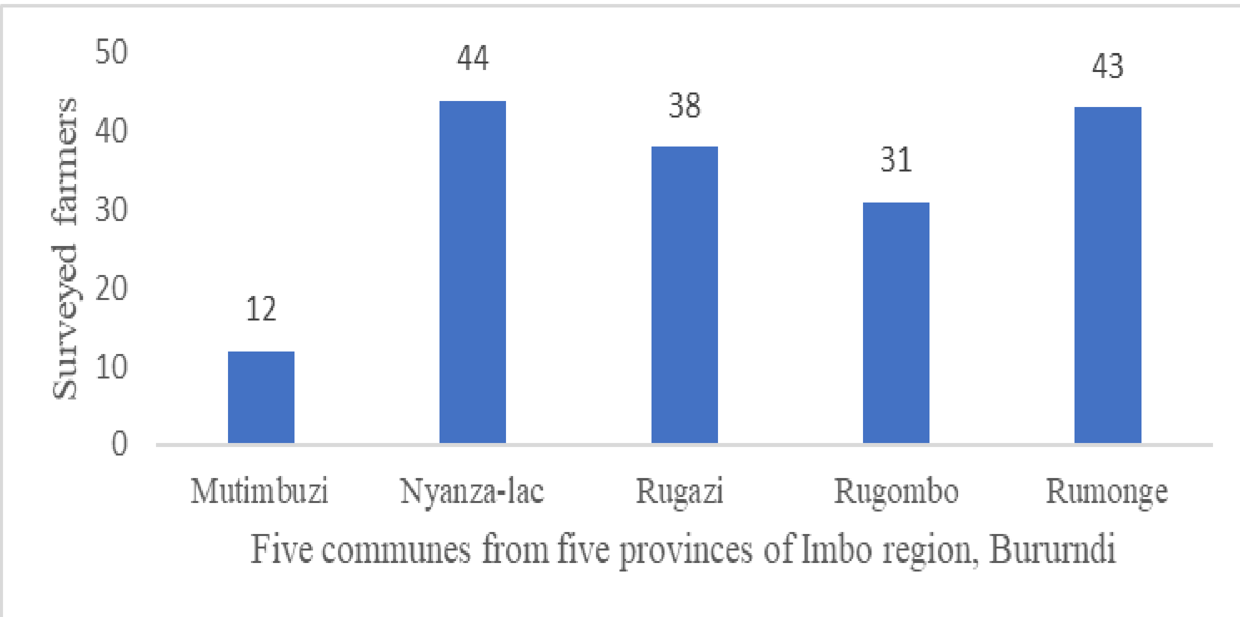

Figure 10: Farmer's distribution in five communes of five provinces in the Imbo region of Burundi (Drawn by Nkurunziza S)

This survey included 23 women (13.7 %) and 145 men (86.3 %). The majority of respondents (66.1 %) were 35-60 years and in this survey, a young farmer had a minimum of 26 years while an old farmer had a maximum of 86 years. Most of the respondents (36.9 %) and (33.3%) were not attended any school or primary level respectively. Of the total study participants, 84.5% were professional farmers, 11.9 % were public services and pastoralists and 3.6 % were private professionals and pastoralists. About 90.5% of respondents were married and 67.3% had experience in cattle rearing ranging from 1 to 5 years. The details of the characteristics of farmers surveyed in this study are summarized in Table 1.

Table 1: Socio-demographic characteristics information of farmers surveyed in the Imbo region of Burundi

Variables	**Number of respondents (*n*=168)**	**Percentage (95% CI)**
	Gender	
Female	23	13.7 (8.5-18.9)
Male	145	86.3 (81.1-91.5)
	Age (years)	
<35 (Minimum: 26)	16	9.5 (5.1-14)
35-60	111	66.1 (58.9-73.2)
>60 (Maximum: 86)	41	24.4 (17.9-30.9)
	Education level	
Not attended any school	62	36.9 (29.6-44.2)
Primary level	56	33.3 (26.2-40.5)
Secondary level	35	20.8 (14.7-27)
University level	15	8.9 (4.6-13.2)
	Profession	
Farmer professional	142	84.5 (79.1-90)
Private professional pastoralists	6	3.6 (0.8-6.4)
Public service pastoralists	20	11.9 (7-16.8)
	Marital status of animal owner	
Married	152	90.5 (86-94.9)
Widow/Widower	14	8.3 (4.2-12.5)
Single	2	1.2 (0.5-2.8)
	Experience in cattle rearing (Years)	
<5	113	67.3 (60.2-74.4)
5-10	36	21.4 (15.2-27.6)
>10	19	11.3 (6.5-16.1)

Results obtained from farmers in this survey showed that 82.7% raise cattle only in their farms and 17.3% raise cattle and small ruminants at the same time in their farms. About 94.6% of participants raise cattle and small ruminants in permanent stables and most of 61.3% raise cattle for a savings source of income and manure source of income. The results of the survey indicated that 99.4% of respondents know snails and 83.9% of respondents confirmed that they always saw snails in terrestrial or aquatic environments. Results showed also 85.1% of respondents don't think that snails can spread diseases that could attack cattle and 99.4% don't think that snails can spread diseases that could attack humans.

Among surveyed farmers, 57.7% of respondents knew the name fasciolosis disease and only 28.6% knew the cause of fasciolosis. This survey showed also that 40.5% of respondents think that fasciolosis can attack cattle and 0.6% of respondents think that fasciolosis can attack humans. Most of the respondents (85.7 %) didn't know that fasciolosis can cause death on the farms and 78% didn't know that fasciolosis can be a major problem in their farms. The detail of knowledge and perception of fasciolosis and its intermediate host among farmers is presented in Table 2.

Table 2: Farmers 'knowledge and perception information regarding fasciolosis and its intermediate host snails

Variables	**Frequency (*n*=168)**	**Percentage (95% CI)**
Knowledge of bovine raising status		
Animal species(Ruminants)		
Cattle	139	82.7 (77-88.5)
Small ruminants and Cattle	29	17.3 (11.6-23)
Type of cattle husbandry practice followed by the farmer		
Permanent stable	159	94.6 (91.2-98.1)
Semi-permanent stable	6	3.6 (0.8-6.4)
Non-permanent stable	3	1.8 (0.2-3.8)
Why do you raise cattle?		
Savings source of income/Manure source of income	103	61.3 (53.9-68.7)

Manure source of income/Savings source of income/Family self-consumption	65	38.7 (31.3-46.1)
Knowledge and perception of the intermediate host of fasciolosis		
Do you know snail, or have you at least heard this name (snail)?		
Yes	167	99.4 (96.7-100)
No	1	0.6 (0-3.3)
Where do you commonly find snails?		
Aquatic environments/In irrigation canals	27	16.1 (10.5-21.6)
Terrestrial environments/Aquatic environments	141	83.9 (78.4-89.5)
Do you think that snails can spread diseases that could attack the cattle?		
Yes	25	14.9 (9.5-20.3)
Don't know	143	85.1 (79.7-90.5)
Do you think that snails can spread diseases that could attack humans?		
Yes	1	0.6 (0-3.27)
Don't know	167	99.4 (96.7-100)
Knowledge and perception of fasciolosis		
Do you know fasciolosis, or have you at least heard this name?		
Yes	97	57.7 (50.3-65.2)
No	71	42.3 (34.8-49.7)
Do you know what causes fasciolosis?		
Yes	48	28.6 (21.7-35.4)
No	120	71.4 (64.6-78.3)
Do you think fasciolosis can attack humans?		
Yes	1	0.6 (0-3.3)
Don't know	167	99.4 (96.7-100)
Do you think fasciolosis can attack cattle?		
Yes	68	40.5 (33.1-47.9)
Don't know	100	59.5 (52.1-67)
Where do you think the cattle get fasciolosis from?		

From drinking water/From feed with fodder	55	32.7 (25.6-39.8)
I don't know	113	67.3 (60.2-74.4)
Farmers' source of information about the transmission of bovine fasciolosis		
Community animal health workers	52	31 (24-37.9)
Nowhere	116	69.1 (62.1-76)
Is the disease fasciolosis a major problem for you on this farm?		
Yes	37	22 (15.8-28.3)
Don't know	131	78 (71.7-84.2)
Does it cause death?		
Yes	24	14.3 (9-19.6)
Don't know	144	85.7 (80.4-91)

The descriptive results obtained from the responses to the questions about farmer's attitudes indicated that 75.6% thought that adopting good practices including watering clean water, feeding clean fodder, respect control measures against diseases including fasciolosis can reduce the risk of fasciolosis infection in cattle herds and 64.9% thought that timely deworming against fasciolosis can reduce cattle morbidity and mortality. Farmers 'attitudes information regarding fasciolosis is presented in Table 3.

Table 3: Famers 'attitudes information towards cattle raising management against fasciolosis

Variables	**Frequency (n=168)**	**Percentage (95% CI)**
Adopting good farm practices can reduce the risk of fasciolosis infection (e.g., watering clean water, feeding clean fodder, respecting control measures, etc.)		
Agree	127	75.6 (69.1-82.1)
No opinion	41	24.4 (17.9-30.9)
Timely deworming against fasciolosis can reduce cattle morbidity and mortality		
Agree	109	64.9 (57.7-72.1)
No opinion	59	35.1 (27.9-42.3)
The risk of fasciolosis infestation can be reduced by always keeping cattle in the shed		
Agree	52	31 (24-37.9)
No opinion	116	69.1 (62.1-76)

Avoidance of mixing different animal species in a single shed can reduce the risk of fasciolosis spread.		
Agree	41	24.4 (17.9-30.9)
No opinion	127	75.6 (69.1-82.1)
The animal is suspected of disease, timely reporting to an animal health professional can reduce animal mortality including fasciolosis		
Agree	37	22 (15.8-28.3)
No opinion	131	78 (71.7-84.2)

From farmers ‘practices regarding cattle raising management, the results from this survey indicated that 97% of respondents use stall feeding during cattle feeding. The majority of 88.1% of respondents found the fodder to feed the cattle in the agricultural land or aquatic environment or at the edge of the rivers or lakes and 61.3% found the water for watering the cattle from tap water. Most 76.2% of respondents used alternatively of albendazole and nitroxynil for deworming against helminthosis infection including fasciolosis in cattle and the results of this survey indicated that all respondents apply deworming against helminthosis infection including fasciolosis every three months. The detail of results obtained from the responses to the questions about farmer's practices is presented in Table 4.

Table 4: Farmers ‘practices towards cattle raising management

Variables	**Frequency (*n*=168)**	**Percentage (95% CI)**
How do you feed your cattle?		
Stall feeding	163	97 (94.5-99.6)
The mix of stall feeding & free grazing	5	3 (0.4-5.6)
Where do you find the fodder to feed the cattle?		
Aquatic environment	20	11.9 (7-16.8)
In the agricultural land/Aquatic environment/At the edge of the rivers or lake	148	88.1 (83.2-93)
Where do you find the water for watering the cattle?		
Tap water	103	61.3 (53.9-68.7)
In the rivers/lake/In irrigation canals	65	38.7 (31.3-46.1)
Which anthelmintics are you commonly using to treat your animals?		

Albendazole/Nitroxinil	128	76.2 (69.8-82.6)
Oxyclozanide(Zanil)/Nitroxinil	40	23.8 (17.4-30.3)
What is the frequency of applying deworming on your farm?		
In three months	168	100%

4.1.2 Profile characteristics of veterinary professionals, their perceptions, and practices information on fasciolosis

A total of 26 veterinary professionals were interviewed and provided complete information during this survey (Figure 11).

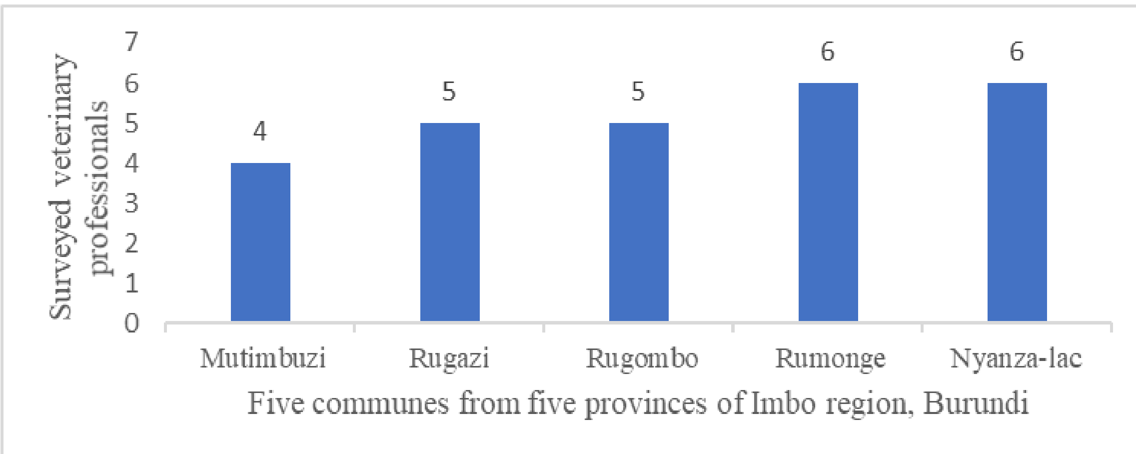

Figure 11: Distribution of veterinary professionals in five communes of five provinces in the Imbo region of Burundi (Drawn by Nkurunziza S)

The results from this study conducted through a survey among veterinary professionals included 1 woman (3.9%) and 25 men (96.2%). The majority of veterinary professionals (80.8 %) were 35-60 years among them a younger had a minimum of 28 years while an older had a maximum of 58 years. The interviewed veterinary professionals (88.5 %) and (11.5%) had an education level of Basic Veterinary Worker (Holder of Diploma obtained from Agricultural Technical Institute of Burundi in veterinary sciences option) and Zootechnical Engineer or bachelor's in animal health and production (Holder of Diploma obtained from University of Burundi in Faculty of Agronomy and Bioengineering in department of animal production and health), respectively.

Among the surveyed veterinary professionals, 38.1% were Zonal veterinary technicians (Basic Veterinary Workers), 19.2% were Commune veterinary technicians (Basic Veterinary

Workers) and 19.2% were Provincial Livestock Officer (Zootechnical Engineer or bachelor's in animal health and production). About 38.5 % of veterinary professionals had experience in a field of more than 10 years, 23.5 % had experience in a field of 5 to 10 years, and 23.5 % had experience in a field of under 5 years. The details of the profile characteristics of veterinary technicians are presented in Table 5.

Table 5: Veterinary professionals 'profile characteristics information in the Imbo region of Burundi

Variables	**Number of respondents (*n*=26)**	**Percentage (95% CI)**
	Gender	
Female	1	3.9 (0.1-19.6)
Male	25	96.2 (80.4-99.9)
	Age (Years)	
<35 (Minimum: 28)	5	19.2 (4.1-34.4)
35-60 (Maximum: 58)	21	80.8 (65.6-95.9)
	Education level	
Basic Veterinary Worker	23	88.5 (69.9-97.6)
Zootechnical Engineer or Bachelor's in animal health and production	3	11.5 (2.5-30.2)
	Profession	
Zonal veterinary technician	16	38.1 (23.4-52.8)
Commune veterinary technician	5	19.2 (4.1-34.4)
Provincial Livestock Manager	5	19.2 (4.1-34.4)
	Years of experience in the field	
<5	8	23.5 (9.3-37.8)
5-10	8	23.5 (9.3-37.8)
>10	10	38.5 (19.8-57.2)

The descriptive results obtained from the responses to the questions addressed to veterinary professionals regarding their perceptions and practices on bovine fasciolosis indicated that among surveyed participants, 62% didn't have a record-keeping book and the majority of 73.1% said that all ruminants including cattle, sheep and goats are frequently attacked by fasciolosis. The results showed also that 69.2% of surveyed participants said that exotic breeds and crossbreds are more frequently attacked by fasciolosis than local breeds. All surveyed veterinary professionals (100%) said that the diagnosis of fasciolosis at the local Veterinary Field Units is made by clinical diagnosis due to the lack of materials and equipment for diagnosis of parasitic infections. Therefore, most surveyed veterinary professionals (92.3%) indicated that they find a high-rate prevalence of bovine fasciolosis in abattoirs when comparing the clinical diagnosis made in the farms and 65.4% indicated that among the diseases that occur often and cause high economic losses and reduce productivity in cattle, fasciolosis is classified at 2nd level.

This study showed also that 50% of surveyed veterinary professionals were using anthelmintics including alternatively albendazole and Nitroxinil, 34.6% were using the alternative of oxyclozanide (Zanil) and Nitroxinil and 15.4% were using Nitroxinil also during deworming against fasciolosis. All surveyed veterinary professionals indicated that they advised the farmers to follow bovine fasciolosis control measures through deworming practices and the use of clean feed, water, and forage but 50% of surveyed indicated that the farmers didn't call them in good time to treat their cattle showing signs of illness. The details of descriptive results from questions of veterinary professionals on their perceptions and practices regarding bovine fasciolosis are presented in Table 6.

Table 6: Perceptions and practices of veterinary professionals on bovine fasciolosis in the Imbo region of Burundi

Variables	**Frequency (*n*=26)**	**Percentage (95% CI)**
Do you have a record-keeping book?		
Yes	10	39 (20-57)
No	16	62 (43-80)
What species of animals are frequently attacked by fasciolosis?		
Cattle	7	26.9 (9.9-44)
Small ruminants/Cattle	19	73.1 (56-90.1)
What breed of cattle are frequently attacked by fasciolosis?		
Local	8	30.8 (13-48.5)
Exotic breed/crossbreed	18	69.2 (51.5-87)

What type of diagnosis is made at the local Veterinary Field Units?		
Clinical diagnosis	26	100%
Where do you find a high-rate prevalence of bovine fasciolosis when comparing the clinical diagnosis made in the farms and cattle slaughtered in abattoirs?		
In abattoirs	24	92.3 (74.9-99.1)
In breeding areas	2	7.7 (1 -25.1)
Among the diseases caused by the parasites that occur often and cause high economic losses and reduce productivity in cattle, at what level can you classify fasciolosis?		
2nd level	17	65.4 (47.1-83.7)
3rd level	6	23.1 (6.9-39.3)
4th level	3	11.5 (0.7-23.8)
Which anthelmintics do you commonly used to treat the cattle against fasciolosis?		
Albendazole/Nitroxinil	13	50 (30.8-69.2)
Oxyclozanide(Zanil)/Nitroxinil	9	34.6 (16.3-52.9)
Nitroxinil	4	15.4 (1.5-29.3)
Do the farmers call in good time to treat their cattle showing signs of illness?		
Yes	13	50 (30.8- 69.2)
No	13	50 (30.8 -69.2)
What control measures do you follow to address the bovine fasciolosis infection?		
Practice of deworming/Advise the farmers to use clean feed, water, and forage	26	100

4.2 Prevalence of bovine fasciolosis assessment

Of the 426 fecal samples examined from cattle, the current study showed 203 cattle (47.7%, CI: 42.9- 52.4) positive for fasciolosis in farms from the study region (Table 7). Of the 467 livers from slaughtered cattle in the study area, 155 livers (33.2%, CI: 28.9-37.5) were found to have liver flukes (Table 7).

Table 7: Overall prevalence of bovine fasciolosis in farms (fecal analysis) and abattoirs (liver necropsy) in the study area of Imbo region, Burundi

In farm (fecal analysis)			In abattoir (liver necropsy)		
Cattle examined	Positive cattle	Overall prevalence % (95% CI)	Cattle examined	Positive cattle	Overall prevalence % (95 % CI)
426	203	47.7 (42.9-52.4)	467	155	33.2 (28.9-37.5)

95% CI: 95% Confidence Interval

Regarding the level of infection based on eggs per gram of feces (epg), this study showed 123 cattle (60.6%) with light infection, 57 cattle (28.1%) with moderate infection, and 23 cattle (11.3%) with heavy infection (Table 8). Regarding the level of infection based on liver fluke burden, the study showed 124 cattle (80%) with mild infection, 12 cattle (7.7%) with moderate infection, and 19 cattle (12.3%) with heavy infection (Table 9).

Table 8: Prevalence of *Fasciola* spp. infection intensity based on eggs per gram of feces (epg) in bovines from the study area (Imbo Region, Burundi)

Severity	Positive cattle	Intensity Class	Prevalence % (95% CI)	Mean epg	Std. Dev. of epg	Minimum	Maximum
Light infection	203	123	60.6(53.9-67.3)	561.3	377.9	100	1600
Moderate infection	203	57	28.1 (21.9-34.3)				
Heavy infection	203	23	11.3 (6.9-15.7)				

95% CI: 95% Confidence Interval, epg: eggs per gram of feces, Std. Dev.: Standard deviation

Table 9: Prevalence of *Fasciola* spp. infection intensity based on number of liver flukes in the liver of slaughtered cattle from abattoirs of the study area (Imbo Region, Burundi)

Severity	Positive cattle	Intensity Class	Prevalence % (95% CI)	Mean liver fluke	Std.Dev. liver fluke	Minimum	Maximum
Mild infection	155	124	80 (73.7-86.3)	23.2	35.9	1	210
Moderate infection	155	12	7.7 (3.5-12)				
Heavy infection	155	19	12.3 (7.1-17.4)				

95% CI: 95% Confidence Interval, Std. Dev.: Standard deviation

4.3 Risk factors associated with bovine fasciolosis assessment

Based on the origin of the fecal samples from Imbo region, the current study showed that 38 cattle (30.7%) in Rugombo commune of Cibitoke province, 36 cattle (40.9%) in Rugazi commune of Bubanza province, 42 cattle (50.6%) in Nyanza-Lac Commune of Makamba province, 32 cattle (56.1%) in Rumonge commune of Rumonge province and 55 cattle (74.3%) in Mutimbuzi commune of Bujumbura rural province were positive for fasciolosis. This study reported a statistical difference (P-Value < 0.05) between the prevalence of fasciolosis according to the origin of the fecal sample ($\chi2$ = 39 and P-value = 6.9e-09 means <0.01) (Table 10).

Table 10: Prevalence of bovine fasciolosis based on farm location (Imbo region, Burundi)

Farm location	Cattle tested	-ve	+ve	prevalence % (95% CI)	$\chi2$	df	P-value
Rugombo	124	86	38	30.7 (22.5-38.8)	39	4	6.9e-09(<0.01)*
Nyanza-lac	83	41	42	50.6 (39.9-61.4)			
Rumonge	57	25	32	56.1 (43.3-69)			
Rugazi	88	52	36	40.9 (30.6-51.2)			
Mutimbuzi	74	19	55	74.3 (64.4-84.3)			

-ve: negative cattle, +ve: positive cattle, 95% CI: 95% Confidence Interval, $\chi2$: chi-square, df: degree of freedom, *: significant difference at p<0.05.

Based on abattoir location, this study showed the prevalence of 53.2% in Rubirizi abattoir of Mutimbuzi commune in Bujumbura Rural Province, 51.2% in Rwaba abattoir of Nyanza-Lac commune in Makamba province, 31.7% in Tr4 abattoir of Rugombo commune in Cibitoke province, 16.8% in Muzinda abattoir of Rugazi commune in Bubanza province and 11.5% in Birimba abattoir of Rumonge commune in Rumonge province. Therefore, in the univariate analysis using the chi-square test, there was a statistically significant (P-value<0.05) difference between abattoir location ($\chi 2$ = 67.6, P-value= 7.2e-16 means <0.01) and the prevalence of Fasciola spp. fasciolosis in cattle (Table 11).

Table 11: Prevalence of bovine fasciolosis based on abattoir location (Imbo region, Burundi)

Abattoir location	Livers examined	-ve	+ve	prevalence % (95% CI)	$\chi 2$	df	P-value
					67.6	4	7.2e-16
Rwaba abattoir in Nyanza-Lac	43	21	22	51.2 (36.2-66.1)			(<0.01)*
Birimba abattoir in Rumonge	87	77	10	11.5 (4.8-18.2)			
Tr4 abattoir in Rugombo	60	41	19	31.7 (19.9-43.4)			
Muzinda abattoir in Rugazi	119	99	20	16.8 (10.1-23.5)			
Rubirizi abattoir in Mutimbuzi	158	74	84	53.2 (45.4-60.9)			

-ve: negative cattle, +ve: positive cattle, 95% CI: 95% Confidence Interval, $\chi 2$: chi-square, df: degree of freedom, *: significant difference at p<0.05.

This study showed that the prevalence of bovine fasciolosis based on sex and age was higher for females (51.6%) than males (28.8%) and was higher for adults 2-4 years (58.1%) than old> 4 years (42.7%) and young<2years (33.9%) cattle. The univariate analysis showed that risk factors such as sex ($\chi 2$ = 12.6, P-value= 0.3e-3 means <0.01), and age ($\chi 2$ = 19.7, P-value= 5.1e-07 means <0.01) were statistically associated with the bovine prevalence of fasciolosis in this study. Furthermore, females (OR = 2.6; IC: 1.5-4.6) and adult cattle 2-4 years (OR= 2.7; IC: 1.7-4.3) were likely to be more at risk of getting bovine fasciolosis in the study area (Table 12). In multivariate logistic regression analysis, this study showed that risk factors such as sex (P-value = 0.01) and age (P-value = 0.02) were statistically associated with the prevalence of bovine fasciolosis (Table 13).

Table 12: Univariate analysis of risk factors associated with bovine fasciolosis

Risks factors	Cattle tested	-ve	+ve	prevalence % (95% CI)	OR (95% CI)	χ2	df	P-value
Sex						12.6	1	0.3e-3 (<0.01)*
Male	73	52	21	28.8(18.4-39.2)	Ref			
Female	353	171	182	51.6 (46.3-56.8)	2.6 (1.5-4.6)			
Breed						2.8	3	0.4
Ankole	69	40	29	42 (30.4-53.7)	Ref			
Crossed	77	44	33	42.9 (31.8-53.9)	1.1 (0.5-2)			
Sahiwal	23	13	10	43.5 (23.2-63.7)	1.1 (0.4-2.8)			
Frisian	257	126	131	50.9 (44.9-57.1)	1.4 (0.8-2.5)			
Age						19.7	2	5.1e-07 (<0.01)*
Young	127	84	43	33.9 (25.6-42.1)	Ref			
Adult	210	88	122	58.1 (51.4-64.8)	2.7 (1.7-4.3)			
Old	89	51	38	42.7 (32.4-52.9)	1.5 (0.8-2.5)			
BCS						3.5		0.1
Poor	72	44	28	38.9 (27.6- 50.2)	Ref			
Medium	127	60	67	52.8 (44.1- 61.4)	1.8 (0.9-3.2)			
Good	227	119	108	47.6 (41.1- 54.1)	1.4 (0.8-2.5)			

-ve: negative, +ve: positive, 95% CI: Confidence Interval, OR: Odds Ratio, χ2: chi-square, df: degree of freedom, *: significant difference at p-value<0.05, BCS: Body Condition Score

Table 13: Multivariate logistic regression model analysis of risk factors associated with bovine fasciolosis

Variables	ß-coefficients	P-value
Intercept	-1.1841	<0.01**
sex	0.7430	0.01*
Age	0.5480	0.02*
Body condition score	0.4791	0.08
Breed	-0.3579	0.21

For cattle raising practices, the risk factors assessed were based on the feeding system, source of fodder, source of water, and anthelmintic used to treat fasciolosis. Therefore, this study showed a higher prevalence of fasciolosis among cattle drinking water from rivers or lake, or irrigation canals (58.6%) than the cattle drinking water from the tap (40.9%). The univariate analysis showed that the source of water for watering the cattle ($\chi2 = 12.7$, P-value= 0.4e-03 means <0.01) was statistically associated with the bovine prevalence of fasciolosis in this study. Furthermore, cattle drinking water from rivers or lake or irrigation canals (OR = 2.1; IC: 1.4-3.1) were likely to be more at risk of getting bovine fasciolosis in the study area (Table 14).

Table 14: Univariate analysis and estimated Odds Ratio (OR) for risk factors associated with bovine fasciolosis based on practices among farmers during cattle raising

Risks factors	Level	-ve	+ve	Prevalence % (95% CI)	OR (95% CI)	$\chi2$	P-value
Feeding system of cattle	Mix of stall-feeding & Free grazing	15	12	44.4(25.7-63.2)	Ref	0.1	0.7
	Stall feeding	208	191	47.9(42.9-52.8)	1.2(0.5-2.5)		
Source of fodder to feed the cattle	Agriculture land/fodder crop	210	191	47.6(42.7-52.5)	Ref	<0.1	0.9
	Aquatic environment	13	12	48(28.4-67.6)	1 (0.5, 2.3)		
	Tap water	156	108	40.9(34.9-46.8)	Ref	12.7	

Source of water for watering the cattle	Rivers/lake/ Irrigation canals	67	95	58.6(51.1-66.2)	2.1 (1.4-3.1)		0.4e-03 (<0.01)*
Anthelmintics are commonly used to treat cattle against fascioliasis	Albendazole/ Nitroxinil	143	119	45.4(39.4-51.5)	Ref	1.4	0.2
	Oxyclozanide (Zanil)/ Nitroxinil	80	84	51.2(43.658.9)	1.26(0.9-1.9)		

-ve: negative cattle, +ve: positive cattle, 95%CI: Confidence Interval, OR: odds ratio, $\chi 2$: Chi-square, *: significant

CHAPTER FIVE

DISCUSSION

5.1 Knowledge, attitudes, and practices of farmers, and perceptions and practices of veterinary professionals on bovine fasciolosis

This study investigated cattle farmer's knowledge, attitudes, and practices, and veterinary professional's perceptions and practices on bovine fasciolosis in the Imbo region of Burundi. The survey data reported in this study is the first documented research describing the knowledge, attitudes, and practices among farmers and veterinary professional's perceptions and practices regarding bovine fasciolosis in the area. The data collected from farmers in this survey regarding socio-demographic characteristics information showed that the majority of respondents were men and most of them were between 35-60 years and illiterate. Of the total surveyed farmers were married with the experience of below 5 years in cattle rearing. The high number of men surveyed in this study could be due to the habits of people of the Imbo region with which large livestock activities raising including cattle are reserved for men while small livestock activities raising including indigenous poultry are reserved for women. In addition, according to their culture, in the household where husband and wife are present, the man is the head of the family and is responsible for providing the information or data regarding large livestock in raising. Similar studies on Zootechnical aspects of dairy cattle raising in Burundi (translated from French) and on population pressure and livestock keeping in Burundi were reported by Hatungumukama *et al*. (2007) and Desiere *et al*. (2015), respectively.

Among farmers surveyed, most of them raised only bovine species without other animal species and practiced zero-grazing (permanent stable). The people of the Imbo region raised bovine species more than other animal species for a long time, people of this region were more focused on agriculture without livestock, and then some projects of the World Bank, FAO, and IFAD supported this region by distribution of improved bovine in the objective of improvement of milk production. The practice of Zero-grazing in the Imbo region is due to the priorities of the Burundi Government. The similar data were reported by MINAGRIE (2014) in General states of Agriculture and Livestock in Burundi and by Desiere *et al*. (2015) in the study of population pressure and livestock keeping in Burundi. The data from this study showed also that most of the surveyed farmers knew the snails and had seen snails in terrestrial or aquatic environments, but most of them didn't think that snails could spread diseases that could attack cattle or humans.

Therefore, the results showed that farmers of the Imbo region had lower-level knowledge and perception of the role of snails regarding fasciolosis dissemination. This low knowledge and perception of the intermediate host of fasciolosis could be due to farmers' low-level education, poor animal health services, extension, and low training status of farmers regarding the importance of arthropods including snails that have an impact on the development of parasites causing infection in animals or human or both. The similar results were reported in the study conducted in the Northwest Province, South Africa by Olaogun et al. (2023) and in the study conducted in Central Vietnam by Quy (2016).

The current study showed that some of the surveyed farmers knew the name fasciolosis, in the local language "UMURAGU", but most of them surveyed didn't know the cause of fasciolosis. Almost all surveyed farmers indicated that they didn't know that human can be attacked by fasciolosis and only a few farmers said that they knew that cattle can be attacked by fasciolosis; other few farmers among surveyed participants said that cattle can get fasciolosis from drinking contaminated water or feeding contaminated fodder by the parasite causing fasciolosis infection. Some farmers among surveyed participants indicated that knowledge regarding the mode of transmission of bovine fasciolosis has been obtained from community animal health workers. Most of the surveyed farmers indicated that they didn't know that fasciolosis was a major problem in their farms and most of them didn't know that fasciolosis can cause death on attacked cattle.

This study showed that farmers of the Imbo Region had knowledge of the name of fasciolosis but showed a low knowledge of its transmission and the problem caused by such disease on the farm; this low knowledge could be due to also the farmers' low level of education, poor animal health services, extension, low training status of farmers regarding the problem of diseases in the herd. In addition, this low knowledge could be due to the neglect of parasitological infections because of the focus on viral and bacterial infections by veterinary services. This neglect of parasitological infections mainly helminthosis infections by the veterinary services could be due to the lack of official data regarding epidemiology and the problems caused in the farms by the parasitic infections. The lack of regular short of building capacities of local veterinary services on the current status of parasitic infections and how to improve farmer's knowledge could contribute to the low knowledge of the farmers. These results are in agreement with the study conducted in the Northern Regions of Cameroon by Zangue *et al*. (2022) and with the study conducted in

Ethiopia by Alemayehu *et al*. (2021). In contrast, our findings are not similar to the results obtained in the study conducted in Far Northern KwaZulu - natal, South Africa by Ngoshe *et al*. (2023).

The results on attitudes and practices of the farmers towards cattle raising management showed that most of the surveyed farmers indicated that they adopt good farm practices by watering and feeding cattle with clean water and fodder and regular control of animals to reduce the risk of fasciolosis infection in cattle and other small ruminants. This attitude of adopting good farm practices could be due to some farmers of the Imbo region understanding the mode of transmission of ascariasis infection and other microbiological infections through contaminated water and food rather than how they knew fasciolosis. In addition, some farmers of the Imbo region had tap water in their households, so they watered cattle with water from the tap. The owner of tap water could share with the neighbor so that some of the surveyed farmers indicated that they used water from the tap. But, by default, some farmers who had taps in their households may use water from the rivers or lake or in irrigation canals for watering cattle due to sometimes water interruptions in taps may be observed and many of them live very close to these areas. This study showed that the farmers of the Imbo region didn't have enough pastures and adopted zero-grazing under the Government priorities; therefore, most of the surveyed farmers found fodder in high quantities from agricultural land or aquatic environment or at the edge of the rivers or lake and then pile up the fodder on a tent during the period of their use because many of them live very close to these areas. In addition, most of the surveyed farmers were using stall feeding during cattle feeding and regularly applied deworming of their farm every three months most of them were using alternatively of albendazole and Nitroxinil and few others were using alternatively oxyclozanide (Zanil) and Nitroxinil.

In this study, the data collected from veterinary professionals regarding socio-demographic characteristics information showed that the majority of respondents were men with the age range of 35-60 years. The most of veterinary professionals surveyed were Basic Veterinary workers (Level of Diploma in animal health and production) who were in public services in the Ministry of Environment, Agriculture and Livestock and most of them had experience in the field of 10 years and above. Among veterinary professionals surveyed, most of them didn't have a record-keeping book of parasitic infections. The lack of the record book could be due to neglect of parasitic infections mainly helminthosis infection which may be caused by the focus on viral and bacterial infections by the local veterinary services, the lack of official data regarding the epidemiology, and the problems caused in the farms by these parasitic infections and the lack of regular short or long term of building capacities of local veterinary services on the current status of parasitic infections including helminthosis infection. Of the total of veterinary professionals surveyed, most of them indicated that all ruminants (cattle, sheep, and goats) were frequently attacked by fasciolosis, and the breed of cattle frequently attacked by fasciolosis were exotic (Frisian and Sahiwal) and crossbreed. The frequency of fasciolosis in ruminants (Cattle, sheep, and goats) could be due to these animals sharing the same veterinary services, same feeder and drinker, same sources of water and fodder, and sometimes share the shed. In the farm, Veterinary services identified fasciolosis using clinical diagnosis due to the lack of equipment and materials for diagnosis of parasitic infections in the local areas. Therefore, most of the surveyed veterinary professionals indicated that they found a high-rate prevalence of bovine fasciolosis in abattoirs during routine meat inspection.

Accordingly, on the routine meat inspection done by veterinary professionals, most of the surveyed participants indicated that fasciolosis in cattle is classified as second level among the diseases caused by parasites that occur often and cause high economic losses and reduce productivity in the Imbo region. These results are in agreement with the study conducted in Southern Espirito Santo by Bernardo *et al*. (2011) and with the study conducted in five districts of Punjab in Pakistan, reported by Khan *et al*. (2009). The local veterinary professionals indicated that most of them commonly used alternatively albendazole and Nitroxinil as anthelmintics against fasciolosis, but few of them used alternatively oxyclozanide (Zanil) and Nitroxinil, and another group used Nitroxinil only. Some of the surveyed veterinary professionals indicated that farmers could call them in good time to treat their cattle showing signs of illness, but others indicated that farmers couldn't call them due to some farmers could treat their cattle against parasitic infections or other infections and sometimes farmers may call them when cattle reach the stage of agony. Despite the low knowledge of farmers on fasciolosis, all veterinary professionals surveyed in the Imbo region indicated that they conducted public awareness among farmers regarding the practice of deworming and advised farmers to use clean water and fodder as control measures against the diseases in livestock including fasciolosis in ruminants.

5.2 Prevalence of bovine fasciolosis assessment

The current study assessed the prevalence of *Fasciola* spp. in cattle using two different approaches in the Imbo Region of Burundi. Results from fecal analysis of cattle from different farms in the region indicate that bovine fasciolosis is endemic with an infection prevalence of 47.7%. This result is similar to prevalence reports in other areas of Africa, such as Central, Southern, and Western provinces of Zambia with 48 % by Phiri *et al*. (2005), Iringa District of Tanzania with 42.1% by Keyyu *et al*. (2006), Mardan District in Pakistan with 42.8% by Khan *et al*. (2020), and in Western Ethiopia by Tulu (2018) with 39.1%. On the other hand, a study conducted by Abebe *et al*. (2018) in Southwestern Ethiopia showed a lower prevalence (21.4%) compared with the one found in the current study, while, a study reported in Kwara State north-central of Nigeria showed a higher prevalence (74.9%) by Elelu *et al*. (2016). The results obtained from cattle slaughtered in abattoirs from the study region, the results indicate that bovine fasciolosis is endemic with a prevalence of 33.2%. These results are higher than the prevalence of 12.3% found in abattoirs during routine meat inspection by veterinary services (Report from General Directorate of Livestock in Burundi 2021). The difference in this prevalence could be due to the non-regular recording of parasitic disease cases during routine meat inspection by veterinary services in abattoirs. In a recent study, Minani *et al*. (2023) reported a 13.0% prevalence of *Fasciola* spp. in slaughtered ruminants at Muyinga (Burundi). The difference between the two prevalences could be due to the magnitude of the sample size used in these two studies.

Despite of livestock system used in the two studies areas, farmers of the Imbo region who live and raise their livestock near the lake, rivers, and the marshes where they grow rice, have a habit of drawing water for watering livestock in these areas without being treated. The latter result is also in the same line as the prevalence of 30.9% reported by Elshraway and Mahmoud (2017) in the abattoir of El-Kharga of Egypt, 30% reported by Kusumarini *et al*. (2020) in Malang District-East Java in Indonesia, and 39.1% reported by Tulu (2018) in Western of Ethiopia using the same method in abattoirs. Other studies in the same line as the current study were reported by Mequaninit (2021) with a prevalence of 35% in the Kombolcha ELFORA abattoir located in North Eastern part of Ethiopia, reported by Bernardo *et al*. (2011) with the prevalence of 24.9% in Southern Espirito Santo and reported by Mungube *et al*. (2006) with the prevalence of 26% in semi-arid coastal of Kenya. The study carried out by Abunna *et al*. (2010) showed that the prevalence (4.0%) in the Saddo municipal abattoir in southern Ethiopia was lower than in the current study. The study conducted by Opio *et al*. (2021) at the Lira Municipality abattoir in Northern Uganda showed that the prevalence (65.7%) was higher than the current study. The overall prevalence of bovine fasciolosis observed in farms and abattoirs was almost the same in the study area. This could be explained by the same origin of the selected cattle in the abattoir which was shared with the cattle selected for fecal samples. Furthermore, they shared the same ecology, climatic, and geographical location, and they received the same type of veterinary service (treatment and prevention).

5.3 Risk factors associated with bovine fasciolosis assessment

This study assessed the potential associated risk factors of bovine fasciolosis through coprological examination. The results indicated that female and adult cattle were more exposed to bovine fasciolosis compared to male and young cattle respectively; which converges with studies reported by Khan *et al*. (2020) and Opio *et al*. (2021). Females are more exposed than males due to poor physiological and immune status and susceptibility between sexes may exist for fasciolosis prevalence as reported by Phiri *et al*. (2005). The lower prevalence among young cattle (<2 years) could be due to the cattle of this age have not started the period of reproduction means that the physiological status is still comfortable, and the immune status is still good. The higher prevalence in adult cattle (2-4 years) could be due to these cattle being in the period of reproduction and sometimes the immune status can be lower. Regarding older cattle, the prevalence is lower due to the cattle can be attributed to the high immunogenicity of the parasite, which aids in the stimulation of acquired immunity in old animals as reported by Opio *et al*. (2021). In addition, the low prevalence in old cattle could be due to severe fibrosis of the liver which weakens the movement of immature flukes, calcification, and stenosis of the bile duct providing an unfavorable environment for adult parasites, resulting in their expulsion as reported by Khan *et al*. (2020).

Regarding farmer's practices during cattle raising, the present study showed that the source of water was a statistically significant association with bovine fasciolosis. This prevalence among sources of water could be due to the farmers who are used to drawing water for watering livestock in the areas including canal irrigation, edge of rivers or lake, and stagnant water because many farmers live and raise the livestock near the lake or rivers or the marshes where they grow rice. Therefore, due to the zoonotic character of fasciolosis, the farmers of the Imbo region are at risk of fasciolosis due to some vegetables including eggplant, tomatoes, and cabbage which are growing in these areas, which can be eaten in raw state or farmers could use to drink untreated water from these areas during rice growth activities. In addition, Children are mainly at risk because they could drink contaminated water while swimming in irrigation channels, rivers, and lake.

CHAPTER SIX
SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

6.1 Summary

The findings from this study conducted in the Imbo Region of Burundi were the assessment of farmers' knowledge, attitudes, and practices, and veterinary professional's perceptions and practices regarding fasciolosis. Among farmers, the findings showed that 57.7 % of farmers recognized fasciolosis (locally known as "UMURAGU"), but only 28.6 % understood its causes. In addition, 40.5 % of farmers knew cattle could be infected while 0.6 % believed humans could be infected by fasciolosis infection. Among veterinary professionals, the findings showed that only 39 % maintained records of parasitic infections. However, 65.4 % identified fasciolosis as one of the top two parasitic diseases in cattle by frequency of occurrence and economic impact.

In addition, the findings of this study were the assessment of the prevalence of bovine fasciolosis in the same study areas where coprological prevalence was 47.7% (42.9-52.4, 95% CI) in farms while postmortem examinations prevalence was 33.2% (28.9-37.5, 95% CI) in abattoir. Furthermore, this study assessed the potential associated risk factors of bovine fasciolosis through coprological examination where the findings indicated that female and adult cattle were more exposed to bovine fasciolosis compared to male and young cattle respectively. However, regarding farmer's practices during cattle raising, the present findings showed that the source of water was a statistically significant association with bovine fasciolosis.

6.2 Conclusions

In conclusion**,** the present study identified that the farmers had low to moderate level knowledge, attitudes, and practices regarding fasciolosis infection and the intermediate host of the parasite which could be due to a lack of awareness among farmers regarding fasciolosis epidemiology and control in ruminants. This study showed also that the public veterinary services had moderate perceptions and practices regarding fasciolosis disease which could be due to a lack of capacity building regarding parasitic infections including fasciolosis epidemiology and control in ruminants.

This study showed a high prevalence of bovine fasciolosis in the Imbo region through microscopic examination of feces from farms and postmortem liver inspection in the abattoirs which could confirm the losses in ruminant production including milk production reduction in the farms and seizure of the liver in abattoirs. In addition, due to the zoonotic character of fasciolosis, the farmers of the Imbo region are at risk of getting a fasciolosis infection due to some vegetables are growing in these areas and can be eaten in the raw state without being treated or farmers could use to drink untreated water from these areas during rice growth activities. The current study also identified that the epidemiology of fasciolosis among animals has shown that risk factors, including age, sex, and livestock management, have a significant influence on the prevalence of such disease where the female and adult cattle were more exposed to bovine fasciolosis. In addition, this study showed that livestock management, including sources of water for watering animals, has a significant influence on the prevalence of fasciolosis.

6.3 Recommendations

Based on the above conclusion, the following recommendations are forwarded to the Ministry of Environment, Agriculture and Livestock and Ministry of Public Health and the Fight against AIDS of Burundi:

i. To establish a program for training sessions in the context of One Health to enhance the awareness and attitude of smallholder farmers, slaughterhouse workers, butchers, and meat sellers on the aspects of the nature of fasciolosis, its importance to public health, the benefits of control and prevention mechanisms of fasciolosis in Burundi and the establishment of a continuity program for capacities building among public veterinary services and public health professionals on epidemiology and control of fasciolosis.

ii. To extend this study to other regions of Burundi to provide more precision of the prevalence of fasciolosis and to carry out *Fasciola* species identification, the malacological study, study on prevalence determination in public health in the context of One Health and then to develop and provide more efforts to practical and effective control strategies to prevent and limit infection and reduce significant health and economic consequences.

iii. To continue monitoring endemic parasitic diseases including fasciolosis to detect changes in its distribution and assess the efficacy and benefits of control measures programs and to initiate and continue to carry out the study in the context of One Health on the distribution of fasciolosis infection between animals and humans as known as most farmers of Burundi keep the livestock in their households.

6.4 Suggestions for further study

Given the zoonotic potential of fasciolosis and its high environmental risk exposure, for better control the magnitude of the problem caused by the disease, this study suggest the following further studies:

i. Occurrence of fasciolosis and associated economic losses in ruminants in Burundi
ii. Molecular epidemiology investigation of fluke species in Burundi;
iii. Ecology and biology investigation of snail intermediate hosts of *Fasciola* species in Burundi.
iv. Evaluation of effectiveness of fasciolicides drugs and its potential resistance in Burundi.
v. Current challenges for fasciolicides treatment in ruminant livestock in Burundi
vi. Comparative analysis of anthelmintic treatments: impact on liver biomarkers and clinical recovery in ruminant livestock with fasciolosis in Burundi;
vii. A human-focused molecular epidemiological study of fasciolosis in Burundi
viii. Strategic control assessment and establishment of fasciolosis in Burundi

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APPENDICES

Appendix 1: Permission to conduct research in the Imbo region of Burundi obtained from the Ministry of Environment, Agriculture and Livestock of Burundi through the Directorate of Veterinary Services (Translated in English)

REPUBLIC OF BURUNDI **Done in Gitega on April 12th,2023**

DELIVRE EN BREVET
Fait à BUJUMBURA
Le........1 2 NOV 2024........

REPUBLIQUE DU BURUNDI
OFFICE NOTARIAL DE BUJUMBURA

Maître Avit SABAYABAYA
NOTAIRE DE BUJUMBURA

MINISTRY OF ENVIRONMENT
AGRICULTURE AND LIVESTOCK

GENERAL DIRECTION OF LIVESTOCK
DIRECTION OF ANIMAL HEALTH

Ref N°:7132/39/NT.D/n.p/2023

To Mr Sylvère NKURUNZIZA

Subject: Permission to carry out A Research for your Master's thesis work .

Dear sir,

With Reference to your letter of April 12, 2023 in which you requested the authorization to carry out a research relating to the epidemiology and control of fasciolosis of the cattle of Imbo Region exactly in the commune of Nyanza Lac in Makamba Province, Rumonge Commune in Rumonge province, Mutimbuzi commune of Bujumbura Rural Province, Rugombo commune of Cibitoke province and Rugazi commune of Bubanza province, I have the honor to confirm my agreement that you are authorized to carry out a such research.

Wishing you a good reception, Please accept, **Dear Sir**, the assurance of my highest consideration.

The Director of Animal Health
Dr. Désiré NTAKARUTIMANA, Signed and sealed

Certification of true and Accurate English Language Translation by:
Title /Company : Global Training and Translation Interpreting Services (GTIS)
Name of the Translator : Pascal Nzitonda

Address /Contact Information :Phone + (257) 79 742 048, Email : n/ipascal79@gmail.com

VU POUR LA LEGALISATION DE
LA SIGNATURE DE Pascal NZITONDA APPOSEE Ci-
Fait à BUJUMBURA
Le........1 2 NOV 2024........

Appendix 2: Permission to conduct research in the Imbo region of Burundi obtained from the Ministry of Environment, Agriculture and Livestock of Burundi through the Directorate of Veterinary Services (Written in French)

REPUBLIQUE DU BURUNDI

MINISTERE DE L'ENVIRONNEMENT,
DE L'AGRICULTURE ET DE L'ELEVAGE

Gitega, le .11./.04./2023

DIRECTION GENERALE DE L'ELEVAGE
DIRECTION DE LA SANTE ANIMALE

Réf 7132/.39.../NT. D/n. p/2023

A Monsieur NKURUNZIZA Sylvère

<u>Objet</u> : Autorisation pour effectuer une recherche pour votre travail de Thèse de Master

Monsieur,

Me référant à votre lettre du 12/04/2023 dans laquelle vous demandiez l'autorisation pour effectuer une recherche relative à l'épidémiologie et le control de la fasciolose chez les bovins dans la région de l'Imbo précisément dans la Commune de Nyanza-lac de la Province de Makamba, Commune Rumonge de la Province de Rumonge, Commune Mutimbuzi de la Province Bujumbura Rural, Commune Rugombo de la Province Cibitoke et Commune Rugazi de la Province Bubanza, j'ai l'honneur de porter à votre connaissance que je marque mon accord.

Vous en souhaitant une bonne réception, je vous prie d'agréer **Monsieur**, l'assurance de ma considération distinguée.

LE DIRECTEUR DE LA SANTE ANIMALE

Dr. Désiré NTAKIRUTIMANA

Appendix 3: The animal level questionnaire used for cattle identification during feces collection

Animal level questionnaire

Survey No (Name of sector followed by the first name of cattle producers)

____

Date of survey (DD/MM/YY)

yyyy-mm-dd

____

1.1 Age (Years)

- ◯ 1. < 2 years
- ◯ 2. 2 – 4 years
- ◯ 3. > 4 years

1.2 Gender

- ◯ 1. Male
- ◯ 2. Female

1.3 Breed

- ◯ 1. Frisian
- ◯ 2. Crossbreed
- ◯ 3. Local
- ◯ 4. Sahiwal

1.4 Body condition

- ◯ 1. Poor
- ◯ 2. Medium
- ◯ 3. Good

1.5 Origin (commune)

- ◯ 1. Nyanza-lac
- ◯ 2. Rumonge
- ◯ 3. Rugombo
- ◯ 4. Rugazi
- ◯ 5. Mutimbuzi

1.6 Origin (Province)

- ◯ 1. Makamba
- ◯ 2. Rumonge
- ◯ 3. Rugombo
- ◯ 4. Rugazi
- ◯ 5. Bujumbura Rural

1.7 Clinical signs

- ☐ 1. Bottle jaw
- ☐ 2. Diarrhea
- ☐ 3. Padded bristles
- ☐ 4. Dyspnea
- ☐ 5. Pale mucous membranes or jaundice
- ☐ 6. Reluctance to move
- ☐ 7. Weight loss
- ☐ 8. Quilted bristles
- ☐ 9. Emaciation
- ☐ 10. 10. Normal

Appendix 4: Farmer's survey questionnaire

KAP survey on fasciolosis disease in cattle among farmers in Imbo region, Burundi

Survey No (The name of the sector followed by the first name of the cattle producer)

___

Date of survey (DD/MM/YY): yyyy-mm-dd

___

___

Interviewer full name :

___

Interviewer education level and qualification :

- ◯ High school diploma
- ◯ Bachelor's degree
- ◯ Master's degree

Interviewer current profession:

___

Section 1: Respondent's Information

1.1 Respondent full Name:

___

1.2 Age (Years):

___

1.3 Gender

- ◯ 1. Male
- ◯ 0. Female

1.4 Education level:

- 0. Not attended any school
- 1. Buddhist studies
- 2. Attending/Attended Non-Formal Education
- 3. Primary level
- 4. Secondary level
- 5. High school
- 6. Undergraduate
- 7. Postgraduate(MSc or PhD)

1.5 Location (Commune)

- 1. Mutimbuzi
- 2. Nyanza-lac
- 3. Rugazi
- 4. Rugombo
- 5. Rumonge

1.6 Location (Province)

- 1. Bubanza
- 2. Bujumbura Rural
- 3. Cibitoke
- 4. Makamba
- 5. Rumonge

1.7 Status of animal owner

- 1. In totality
- 0. In part

1.8 Profession

- 1. Farmer
- 2. Private professional
- 3. Public service

1.9 Marital status of animal owner

- 1. Married
- 2. Widow/Widower
- 3. single
- 4. Divorce

1.10 Is the owner of the animal the head of the household?

- ◯ 1. Yes
- ◯ 0. No

1.11 Experience in cattle rearing (Years):

____

Section 2 : Knowledge and perception of fascioliasis and its intermediate host

2.1. Knowledge on bovine breeding status

2.1 1 Animal species (Ruminants)

- ☐ 1. Cattle
- ☐ 2. Goat
- ☐ 3. Sheep

2.1.2 Housing system

- ◯ 1. Cattle, goats, and sheep mixed
- ◯ 2. Cattle, goats, and sheep separate

2.1.3 Type of cattle husbandry practice followed by the farmer

- ◯ 1. Permanent stable
- ◯ 2. Semi-permanent stable
- ◯ 3. Non-permanent stable

2.1.4 Cattle breed

- ☐ 1. Local
- ☐ 2. Crossbreed
- ☐ 3. Purebreed

2.1.5 Cattle age

- ☐ 1. Below 1 year
- ☐ 2. Between 1-2years
- ☐ 3. Between 2-3 years
- ☐ 4. Between 3-4 years
- ☐ 5. Above 4 years

2.1.6 Why do you raise cattle?

- ☐ 1. Savings source of income
- ☐ 2. Manure source of income
- ☐ 3. Family self-consumption

2.2 knowledge and perception on intermediate host of fasciolosis

2.2.1 Do you know snail or have you at least heard this name(snail)

- ○ 1. Yes
- ○ 0. No

2.2.2 Farmers' source of information about snail

- ☐ 1. Community animal health workers or para- veterinarian
- ☐ 2. Radio
- ☐ 3. TV
- ☐ 4. Hill or community leaders
- ☐ 5. Other sources (neighbors, relatives, and friends)
- ☐ 6. Brochures/Posters (provided by government or NGOs)
- ☐ 7. View in nature
- ☐ 8. Newspaper
- ☐ 9. Media (Including social media)
- ☐ 10. Nowhere

2.2.3 Have you seen a snail?

- ○ 1. Yes
- ○ 0. No

2.2.4 Where do you commonly find snails?

- ☐ 1. Terrestrial environments
- ☐ 2. Aquatic environments3. In the pastureland
- ☐ 4. In the agriculture land
- ☐ 5. At the edge of the rivers
- ☐ 6. In irrigation canals
- ☐ 7. On the land around the stable
- ☐ 8. In the forests
- ☐ 9. All of the above
- ☐ 10. Don't know

2.2.5 Which places do you think you find the snails most commonly?

- ☐ 1. Warm places
- ☐ 2. Cold places
- ☐ 3. Both places
- ☐ 4. Don't know

2.2.6 Which season do you commonly see the snails in the environments?

- ☐ 1. During long rainy
- ☐ 2. During dry season
- ☐ 3. During short rainy
- ☐ 4. Throughout the year
- ☐ 5. Don't know

2.2.7 Do you think cattle can get diseases from the snails?

- ○ 1. Yes
- ○ 2. No
- ○ 3. Don't know

2.2.8 Do you think humans can get diseases from the snails?

- ○ 1. Yes
- ○ 2. No
- ○ 3. Don't know

2.2.9 Have you heard of any snail-borne diseases in cattle?

- ○ 1. Yes
- ○ 0. No

2.2.10 For at least one or all of these last questions) , from where did you hear about the information?

- ☐ 1. Community animal health workers or para- veterinarian
- ☐ 2. Radio
- ☐ 3. TV
- ☐ 4. Hill or community leaders
- ☐ 5. Other sources (neighbors, relatives, and friends)
- ☐ 6. Brochures/Posters (provided by government or NGOs)
- ☐ 7. Livestock wholesalers or traders
- ☐ 8. Newspaper
- ☐ 9. Media (Including social media)
- ☐ 11. Nowhere

2.3 Knowledge and perception on fasciolosis

2.3.1 Do you know fasciolosis, or have you at least heard this name?

- ○ 1. Yes
- ○ 0. No

2.3.2 Do you know what cause fasciolosis?

- ○ 1. Yes
- ○ 0. No

2.3.3 Do you think fasciolosis can attack human?

- ○ 1. Yes
- ○ 2. No
- ○ 3. Don't know

2.3.4 Do you think fasciolosis can attack cattle?

- ○ 1. Yes
- ○ 2. No
- ○ 3. Don't know

2.3.5 Where do you think the cattle get fasciolosis from? Respondents can have more than one answer.

- ☐ 1. From drinking water
- ☐ 2. From feed with fodder
- ☐ 3. From the grazing land
- ☐ 4. From the bedding materials
- ☐ 5. From the forest
- ☐ 6. I don't know

2.3.6 Farmers' source of information about fasciolosis disease.

- ☐ 1. Community animal health workers or para- veterinarian
- ☐ 2. Radio
- ☐ 3. TV
- ☐ 4. Hill or community leaders
- ☐ 5. Other sources (neighbors, relatives, and friends)
- ☐ 6. Brochures/Posters (provided by government or NGOs)
- ☐ 7. Livestock wholesalers or traders
- ☐ 8. Newspaper
- ☐ 9. Media (Including social media)
- ☐ 10. Nowhere

2.3.7 Have you observed the Presence of a disease with the following symptom?

- ☐ 1. Bottle jaw
- ☐ 2. Diarrhea
- ☐ 3. Emaciation
- ☐ 4. None

2.3.8 Is the disease fasciolosis a major problem for you on this farm?

- ◯ 1. Yes
- ◯ 2. No
- ◯ 3. Don't know

2.3.9 Is it present now?

- ◯ 1. Yes
- ◯ 2. No
- ◯ 3. Don't know

2.3.10 How frequently does it occur?

- ◯ 1. Every year
- ◯ 2. Always there
- ◯ 4. Don't know

2.3.11 When does it occur normally?

- ◯ 1. During long rainy
- ◯ 2. During dry season
- ◯ 3. During short rainy
- ◯ 4. Don't know

2.3.12 How does it affect cattle ?

- ◯ 1. The entire herd
- ◯ 2. Proportion
- ◯ 3. Single one
- ◯ 4. Don't know

2.3.13 In cattle which age group is highly affected?

- ☐ 1. Below 2 years
- ☐ 2. Between 2-4years
- ☐ 3. Above 4 years
- ☐ 4. All age group
- ☐ 5. Don't know

2.3.14 Does it cause death?

- ◯ 1. Yes
- ◯ 2. No
- ◯ 3. Don't know

2.3.15 Which type of cattle breed do you know gets mostly infested?

- ☐ 1. Local breed
- ☐ 2. Crossbreed
- ☐ 3. Purebreed
- ☐ 4. Don't know

2.3.16 Does it influence milk production and body weight gain?

- ◯ 1. Yes
- ◯ 2. No
- ◯ 3. Don't know

2.3.17 Is it necessary to give a vaccine?

- ◯ 1. Yes
- ◯ 2. No
- ◯ 3. Don't know

Section 3: Attitudes of the farmers (Start asking each question by "Do you agree?)

3.1 Adopting good farm practices can reduce the risk of fasciolosis infestation (e.g., regularly sweep cattle sheds, watering clean water, feeding clean fodder, regular control of animals, etc.)

- ◯ 1. Strongly agree
- ◯ 2. Agree
- ◯ 3. Strongly Disagree
- ◯ 4. Disagree
- ◯ 5. No opinion

3.2 Timely deworming against fasciolosis can reduce cattle morbidity and mortality.

- ◯ 1. Strongly agree
- ◯ 2. Agree
- ◯ 3. Strongly Disagree
- ◯ 4. Disagree
- ◯ 5. No opinion

3.3 The risk of fasciolosis infestation can be reduced by always keeping cattle in the shed.

- ◯ 1. Strongly agree
- ◯ 2. Agree
- ◯ 3. Strongly Disagree
- ◯ 4. Disagree
- ◯ 5. No opinion

3.4 Avoidance to mix different animals' species in the single shed can reduce the risk of fasciolosis spread.

- ◯ 1. Strongly agree
- ◯ 2. Agree
- ◯ 3. Strongly Disagree
- ◯ 4. Disagree
- ◯ 5. No opinion

3.5 When an animal is suspected of fasciolosis, timely reporting to an animal health professional can reduce animal mortality.

1. Strongly agree
2. Agree
3. Strongly Disagree
4. Disagree
5. No opinion

Section 4: Farmers' Practices

4.1 How do you feed your cattle ?

1. Stall feeding
2. Mix of stall feeding & free grazing
3. All-time free grazing
4. Mix of stall feeding & tethered grazing
5. Mixture of the above practices

4.2 Where do you find the fodder to feed the cattle

1. In the pastureland
2. In the agriculture land
3. Aquatic environment
4. In the forests
5. At the edge of the rivers or lake
6. At the edge of irrigation canals
7. All of the above

4.3 Where do you find the water for watering the cattle

1. Aquatic environments
2. In the rivers or lake
3. Tap water
4. In irrigation canals5. All of the above

4.4 If your cattle shed's floor is concrete, how often do you wash the flooring of your cattle shed?

1. Daily
2. Weekly
3. Fortnightly
4. Monthly
5. Never

4.5 What do you do when your animals get sick?

- ◯ 1. Treat at home
- ◯ 2. Vet. Clinic

3.Others (specify):

____

4.6 What is the local name for treatment against fasciolosis?

____

4.7 Do you use anthelmintics for the treatment?

- ◯ 1. Yes
- ◯ 0. No

4.8 Which anthelmintics are you commonly using to treat your animals?

- ☐ 1. Albendazole
- ☐ 2. Nitroxinil
- ☐ 3. Oxyclozanide(Zanil)
- ☐ 4. closantel
- ☐ 5. All of the above

4.9 Are the anthelmintics used in the treatment or deworming against bovine fasciolosis effective?

- ◯ 1. Yes
- ◯ 2. No
- ◯ 3. Don't know

4.10 How many times did each animal (Showing the symptoms of fasciolosis) get vets since last year?

- ◯ 1. Don't know
- ◯ 2. Once
- ◯ 3. Several times

4.11 Do you have anthelminthics now in stock?(If yes, can you show please?)

- ◯ 1. Yes
- ◯ 0. No

4.12 How do you use it?

- ◯ 1. You yourself
- ◯ 2. Vets
- ◯ 3. Drug smugglers

4.13 What control measures do you follow on your farm to address the bovine fasciolosis infestation problem?

- ☐ 1. Practice of deworming
- ☐ 2. Don't let the animals out for grazing
- ☐ 3. Adopt good farm practices

4.14 What is the frequency of applying of deworming on your farm?

- ○ 1. In two weeks
- ○ 2. Monthly
- ○ 3. Fortnightly
- ○ 4. In three months
- ○ 5. In year
- ○ 6. None

Appendix 5: Veterinary professional's survey questionnaire

Veterinary Professional Survey Questionnaire (Veterinary technicians and para-veterinary)

Survey No (Name of sector or zone or district or province followed by the first name of the surveyed)

____

Date of survey (DD/MM/YY)

yyyy-mm-dd ______________

____

Interviewer full name

____

Interviewer's education level and qualification

◯ 1. Bachelor

◯ 2. MSc

◯ 3. PhD

Interviewer current profession

____

Section 1: Veterinary professional profile

1.1 Respondent full Name:

____

1.2 Age (Years):

____

1.3 Gender:

◯ 1. Male

◯ 2. Female

1.4 Education level:

- ◯ 1. Veterinarian technicians (Basic Veterinary Worker)
- ◯ 2. Bachelor's in animal health and production(Para-veterinary)
- ◯ 3. Veterinarian
- ◯ 4. Master's in veterinary science
- ◯ 5. Center for teaching professions in the field of the veterinary profession

1.5 Location (Commune)

- ◯ 1. Mutimbuzi
- ◯ 2. Nyanza-lac
- ◯ 3. Rugazi
- ◯ 4. Rugombo
- ◯ 5. Rumonge

1.6 Location (Province)

- ◯ 1. Bubanza
- ◯ 2. Bujumbura Rural
- ◯ 3. Cibitoke
- ◯ 4. Makamba
- ◯ 5. Rumonge

1.7 Profession

- ◯ 1. Zonal veterinary technician
- ◯ 2. Commune veterinary technician
- ◯ 3. Provincial Livestock Officer
- ◯ 4. Community animal health workers

1.8 Years of experience in the field

- ◯ 1. 1-5 years
- ◯ 2. 5-10 years
- ◯ 3. >10 years

Section 2: Perception and practices about bovine fasciolosis and potential reasons for the failure to control such disease.

1.1 **Do you have a record keeping book?**

- ◯ 1. Yes
- ◯ 0. No

2.2 Do you record all diseases reported to you, including bovine fascioliasis?

- ◯ 1. Yes
- ◯ 0. No

2.3 Do you have records of bovine fasciolosis cases for the last year (From breeding areas and abattoirs)?

- ◯ 1. Yes
- ◯ 0. No

2.4 Do you have historical records of cases of bovine fasciolosis from more than one year ago (From breeding areas and abattoirs)?

- ◯ 1. Yes
- ◯ 0. No

2.5 What species of animals are frequently attacked by fasciolosis?

- ☐ 1. Cattle
- ☐ 2. Sheep
- ☐ 3. Goat

2.6 What breed of cattle are frequently attacked by fasciolosis?

- ☐ 1. Local
- ☐ 2. Crossbreed
- ☐ 3. Purebreed

2.7 How do you usually procedure when you have a case of bovine fasciolosis ?

- ☐ 1. Report to superior animal health manager
- ☐ 2. Apply control measures only

2.8 What type of diagnosis is made at the local Veterinary Field Units?

- ☐ 1. Clinical diagnosis
- ☐ 2. Microscopy diagnosis

2.9 Where do you find a high rate of bovine fasciolosis when comparing the clinical diagnosis made in breeding areas and cattle slaughtered in abattoirs in your veterinary professional areas?

- ☐ 1. In breeding areas
- ☐ 2. In abattoirs
- ☐ 3. Don't know

2.10 According to your observation, cattle can die from the attack of fasciolosis?

- ◯ 1. Yes
- ◯ 0. No

2.11 Among the diseases that occur often and cause high morbidity in cattle in your area of veterinary professional, at what level can you classify fasciolosis?

- ◯ 0. 1st level
- ◯ 1. 2nd level
- ◯ 2. 3rd level
- ◯ 3. 4th level

2.12 Among the diseases that occur often and cause high economic losses and reduce productivity in cattle in your area of veterinary professional, at what level can you classify fasciolosis.

- ◯ 1. 1st level
- ◯ 2. 2nd level
- ◯ 3. 3rd level4. 4th level

2.13 Which anthelmintics do you commonly used to treat the cattle against fasciolosis?

- ☐ 1. Albendazole
- ☐ 2. Nitroxinil
- ☐ 3. Oxyclozanide(Zanil)
- ☐ 4. closantel

Any other, please specify:

____

2.14 Is there a traditional treatment against bovine fasciolosis that farmers use in your veterinary professional area?

- ◯ 1. Yes
- ◯ 0. No

2.15 Are the anthelmintics used in the treatment or deworming against fasciolosis effective?

- ◯ 1. Yes
- ◯ 2. No
- ◯ 3. Don't know

2.16 What do you think about the ineffectiveness of the anthelmintics you use?

- ☐ 1. Resistance among parasites
- ☐ 2. Non-compliant anthelmintics (Inadequate chemical composition of anthelmintics) 3. Don't know

2.17 What do you think on the cause of the resistance among parasites?

- [ ] 1. Genetic diversity of parasite
- [ ] 2. Inadequate use of antiparasitics.
- [ ] 3. Don't know

2.18 Are the anthelmintics used in the treatment available in the favorable time and space?

- ◯ 1. Yes
- ◯ 0. No

2.19 Do the producers call in good time to treat their cattle showing signs of illness?

- ◯ 1. Yes
- ◯ 0. No

2.20 what control measures do you follow to address the bovine fasciolosis infestation problem?'

- [ ] 0. Practice of deworming
- [ ] 1. Advise the farmers not to let animals out for grazing.
- [ ] 2. Treat the infected cattle
- [ ] 3. Advise the farmers to use clean feed water and forage

Any other, please specify

____

2.21 Do you think vaccination program can be an effective measure to control bovine fasciolosis compared to the measures currently used in veterinary profession?

- ◯ 1. Yes
- ◯ 0. No

2.22 What are the main barriers to the control or prevention of bovine fasciolosis in your veterinary professional areas ?

- [ ] 0. Clinical diagnosis failure
- [ ] 1. Lack of diagnostic laboratory for diseases (including helminthosis) close to our area of action
- [ ] 2. Lack of enough knowledge and materials for snail control
- [ ] 3. Incompetence of farmers in the implementation of advice on good breeding practice
- [ ] 4. Lack of vaccines against bovine fascioliasis
- [ ] 5. Poverty among farmers leading to inability to pay for treatment.
- [ ] 6. None

Appendix 6: Paper from this thesis work published in a peer-reviewed journal

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RESEARCH

Prevalence and associated risk factors assessment of bovine fasciolosis in the Imbo Region, Burundi

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Abstract

Fasciolosis is a zoonosis that limits the productivity of ruminants worldwide, but there is a lack of information on its occurrence in Burundi. Therefore, this study aimed to fill the information gap by determining the prevalence and risk factors associated with bovine fasciolosis in the Imbo Region of Burundi. Two prevalence studies were conducted in parallel in the five communes of the five provinces in the Imbo region. In the first study, a total of 426 fecal samples were collected from randomly selected cattle farms and microscopically examined to determine *Fasciola* egg burden. Survey data on cattle husbandry were collected from owners of these cattle and analyzed to determine the risk factors for bovine fasciolosis. In the second study, 467 cattle were randomly selected in abattoirs and their livers were examined postmortem to determine liver fluke burdens. Data were entered separately into Microsoft Excel and analyzed using R software. The overall prevalence of bovine fasciolosis was 47.7% (42.9–52.4, 95% CI) for microscopic examination and 33.2% (28.9–37.5, 95% CI) for postmortem examinations. The majority of positive cattle (60.6%) had light intensity infections as determined by eggs per gram of feces (epg). Postmortem examinations corroborated these results and indicated that 80% of cattle had light intensity infections. Chi-square analysis showed a statistical association with the presence of bovine fasciolosis and the age, sex, and origin of cattle and the practices of cattle owners ($P < 0.05$).

Keywords Fasciolosis · Cattle · Prevalence · Risk factors · Burundi

Introduction

Fasciolosis is a helminth infection of cattle, buffaloes, sheep, goats, horses, and human of all ages caused by ingestion of encysted metacercaria of liver flukes from the genus *Fasciola* (Ardo et al. 2014; Figtree et al. 2015; Najib et al. 2020). Livestock acquire infection through ingestion of metacercaria that are attached to forage or by drinking water contaminated by metacercaria attached to soil particles or vegetative debris (Irsik et al. 2008). Humans get infected not directly from livestock, but through ingestion of encysted metacercaria that are attached on raw vegetables or certain aquatic plants. The infection may also be acquired through consumption of water contaminated by encysted metacercaria, or food items washed with such water (Ibrahim 2017) or consumption of contaminated water of human sewage (Aleixo et al. 2015). According to the World Health Organization (WHO), human fasciolosis is classified as a Neglected Tropical Disease (NTD) (Ai et al. 2017; Abah et al. 2019). Human fasciolosis is a major

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