DETECTION AND MOLECULAR EPIDEMIOLOGY OF *ECHINOCOCCUS spp* IN THE WILD AND DOMESTIC CARNIVORES IN THE WILDLIFE-LIVESTOCK INTERFACE AREAS OF THE SOUTH LUANGWA NATIONAL PARK IN MFUWE OF MAMBWE DISTRICT OF ZAMBIA

BY

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A Dissertation Submitted to The University of Zambia in Partial Fulfilment of The Requirements for The Award of The Degree of Master of Science in One Health Analytical Epidemiology.

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DECLARATION

I, Moses Nyirongo, here by declare that the work presented in this dissertation is my own original work. It is being submitted for the Degree of Master of Science in One Health Analytical Epidemiology at the University of Zambia, Lusaka. It has not been submitted for at this institution or any other University.

Signature: …………………………………….

Date: ………………………………………….

CERTIFICATE OF APPROVAL

This dissertation of **Moses Nyirongo** has been approved in partial fulfilment of the requirements for the degree of Master of Science in One Health Analytical Epidemiology at the University of Zambia

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ABSTRACT

Echinococcosis is a zoonotic disease with specific genotypes or strains caused by parasites of the genus Echinococcus. The close relationship between humans and animals provides chances of transmission of this zoonotic disease. Globally, there may be more than one million people living with these diseases at any given point in time, making echinococcosis as a major public health concern, more especially in developing countries with meagre economic resources. However, there are major constraints when it comes to prevention measures which are exacerbated by more emerging fatal zoonotic infections. The aim of this study was to determine the prevalence of *Echinococcus spp.* in the wild and domestic carnivores in the wildlife-livestock interface areas. A cross-sectional study was conducted from March 2019 – January 2020. A total of 631 stool samples from wild carnivores were collected from the South Luangwa National Park game management area and 442 stool samples were domestic dogs collected from six zones of Mfuwe in Mambwe District. The samples were subjected to microscopic isolation of taeniid eggs and DNA extraction for sequencing at University of Zambia, School of Veterinary Medicine laboratory. Up to an average of 20 taeniid eggs per positive faecal sample were picked and lysed. Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) and sequencing were performed using Nicotinamide Adenine Dinucleotide (NAD) nest and Big dye standard protocol. The proportion of stool samples of wild carnivores which were found with taeniid eggs was 1.3% (8/631) and domestic dogs was 0% (0/334). Among the wild carnivore samples, 14.4 % (17/118) of the lion stool samples were found to be positive with *Echinococcus spp*. In the phylogenetic tree of *Echinococcus* which was constructed from the mitochondrial genes, *E. felidis* was positioned as a sister taxon of *E. granulosus* sensu stricto. The obtained sequences of the mitochondrial nad1 gene showed 98% identity to previously published sequence of *E. felidis* (MG 271924) in Kenya. All DNA fragments (<200bp) showed 98% similarity with *E. felidis* sequences from Kenyan dog's (MG271925) and lions in Uganda (EF558357). The results of this study indicated that *Echinococcus felidis* is prevalent in canid wildlife while there is no evidence in dogs. However, this does portray a potential risk given that there is an interaction among humans, domestic dogs and wildlife as they share the same habitat. Therefore, there is a need to create a platform for information, education and communication in the game areas about the dangers of this infection to increase preventive and control measures.

DEDICATION

This work is dedicated to my wife, children, and friends who made it possible for me to undertake this research.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABBREVIATIONS

DNA – Deoxyribonucleic acid

PCR – Polymerase chain reaction

RFLP – Restriction fragment length polymorphism

nad1 – NADH dehydrogenase subunit 1 (mitochondrial gene)

bp – Base pair

µL – Microliter

UV – Ultraviolet

SG – Specific gravity

$ZnCl_2$ – Zinc chloride

TAE – Tris-acetate-EDTA (buffer)

GMA – Game Management Area

SLNP – South Luangwa National Park

DNPW – Department of National Parks and Wildlife (Zambia)

WHO – World Health Organization

mtDNA – Mitochondrial DNA

Sanger seq. – Sanger sequencing

CHAPTER ONE: INTRODUCTION

1.1. Background

Echinococcosis is a cosmopolitan illness that is a major public health concern around the world, with both the cystic echinococcosis (CE) and alveolar echinococcosis (AE) variants being endemic (Wen et al., 2019). Generally, CE is recognized to occur in all of the eastern and most of the southern African countries, but in central and western Africa, the parasite appears to be limited to the (drier) Sahelian zone with its pastoralist cultures (Eckert et al., 2001). Reports from the Western countries state that CE is a re-emerging disease as it has the tendency of disappearing and appearing (Romig et al., 2006). Both cystic and alveolar echinococcosis cause a significant disease burden worldwide, however they receive little or no attention in some circumstances. However, there may be more than 1 million people living with these diseases at any given time, making echinococcosis a major public health concern, particularly in underdeveloped countries with limited economic resources (Nakao et al., 2013).

According to reports, *Echinococcus* spp. has been described in canid wildlife and domestic canids (Alvarez et al., 2014; Thompson., 2008; Romig et al., 2006). In comparison, the occurrence of *Echinococcus spp.* in wild mammals is largely known from surveys conducted 50 years or more ago. Such surveys yield a wide list of definitive and intermediate host species. However, data from most countries where surveys have been conducted suggest a complex situation. The sylvatic transmission of *E. felidis* involving solely wild mammals coexists with domestic lifecycles of *E. granulosus sensu stricto*, resulting in spill-over to wild animals (Macpherson and Wachira, 199; Kagendo et al. 2014).Most available information from Southern Africa indicate mainly the prevalence in livestock (Verster, 1965).

Gough (1908) was the first to describe the presence of *Echinococcus spp.* in a wild animal when he successfully infected a silver-backed jackal. *Echinococcus* worms were also discovered in the African wild dog (*Lycaon pictus*), black-backed jackal (*Lupulella mesomelas*), cape fox (*Vulpes chama*), lion (*Panthera leo*), African wild cat (*Felis lybica*), plains zebra (*Equus quagga*), hippopotamus *(Hippopotamus amphibius*), warthog (*Phacochoerus africanus*), impala (*Aepyceros melampus*), greater kudu (Tragelaphus *strepsiceros*), and African buffalo (*Syncerus caffer*) (Basson et al., 1970; Boomker et al., 1989; McCully et al., 1967; Verster and Collins 1966; Young, 1975; Ortlepp, (1937). There was no additional attempt to distinguish between the *Echinococcus* *species* implicated in the bulk of the hydatid cysts from intermediate hosts, and it was assumed that the inclusive taxon *Echinococcus granulosus* was solely responsible. Hydatid cysts are now recognized as metacestodes found in a variety of *Echinococcus species* and ‘strains. The genus is now being revised, and *E. granulosus (sensu lato)* has been divided into several distinct species, which are discussed in other reports (McManus et al., 2002; Nakao et al., 2007; Saarma et al., 2009). The majority of these taxa, namely *E. granulosus* (*sensu stricto*), *Echinococcus equinus, Echinococcus ortleppi,* and *Echinococcus canadensis* or *Echinococcus intermedius* (Thompson, 2008), have already been recorded from the African continent, and early results from genetic surveys show a complicated epidemiological scenario in which many types of *Echinococcus* are transmitted concurrently in the same locations and, in some cases, the same host species (Maillard et al., 2007). *Echinococcus felidis* is the most recent addition to the taxonomic list which was recently characterized and verified as a separate species. It is closely related but obviously distinct from *E. granulosus sensu stricto* (Hüttner et al., 2008).

Echinococcus spp. infects a wide range of wildlife and domestic herbivores (Wasserman et al., 2015). The eggs are well adapted to live in the environment for as long as a year in cool moist conditions but are susceptible to desiccation (Maillard et al., 2007; Hüttner et al., 2008). Fresh eggs are gluey and may adhere to the fur of definitive hosts enabling their spread. The intermediate host ingests the eggs incidentally while grazing, foraging, or drinking. The eggs hatch in the small intestine, become larvae that penetrate the gut wall, and carried in the circulatory system to several organs. In these organis, the cysts called hydatid cysts or metacestodes, are formed (Maillard et al., 2007; Hüttner et al., 2008).The cysts, which contain larvae, either comprise fluid filled bladders, which contain larval pre-tapeworms (protoscolices), and cause the disease cystic echinococcosis due to *E. granulosus* or alternatively, for *E. multilocularis* a multivesiculated lesion or mass containing protoscolices that grows rapidly by exogenous budding and causes alveolar echinococcosis in rodents and other small mammals (Maillard et al., 2007; Hüttner et al., 2008).

An understanding of molecular epidemiology of echinococcosis is a big step ahead in making it a priority disease to limit its transmission. Robust surveillance data is as well fundamental to show the burden of disease and to evaluate progress and success of control programmes (Lahmar et al., 2009). However, as for other neglected tropical diseases (NTDs) which are focused on underserved populations and remote areas, data is especially scarce and will need more attention if control programmes are to be implemented and measured (Lahmar et al., 2009).

The geographic distribution of individual *E.granulosus* genotypes is variable and is an area of ongoing research. The lack of accurate case reporting and genotyping currently prevents any precise mapping of the true epidemiological picture of *echinococcus spp* (Lahmar et al., 2009). However, genotypes G1 and G3 (associated with sheep) are the most reported at present and broadly distributed. In North America, *E.granulosus* is rarely reported in two States (Canada and Alaska), and a few human cases have also been reported in Arizona and New Mexico in sheep-raising areas(Lahmar et al., 2009). In the United States, most infections are diagnosed in immigrants from countries where cystic echinococcosis is endemic. Some genotypes designated "*E. canadensis,* genotypically regarded as (G6, G7)" occur broadly across Eurasia, North and South America while some others seem to have a northern holarctic distribution (genotypes G8, G10). *Echinococcus Multilocularis* occurs in the northern hemisphere, including central and northern Europe, Central Asia, northern Russia, northern Japan, north-central United States, Northwestern Alaska, and Northwestern Canada. In North America, *Echinococcus multilocularis* is found primarily in the north-central region as well as Alaska and Canada. Rare human cases have been reported in Alaska, the province of Manitoba, and Minnesota. Only a single autochthonous case in the United States (Minnesota) has been confirmed to be *E. Oligarthrus* which occurs in Central and South America (Deplazes et al., 2017; Avcioglu et al., 2016; Avcioglu et al., 2017;Emily et al., 2013; Robbins, 2018; Schurer et al., 2018).

Africa has not been spared from Echinococcosis. *Echinococcus canadensis* genotype G6 and 7 have been reported in Eastern Africa. According to Kagendo et al. (2014), numerous records exist on the molecular identity of *Echinococcus spp.* from domestic animals and humans in Kenya. However, no such information is available for wild mammals. Wasserman et al., (2015), stated that various surveys, mainly from eastern and southern Africa, have been conducted in which two species of jackal, cape fox, African wild dog, spotted hyena, wild cat and lion were identified as definitive hosts, while cysts were found mainly in zebra, bush pig, warthog, hippopotamus, giraffe, buffalo and at least ten species of other bovids ("antelopes"). In Kenya Multiple infections were also recorded in some samples (Mulinge et al., 2018). *Echinococcusgranulosus s. s.* (G1-G3) and *E. canadensis* (G6/7) coinfections occurred in seven and one faecal samples from Turkana and Maasai Mara, respectively. *Echinococcus granulosus s. s.* and *E. ortleppi* infections were recorded in one faecal sample from Turkana.

One faecal sample from Turkana contained eggs of three taxa namely, *E. granulosus s. s.*, *E. ortleppi*, and *E. canadensis* (G6/7) (Mulinge et al., 2018). The prevalence of *Echinococcus* spp. infection in dogs was also reported in different regions of Kenya (Mulinge et al., 2018). The presence of molecular identities of isolates of *Echinococcus spp*. were reported in the Southern Africa Development Community (SADC) countries (Namibia and South Africa), (Obwallar et al., 2004; Magoye et al., 2013; Wassermann et al., 2015; Addy et al., 2017; Halajian et al., 2017).

1.2. Statement of the problem

Zambia is among the Southern African countries that have not taken this zoonotic infection as a priority. Lack of prioritising of this disease could be because of limited data on its prevalence in the country. Though Banda et al. (2012) established the existence of hydatidosis in cattle and humans in the western province of Zambia, the study did not investigate prevalence of *Echinococcus* in wildlife, let alone in wildlife-livestock interface areas hence the need to conduct the current study. The knowledge generated from this study would help in designing control programmes that could help to reduce transmission of echinococcosis in humans and animals.

A major obstacle to detailing the actual epidemiological situation of echinococcosis in Zambia is the absence of precise reporting of cases as well as genotyping of *Echinococcus granulosus*. The frequency of *Echinococcus* in wildlife and wildlife-livestock interface regions is still poorly understood, notwithstanding earlier research in Zambia's western province establishing the existence of hydatidosis in people and cattle (Phiri et al., 2002; Mwape et al., 2012). The insufficient availability of data impedes the establishment of focused control strategies aimed at reducing the spread of echinococcosis between people and animals. Effective control programme design and execution in preventing the spread of this zoonotic disease are hindered in the absence of an in-depth comprehension of the distribution and frequency of *Echinococcus* genotypes in different host populations.

The dearth of data on the occurrence and spread of the disease in various ecological situations could be the reason why echinococcosis has not received as much attention as it should in Zambia's public health agenda. Although Banda et al. (2012) provided insight into the presence of hydatidosis in particular areas, the dearth in the evidence evaluating the frequency of *Echinococcus* in the wildlife-livestock interface highlights the critical need for more research. To successfully minimize the impact of *Echinococcus* on both human and animal populations in Zambia, evidence-based prevention, control, and treatment measures must consider the dynamics of echinococcosis transmission in these critical places.

1.3. Rationale for the study

It was imperative that a study be conducted on the precise case reporting and genotyping of *Echinococcus granulosus* genotypes in Zambia for several reasons. First, for public health measures to be effective, it is imperative to comprehend the epidemiology of echinococcosis. This might allow for focused control strategies which are aimed at lessening the parasite's transmission between humans and animals. This could be done through the acquisition of precise data on the proportion and distribution of Echinococcus genotypes in various host groups, such as wildlife and domesticated animals.

One possible explanation for echinococcosis lack of prominence on Zambia's public health agenda might be the dearth of thorough data on disease. Detailed statistics are desperately needed to inform evidence-based practice and assist in reducing the echinococcosis burden. Investigating echinococcosis occurrence and distribution can help develop One Health concepts and support international efforts to combat newly emerging infectious diseases.

1.4. Research question.

1. What is the prevalence of *Echinococcus spp.* infections among wild carnivores in the South Luangwa National Park?
2. What is the extent of *Echinococcus spp.* infections in domestic carnivores within the wildlife-livestock interface areas of South Luangwa National Park?

1.5. General objective

To detect and identify *Echinococcus spp.* circulating in the wildlife of the South Luangwa National Park and domestic carnivores in the wildlife-livestock interface areas in Mfuwe of Mambwe district.

1.6. Specific objectives

1. To assess whether wild carnivores of the South Luangwa National Park are infected with *Echinococcus* tapeworms.

2. To investigate if domestic carnivores (dogs) in the wildlife-livestock interface areas in Mfuwe district are infected with *Echinococcus* tapeworms.
3. To identify the detected *Echinococcus spp.* using molecular methods

CHAPTER TWO: LITERATURE REVIEW

2.1. Parasite background

The history of echinococcosis in Europe includes a period of over 2000 years. Eckert (2007), mentioned in ancientness metacestodes (hydatids) of *Echinococcus granulosus*, the causative agents of Cystic Echinococcosis (CE), were observed in animals and humans. Alveolar Echinococcosis (AE), caused by metacestodes of *E. multilocularis*, was recognized as a disease entity only in the middle of the 19th century. It took around 100 years until it was undoubtedly clarified and understood that CE and AE are not caused by a lone *Echinococcus* species, but by *E. granulosus* and *E. multilocularis*, respectively (Eckert, 2007).

In the 20thcentury significant progress had been achieved in echinococcosis research, including diagnosis, epidemiology, therapy, immunology, molecular biology, and other fields. Pierre Simon Pallas (1766) projected that these hydatid cysts found in infected humans were larval stages of tapeworms. Goeze (1782) precisely described the cysts and the tapeworm heads. The *E. granulosus* was perfectly described by Batsch (cited by Christopher et al., 2021). In the 1850s, Siebold (cited by Christopher et al., 2021) showed over a series of experiments that *Echinococcus* cysts cause adult tapeworms in dogs. Further, in 1863, *E. Multilocularis* was identified by Rudolf Leuckhart (cited by Christopher et al., 2021).

In the early1900s, the more distinctive features of *E. granulosus* and *E. multilocularis*, their life cycles and how they cause disease were fully defined as more people began exploring (Banda et al., 2012). Echinococcosis is an emerging and re-emerging zoonotic parasitic disease caused by the cestode species of the genus *Echinococcus*. It is one of the most significant neglected tropical diseases with a global distribution including Europe, Asia, Africa, South America, Canada and Australia (Altintas, 2003; Budke et al., 2006; Moro and Schantz, 2006).

Reports have shown that hydatidosis is a disease of increasingly public health and socio-economic concern and is presently considered as an emerging or a re-emerging disease. Its geographical distribution is greater than previously believed (Thompson and McManus, 2002; Torgerson and Budke, 2003; Dakkak, 2010). Reports in different parts of sub-Saharan Africa have shown a diverse distribution of cystic echinococcosis (Magambo et al., 2006). Cystic echinococcosis causes severe disease and possible death in humans, as well as economic losses from treatment costs, lost wages and livestock associated production losses (Budke et al., 2006). It is one of the most significant helminthic diseases of livestock that has mutually economic and public health implication. It is associated with severe morbidity and disability (Getaw et al., 2010). However, CE and AE remain the real problems as in many endemic regions resources and structures are lacking for effective surveillance and control of these zoonoses threatening humans.

2.2. Aetiology of echinococcosis

Echinococcosis is caused by tape worms of the genus *Echinoccoccus. Echinococcus* belongs to the phylum of Platyhelminthes, in the class Cestoda, order Cyclophyllidea, family Taeniidae and from the genus *Echinococcus* (Niilo, 1969).According to Da Silva (2010), there are other types of *Echinococcus* species described apart from *E. Granulosus* and *E. multilocularis,* including *E. oligarthrus*, *E. shiquicus*, and *E. vogeli*. These species are taxonomically relevant but only *E. granulosus* and *E. multilocularis* are the most pathogenic to humans and other domestic animals (Da Silva (2010).

The World Health Organization (WHO) proposed the designation of CE for the disease caused by *E. granulosus* and AE for the disease caused by E. multilocularis to distinguish these two most pathogenic species. In addition, a third form of echinococcosis caused by *E. oligarthrus* and *E. vogeli* called *polycystic echinococcosis* (PE) is also recognised (Raether and Hanel, 2003; Eckert, 2004; Macpherson et al., 2003; Eckert and Deplazes, 2004). Ortlepp (1937) identified *Echinococcus felidis* from the South African lion, *Panthera leo*. The conspicuous rugosity of the rostellar hooks, as well as its presence in a *felid* as a definitive host, was used to describe it as a new species. In 1963, Rausch and Nelson identified it to be similar with *E. granulosus,* but Vester (1965) re-examined its morphology and noted its genital hole in the mature segment of the lion parasite (Rausch, 1967). The 'lion strain' is now thought to be a type of *E. granulosus* with an unknown taxonomic rank that spreads between lions and big wild animals in Africa (Macpherson and Wachira, 1993).

2.3. The parasite

2.3.1. Parasite morphology

These parasitic tapeworms undergo various developmental stages which include: eggs; larvae (also known as hydatid); and adults. The adult *Echinococcus granulosus* worms are small of about 2-6mm long. The body is divided into three regions: the scolex (anterior), the neck and the strobila (posterior), which is sub-divided into proglottids or segmented reproductive units.

Thompson and McManus (2001), stated that, the scolex has four lateral suckers (specialized structures used to attach to the intestinal lining of the definitive host) as seen in Figure 2.1. The scolex also has a rostellum which is non-retractable and armed with a double crown of 28-50 recurved hooks. The hooks, arranged in one large row, and one small row range from 24.9-34µm and 20.4-30µm, respectively.

Numerous infective stages (protoscolices, apparent as invaginated scolices already containing suckers and hooks) are produced by the encysted larval (metacestode) stage which is known as a bladder-worm or hydatid. They will either be generated directly from the germinal layer of the cyst wall or by forming brood sacs (hydatid sand) by endogenous (internal) or exogenous (external) budding of the germinal layer (Thompson and McManus, 2001). *Echinococcus granulosus* forms fluid-filled unilocular cysts with endogenous budding of brood capsules. *Echinococcus vogeli* forms fluid-filled polycystic cysts with exogenous budding, and *Echinococcus multilocularis* forms fluid-free multilocula or alveolar cysts with exogenous budding (Sweatman et al., 1963; Belding, 1965). The different developmental stages in *Echinococcus granulosus* and *E. multilocularis* are illustrated in Figure 2.1.

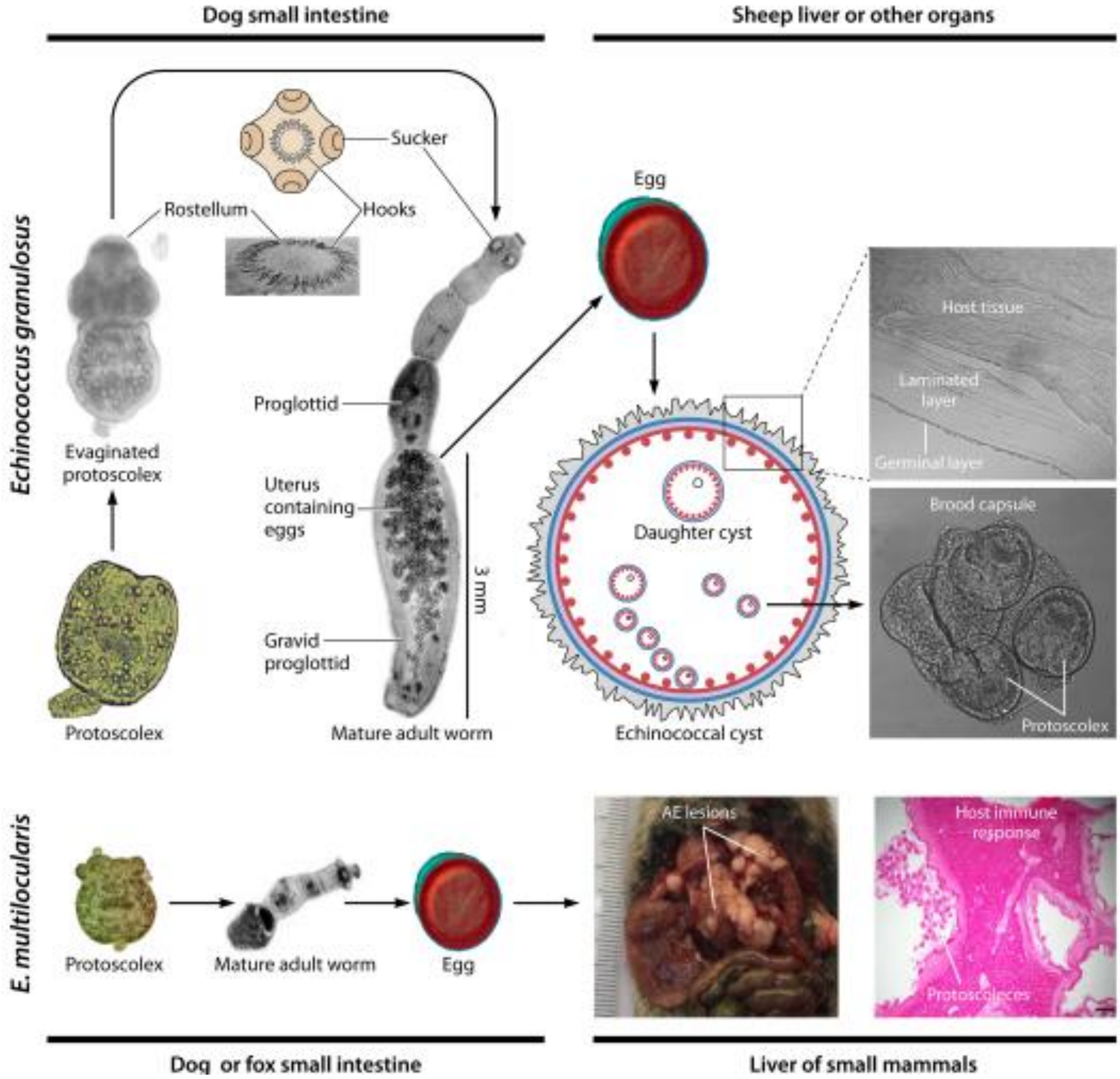

Figure 2.1: Different developmental stages in *Echinococcus granulosus* and *E. multilocularis*. Growth of the larval cyst is unlimited, and it can, for *E. granulosus*, grow to 30 cm or more in humans, while the adult worm, egg, and protoscolex are limited in size and shape. (Source: Wen et al., 2019).

2.3.2. Morphology of *Echinococcus* eggs.

The presence of adult worms in the intestine can be confirmed by the presence of *Echinococcus* segments and/or ova in the faeces. The size (2.0–3.0 mm) and shape of the egg, which is ovoid, are used to identify it. The eggs have a thick shell with radial striation (embryophore). The hexacanth embryo's six hooks distinguish it from pollen grains and other debris (Thompson 2006).

2.4. Host range.

The definitive hosts of *Echinococcus granulosus* are canids, both wild and domestic. Natural intermediate hosts vary by genotype. Intermediate hosts for zoonotic species/genotypes are often ungulates such as sheep and goats (E. granulosus sensu stricto), cattle ("E. ortleppi"/G5), camels ("*E. canadensis*"/G6), and cervids ("*E. canadensis*"/G8, G10) (CDC, 2019).

Many reports of *E. felidis* remained unidentified from a vast range of African animals, due to a lack of diagnostic criteria, primarily genetic characterization, and misinterpretation with sympatric *Echinococcus spp.* (Macpherson and Wachira, 1993). Echinococcosis is a major public health concern in endemic places across the world, particularly in Africa, where more than one species of intermediate host exists, and transmission cycles can interact. As a result, *E. felidis* infections with other *Echinococcus spp.* may be sympatrically overlapping in understudied areas, allowing them to be overlooked.

E. felidis' explicit status was not identified until recently. Huttner et al. (2008) demonstrated its validity using genetic categorization and phylogenetic position (Huttner et al., 2008). The *felids* are definitive hosts for the mysterious 'lion strain', although it is unknown which sympatric wild ungulates serve as intermediate hosts in *E. felidis'* life cycle. The many characteristics of *E. felidis* like *E. shiquicus*, there is insufficient information regarding human infection, hydatid features, and intermediate hosts. As a result, the zoonotic potential and public health risk among circulating isolates, particularly those with concurrent illnesses, should be taken very seriously (Huttner et al., 2008). A list of *E. felidis and E. shiquicus* are presented in Table 2.1.

Table 2.1: A list of *E. felidis and E. shiquicus* (Zoonotic)

Hydatid Characters	**Infectivity to Humans**	**Genetic similarity**	**Distribution**	**Intermediate host**	**Definitive host**	**Species**
Unilocular	Uncertain	*Echinococcus multilocularis*	Tibetan Plateau	Pika	Tibetan fox	*E. shiquicus*
Unknown	Uncertain	*Echinococcus granulosus*	Africa	Warthog (possibly zebra, wildebeest, bush pig, buffalo, various antelope, giraffe, hippopotamus)	Lion	*E. felidis*

The *E. multilocularis'* principal definitive host species are foxes, i.e. red foxes (*Vulpes vulpes*). Other canids, such as domestic dogs, wolves, and raccoon dogs (*Nyctereutes procyonoides*), are capable definitive hosts. Many rodents can act as intermediate hosts, but those from the Arvicolinae subfamily (voles, lemmings, and similar rodents) are the most common (CDC, 2019).

The *E. vogeli's* natural definitive host is the bush dog (*Speothos venaticus*); however, it might also be domestic dogs. Pacas (*Cuniculus paca*) and agoutis (*Dasyprocta spp.*) are intermediate hosts. *E. oligarthrus* relies on wild Neotropical *felids* (such as *ocelots*, *pumas*, and *jaguarundis*) as definitive hosts, with a wider range of rodents and lagomorphs serving as intermediate hosts (CDC, 2019). The host range of *Echinococcus* by species are presented in Table 2.2. Further, the host range of Echinococcus by region is presented in Table 2.3**.**

Table 2.2: Host range of *Echinococcus* by Species

Echinococcus species	Host range (Definitive)	Source
E. granulosuss.s	Sheep	Eckert and Deplazes, 2004
	Tasmanian sheep	Oksanen et al., 2016
	Buffalo	CDC, 2019
	Cattle	
E. equinus	Horse	Oksanen et al., 2016
		CDC, 2019
E. ortlepi	Cattle	Oksanen et al., 2016
	Camel-pig	CDC, 2019
E. canadensis	American cervid	Oksanen et al., 2016
	Variant pig	CDC, 2019
E. Multicularis	Red foxes	Eckert and Deplazes, 2004
		Oksanen et al., 2016
E. ortleppi	Cattle	Dinkel et al. (2004)
	Cattle	Banda et al. (2020)
	Wild dog	Lopez-Ney and Solar Planas (1943)
	Jackals	Mbaya et al. (2014)
E. granulosus	Adult div. Camdae	Verster, (1965)
E. felidis	Lion	Hutter et al. (2008)
	Spotted hyenas, Lion	Dorothy et al., (2014)
	Dog	Mulinge et al. (2018)
	Hippo	Ali et al. (2017)
	Lion	Huttner and Romig, (2009)
E. equines	Lion	Kagendo et al., (2014)
	Black-Backed Jackal	Wassermann et al., (2015)

Table 2.3: *Echinococcus* by Region

Echinococcus species	Region	Source
E. granulosuss.s	Mediterranean, south- America, Central-Asia (G3-Water Buffalo) –Italy	Eckert and Deplazes, 2004 Oksanen et al., 2016 CDC, 2019
E. equinus	Rare and sporadic (except England) non-infective to humans	Oksanen et al., 2016 CDC, 2019
E. ortlepi	(Central Europe) infrequent low infectivity to humans	Oksanen et al., 2016 CDC, 2019
E. canadensis	(Central and Eastern Europe) wide host range; pigs, goats and camel not sheep	Oksanen et al., 2016 CDC, 2019
E. Multicularis	Europe, North America and Eurasia	Eckert and Deplazes, 2004 Oksanen et al., 2016
E. ortleppi	Sudan	Dinkel et al. (2004)
	Zambia	Banda et al. (2020)
	South Africa	Lopez-Ney and Solar Planas (1943)
	Kenya	Mbaya et al. (2014)
E. granulosus	South Africa	Verster, (1965)
E. felidis	South Africa	Hutter et al. (2008)
	Kenya	Dorothy et al., (2014)
	Kenya	Mulinge et al. (2018)
	South Africa	Ali et al. (2017)
	Uganda	Huttner and Romig, (2009)
E. equines	Eastern and Southern Africa	Kagendo et al., (2014)
	Namibia	Wassermann et al., (2015)

2.5. The life cycle of *Echinococcus spp.*

The adult *Echinococcus granulosus* resides in the small intestines of definitive hosts (dogs or other canids) as shown in Figure 2.2**.** Gravid proglottids discharge eggs that are passed in the faeces. Under favourable conditions, eggs of *E.granulosus* remain viable for several months in pastures or gardens and on household items (Banda et al., 2020). *Echinococcus granulosus* eggs can survive for weeks under wide temperature ranges but they cannot survive for a long time when exposed to direct sunlight and dry conditions. The eggs may also stick to hands when a person handles an infected dog, cat, wild animal, or its carcass, and may then be transferred to the mouth via the hands, (Banda et al., 2020) After ingestion by a suitable intermediate host such as sheep, goat, porcine, cattle, horses or camel, the egg hatches in the small intestines and discharges an oncosphere that enters the duodenal wall and migrate through the circulatory system into different organs, peculiarly the liver and lungs (Eckert, Conraths, and Tackmann, 2000). In these organs, the oncosphere unravels into a cyst that enlarges gradually, producing protoscolices and daughter cysts that fill the sac interior. The definitive host becomes infected by ingesting the cyst-containing organs of the contaminated intermediate host. After ingestion of these contaminated organs, the protoscolices evaginate, attaches to the enteric mucosa, and develop into adult stages in 32 to 80 days (Banda et al., 2020).

The same life cycle happens with *E. multilocularis*, with the following dissimilarity: the definitive hosts are foxes, and to a lesser extent dogs, cats, coyotes and wolves; the intermediate host are diminutive rodents; and larval product (in the liver) relic indefinitely in the proliferative stage, effect in invasion of the enclosing tissues (Eckert and Thompson, 2017). For *E. vogeli*, the definitive hosts are bush and domestic dogs, whereas the intermediate hosts are rodents, and the larval stage (liver and other organs) unravels both externally and internally, resulting in manifold vesicles (Eckert and Thompson, 2017). *Echinococcus oligarthrus* has a life cycle that entwines wild felids as ultimate hosts and rodents as intermediate hosts. Humans become infected by accidentally ingesting eggs, with the resulting discharge of oncospheres in the intestine and the growth of cysts in various organs (Eckert and Thompson, 2017). The Life cycles of *Echinococcus spp* is presented in Figure 2.2**.**

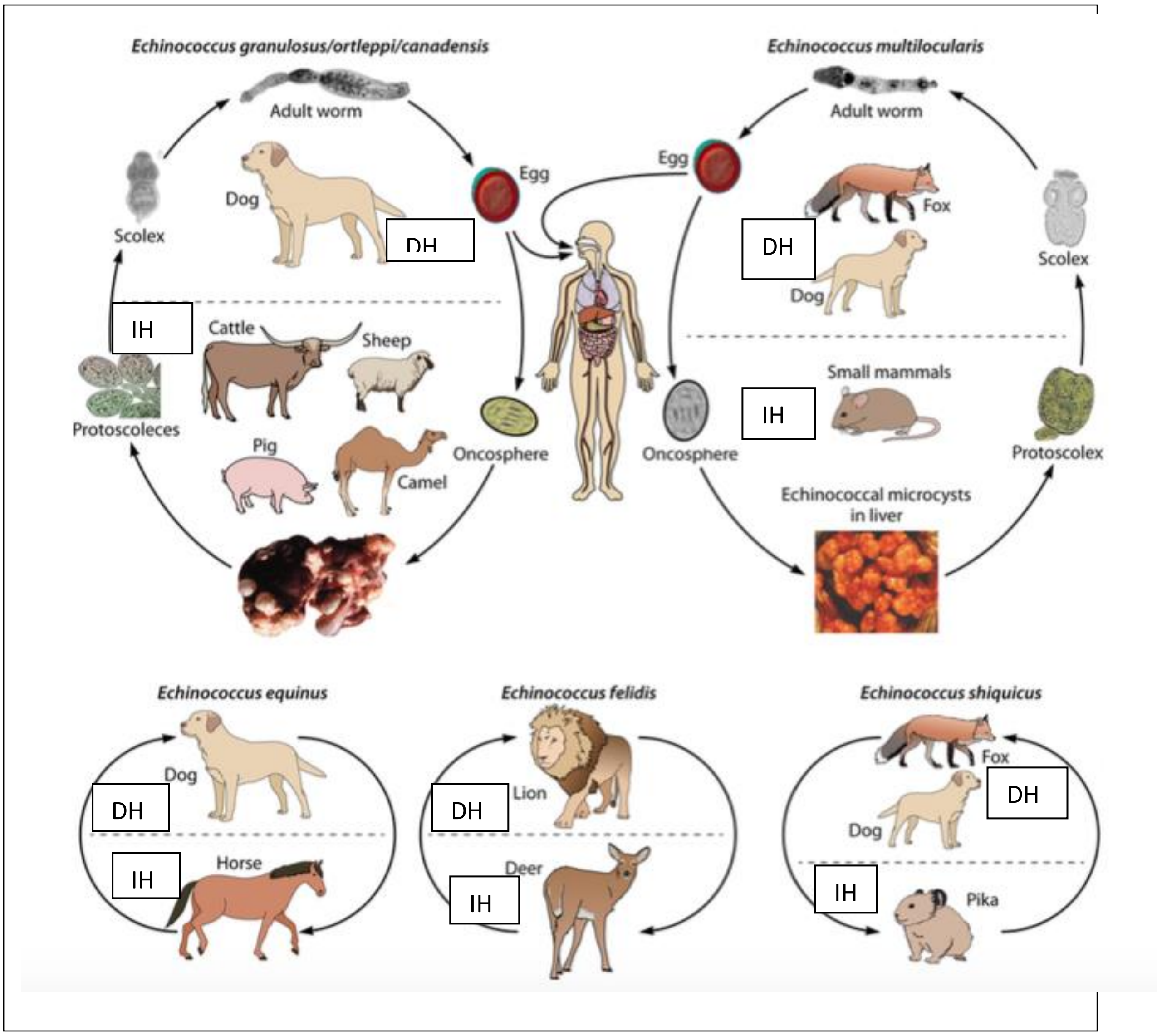

Figure 2.2: Life cycles of *Echinococcus spp.*

2.6. Geographical distribution of Echinococcus species

Cystic echinococcosis occurs on every continent with the exception of Antarctica. Cystic echinococcosis is caused by *Echinococcus granulosus, Echinococcus ortleppi, Echinococcus canadensis,* and *Echinococcus intermedius,* which are found globally. *E. granulosus s.l.* is notably widespread in China and Central Asia, South America, North and East Africa, and Australia; in Europe, another endemic region, it is mainly prominent in the Mediterranean and Eastern nations (Tamarozzi et al., 2020).

Alveolar echinococcosis is restricted to the region on the northern hemisphere, namely China, the Russian Federation, and nations in continental Europe and North America (CDC, 2019). *E. multilocularis*, the causative agent of AE has been found in red foxes in more than 21 countries, and they include Denmark, Slovenia, Sweden, Austria, Belgium, Croatia, Hungary, Italy, the Netherlands, Romania, and Ukraine, Czech Republic, Estonia, France, Germany, Latvia, Lithuania, Poland, Slovakia, Liechtenstein, and Switzerland. However, *E. multilocularis* has been found in Arctic foxes from the Arctic Archipelago of Svalbard, Norway (Oksanen et al., 2016).

Aregawi et al. (2024), in their systematic review, documented the presence of *Echinococcus ortleppi* and *Echinococcus granulosus* in Sudan. They also reported *E. granulosus* and *E. canadensis* in Ethiopia, while in Tanzania, they identified *E. ortleppi*, *E. granulosus*, and *E. canadensis*. Additionally, *Echinococcus felidis* was first described in Africa by Ortlep (1937) in South Africa. Further studies have been conducted in Eastern African countries which have documented these species, including Kenya and Uganda, as reported by Kagendo et al. (2014), Mulinge et al. (2018), and Huttner et al. (2009). The global distribution of *Echinococcus granulosus sensu lato*, responsible for cystic echinococcosis (CE), and *Echinococcus multilocularis*, responsible for alveolar echinococcosis (AE) in humans is presented in Figure 2.3**.**

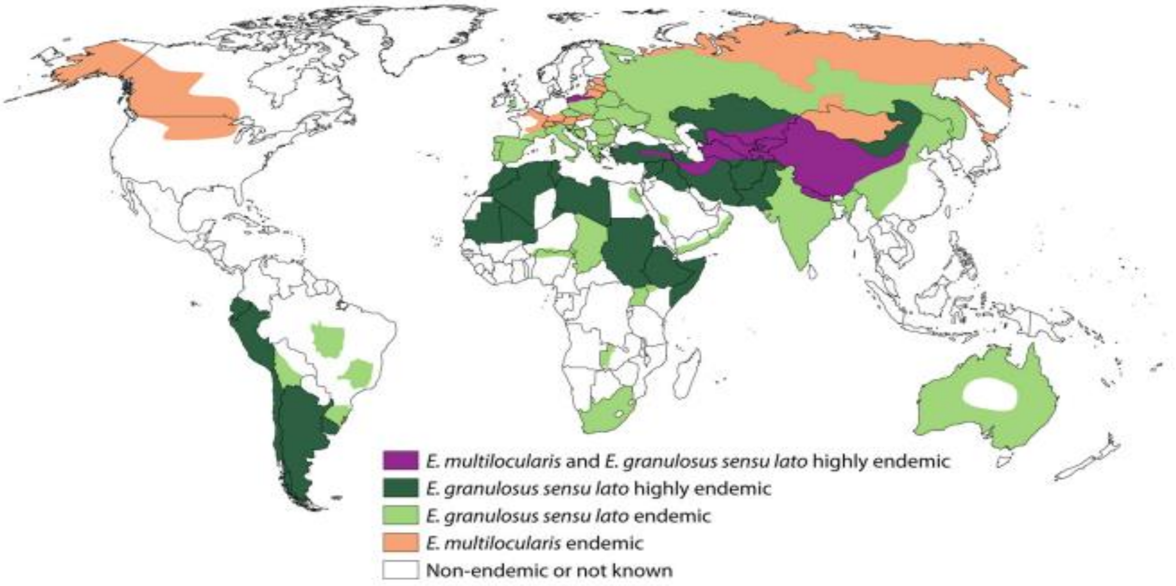

Figure 2.3: Global distribution of *Echinococcus granulosus sensu lato*, responsible for cystic echinococcosis (CE), and *Echinococcus multilocularis*, responsible for alveolar echinococcosis (AE) in humans. (Source: Wen et al., 2019).

2.7. Prevalence of cystic echinococcosis (CE) in Africa.

Cystic echinococcosis (CE) is extremely common in Africa, particularly in northern and eastern Africa, where data is more abundant than in other regions (Kagendo et al., 2014). *Echinococcus granulosus* sensu stricto (G1, G3) accounted for 74.45 percent of total East African isolates, *Echinococcus canadensis* (G6/7) accounted for 60.3 percent and 97.4 percent in North and West Africa, respectively, and 81.3 percent of *Echinococcus ortleppi* (G5) were recorded for Southern Africa (Kagendo et al., 2014). The high prevalence of these species highlights the significant burden of CE across the continent, particularly in regions with extensive livestock farming and close human-animal interactions. Furthermore, the genetic diversity of *Echinococcus* species in Africa underscores the need for region-specific control strategies to effectively mitigate the transmission and impact of this zoonotic disease.

A survey conducted in Kenyan national parks documented an overall prevalence of 60% for *Echinococcus* infections, confirmed through host-specific RLFP-PCR analysis of the cytochrome B gene (Kagendo et al., 2014). This study represents the first molecular investigation into the distribution of *Echinococcus* species within Kenyan wildlife. The presence of *Echinococcus felidis* was confirmed in lions, spotted hyenas, and dogs, with spotted hyenas being reported as hosts for the first time. Additionally, lions and hyenas were newly identified as definitive hosts for *Echinococcus granulosus* sensu stricto, while the role of leopards in the transmission cycle remains unclear (Kagendo et al., 2014; Mulinge et al., 2018; Addy et al., 2012).

According to Wassermann et al. (2015), the Etosha region represents the southern and western limit of the distribution range of plains zebras (*Equus quagga*), whereas the northern subspecies of mountain zebras (*Equus zebra hartmannae*) are more prevalent in Namibia, suggesting a broader distribution of *Echinococcus equinus*. In northern South Africa's Kruger National Park, 60% of plains zebras were found to harbor *Echinococcus* cysts, which were experimentally used to infect lions, demonstrating their viability (Young, 1975). Although this does not definitively confirm the presence of *E. equinus* in Kruger National Park, it indicates that the sylvatic lifecycle of *E. equinus* may be widespread across East Africa. Additionally, Hüttner et al. (2015) reported a 72% fecal prevalence of *Echinococcus felidis* in lion samples from a Ugandan national park. While limited data exist on the pathogenicity of *E. felidis* in humans, evidence of interspecies transmission has been documented, including infections in dogs, raising concerns about its zoonotic potential. Consequently, *E. felidis* may pose a significant risk to pastoral communities in East Africa, where close human-animal interactions are common (Hüttner et al., 2008).

2.8. Economic and public health importance of echinococcosis

Echinococcosis is a zoonosis and one of the most prevalent neglected tropical diseases (NTDs) worldwide listed by the World Health Organization (WHO) (Eckert et al., 2001). It is one of the most important helminthic diseases with a cosmopolitan distribution including Europe, Asia, Africa, South America, Canada and Australia (Altintas, 2003). *Echinococcus* is benign in the intestine of the carnivorous definitive host (Thompson and McManus, 2001). They have been recognized as the most important helminthic zoonoses and of great economic and public-health significance in developing countries (Eckert et al., 2000). Cystic echinococcosis not only causes severe disease and possible death in humans but also results in economic losses from treatment costs, lost wages and livestock-associated production losses (Budke et al., 2005).

Rates of human cystic echinococcosis infection ranging from less than 1 per 100,000 to more than 200 per 100,000 in certain rural populations where there is close contact with domestic dogs have been reported. Incidence of human alveolar echinococcosis is usually < 0.5 per 100,000 but maybe >100 per 100,000 in certain communities (Wen et al., 2019). Both cystic echinococcosis and alveolar echinococcosis represent a substantial disease burden. Worldwide, there may be over one million people who have these diseases at any given time (WHO, 2015). These people experience severe clinical syndromes which are life-threatening if not treated. Even with treatment, people often face a reduced quality of life (OMS/OIE, 2002).

Cystic echinococcosis accounts on average for 2.2% postoperative mortality rate for surgical patients and about 6.5% of cases relapse after an intervention, thus requiring prolonged recovery time (WHO, 2019). The 2015 WHO Food borne Disease Burden Epidemiology Reference Group (FERG) estimated that echinococcosis caused about 19300 deaths and 871000 disability-adjusted life-years (DALYs) globally each year. Annual costs associated with cystic echinococcosis are estimated to be US$ 3 billion for treating cases and losses to the livestock industry (WHO, 2019).

Echinococcosis in humans and animals has both an economic, medical and public health importance in many parts of the world (Moro and Schantz, 2009). For instance, the cost of human health and animal losses was estimated at US $ 60 million per annum in the North African countries (Budke et al., 2006). Hydatidosis in animals is an economic burden making it hard to produce bulk and quality meat, milk and wool. In addition, cystic echinococcosis

(CE) significantly impacts livestock productivity by causing a decline in fertility and leading to the condemnation of infected organs (Paul, Torgerson, and Budke, 2003). For instance, in regions such as Uruguay, annual economic losses due to organ condemnation and reduced livestock production were estimated at approximately US$ 6.2 million (Torgerson, 2003). In humans, the disease can manifest with severe clinical outcomes, occasionally proving fatal, while treatment is often prolonged, complex, and costly, further exacerbating its public health burden.

2.9. Epidemiology and risk factors of echinococcosis

The larval stage of *Echinococcus granulosus* is widely known to influence the infection of Cystic echinococcosis (CE). Cystic echinococcosis is prevalent in most parts of Africa (Kagendo et al., 2014), Europe, Asia, the Middle East, Central, and South America, and in rare cases, North America (Zhenghuan, Xiaoming, and Xiaoqing, 2008). Transmission to dogs occurs when there is an introduction of the organs of other animals that enclose hydatid cysts. The cysts will grow into adult tapeworms in the dog and later shed tapeworm eggs through faeces which defile the ground (Eckert et al., 2000).

Dogs are the world's best-adapted canids for human habitation (Dohoo et al., 1998; Ugbomoiko et al., 2008). They have contributed to their owners' physical, social, and emotional wellbeing, especially children who often are most vulnerable to exposure. Molyneux (2004) argued that, despite the positive effects of the incident, however, close ties between dogs and men are not only a significant threat to public health, but also to the infectious parasite (including *Echinococcus*) which can be transmitted to man or domestic animals. Dogs contain an overwhelming number (Ugbomoiko et al., 2008). Getaw et al. (2010), stated that some traditionally deep-rooted activities were described as factors linked to the spread and high prevalence of the disease in certain areas. These factors include the wide-ranging killing of animals in the backyard, the associated lack of stringent meat inspections, the long-standing habit of feeding domesticated dogs with offal, and the ensuing contamination of pasture. The transmission of *Echinococcus granulosus*, the causative agent of cystic echinococcosis (hydatid disease), is influenced by a combination of behavioural, environmental, and socioeconomic risk factors (Craig et al., 2015). Occupational exposure, particularly among pastoral communities, plays a significant role, as individuals in these settings often have close contact with livestock and dogs, which serve as definitive hosts for the parasite. Dogs, in particular, are critical in the transmission cycle, as they become infected by consuming contaminated offal from intermediate hosts (e.g., sheep, goats, or cattle) and subsequently shed Echinococcus eggs in their feces, contaminating the environment (Craig et al., 2015). Poor education and limited awareness of hygiene practices further exacerbate the risk, as individuals may not recognize the importance of handwashing or proper food preparation to prevent ingestion of infectious eggs (Craig et al., 2015). Dietary habits, such as consuming raw or undercooked meat from infected animals, also contribute to transmission. Additionally, age and sexual behaviours may influence exposure patterns, with certain demographic groups being more vulnerable due to their roles in animal husbandry or food preparation (Craig et al., 2015). Finally, the use of untreated drinking water from sources contaminated with Echinococcus eggs can serve as a direct route of infection. Together, these risk factors create a complex web of transmission dynamics that sustain the life cycle of E. granulosus and perpetuate the burden of cystic echinococcosis in endemic regions (Craig et al., 2015; McManus et al., 2003).

Molecular epidemiology (ME) is a science that employs molecular or genetic markers to track illness progression in a community and better understand transmission, as well as the population structure and evolution of bacterial diseases. Phylogenetic study of molecular markers allows for the assessment of genetic relatedness of strains from different origins, geographic areas, and/or time periods, as well as the inference of evolutionary connections. Molecular epidemiology has evolved dramatically over the last several decades as DNA-based molecular typing tools have advanced (Xing et al., 2015).

Molecular epidemiology has transformed our knowledge of infectious disease transmission. It provides laboratory and analytical methods for characterizing infectious agents in research into the origin of infectious illnesses (Xing et al., 2015). Molecular epidemiology is therefore a valuable resource in both outbreaks and continuous surveillance operations, notably in the research of emerging infectious illnesses. Molecular epidemiology plays a crucial role in both human and domestic animal health, and increasingly with infectious agents of wildlife in the context of disease outbreak and conservation (Xing et al., 2015). This is true for infectious disorders such as echinococcosis, where the aetiological agents must be identified and characterised, as well as the host range. Such an approach has been particularly effective with the causal agents of echinococcosis (Thompson, 2013). This is increased if many *Echinococcus species*, or 'strains'/genotypes, are suspected to be present ME will give a workable and useful taxonomy and then finding transmission patterns in endemic locations (Thompson, 2017).

Molecular epidemiology has contributed significantly to our understanding of the genus *Echinococcus's* wide genetic and phenotypic heterogeneity (Thompson, 2017; Romig et al., 2016). This has resulted in the construction of a good taxonomy which is supported by biological evidence, as well as the development of relevant genetic techniques for epidemiological research and transmission pattern elucidation. Molecular tools have mostly used PCR-based methods and sequencing of mitochondrial and nuclear DNA sequencing has been the most often used molecular techniques (Lymbery, 2017; Kinkar et al., 2017).

Molecular Epidemiology investigation discovered *E. granulosus* in Mazandaran Province in Iran, as the only species in livestock and people (Gorgani, 2018). Molecular Epidemiology (ME) study in Riyadh discovered *E. granulosus* in sheep as well as camels (Metwally et al., 2018). Cattle have been thought to play a modest influence in the spread of *E. granulosus*. However, recent ME investigations in Sudan and Ethiopia found that cattle are the most significant intermediate host for *E. granulosus, E. ortleppi,* and *E. intermedius* (Ahmed et al., 2018; Terefe et al., 2019).

Recent ME studies have shown that transmission of *E. equinus* and *E. ortleppi* still happen in Turkey (Kesik et al., 2019), Africa, South America, and portions of Europe (Addy et al., 2016). Interestingly, the first ME investigation on echinococcosis in Bhutan found local transmission of both *E. ortleppi and E. granulosus* (Thapa et al., 2016).

Echinococcus has long been recognized as separate from E. granulosus in cycles including wild and farmed cervids, moose (*Alces alces*), elk (*wapiti*) (*Cervus elephas*), reindeer (*caribou*) (*Rangifer tarandus*), domestic pigs, and camels as intermediate hosts (Thompson, 2017). ME investigations first demonstrated that four different *Echinococcus* genotypes, G6, 7, 8, and 10, are maintained during these life cycles (Lymbery et al., 2014). These findings supplemented morphological descriptions of adult parasites of cervid, pig, and camel origin (Lymbery et al., 2014). Although molecular tools have proven useful in confirming the genetic distinctness of these forms, and subsequent ME studies have revealed different host ranges and geographic distributions, nomenclatural issues have hampered a clear understanding of their transmission cycles (Lymbery et al., 2014).

Molecular surveillance in Argentina using mitochondrial and nuclear markers has recently expanded the host range of *E. oligarthra* to include agoutis (*Dasyprocta azarae*), ocelot (*Leopardus pardalis),* and puma (*Puma concolor*) (Arrabal et al., 2017), as well as demonstrating the existence of two genetically distinct populations of *E. oligarthra* maintained and transmitted in sylvatic cycles in Argentina.

Molecular Epidemiology research conducted in Africa and found an intriguing and more difficult scenario than previously anticipated. Initially, *E. granulosus* was thought to be the most widespread species, with *E. ortleppi* appearing to have a considerably more localized range. Although infections in lions have been recognized for many years, adult worms from lions were only recently characterized using genetic methods and proved to be a different species, *E. felidis* (Hüttner et al., 1937). Interestingly, recent ME investigations have shown that *E. felidis* is not limited to the sylvatic cycle and has been found in domestic dogs in Kenya (Mulinge et al., 2018). ME research has also shown infections with *E. granulosus*, *E. intermedius,* and *E. ortleppi*. Interestingly, recent ME investigations have shown that *E. felidis* is not limited to the sylvatic cycle and has been found in domestic dogs in Kenya (Mulinge et al., 2018). ME investigations have also shown that dogs develop infections with *E. granulosus, E. intermedius,* and *E. ortleppi,* as well as occasional mixed infections (Mulinge et al., 2018).

Another test that can be performed is Loop-mediated isothermal amplification (LAMP) which is a single tube technique used to amplify DNA. It's an alternative for detecting echinococcosis disease done at a constant temperature and does not require a thermal cycler compared to PCR. In addition, LAMP is much simpler with a minimal cost and can be used in the field for diagnosis or at the point of health care by physicians. It has been observed that LAMP is less sensitive than PCR to inhibitors in composite samples such as blood (Eckert et al., 2001). The other diagnostic tool is the probe Southern Hybridization test for detecting specific DNA sequences in DNA samples (Southern, 1975). Besides eggs, the possibility of hydatid cyst fluid and urine as a sample for molecular diagnosis of CE has been tried (Jenkins et al., 2006).

Epidemiological studies were instrumental in the identification and description of transmission cycles involving more than one *Echinococcus* species. While morphological studies can be used to distinguish adult worms from different species, this approach is often not practical given the difficulty of recovery of adult specimens and related risks to public health. Molecular tools also make it possible to recognize species from eggs in the faeces. For instance, during an *E. coli* faecal survey in the Southern Italian Alps, *E. granulosus* was found in wolves and dogs (Thompson, 2020). Massolo (2018), reported that *Multilocularis* eggs were found in four faecal samples from at least two shepherd dogs and five wolf faecal samples, which was unexpected. The current taxonomy of *Echinococcus* is presented in Table 2.4 and Table 2.5.

Table 2.4: Current taxonomy of *Echinococcus.*

Species	**Strain/Genotype**	**Known Intermediate Hosts**	**Known Definitive Hosts**	**Infectivity to Humans**	**Disease**
Echinococcus granulosus	Sheep/G1	Sheep (cattle, pigs, camels, goats, macropods)	Dog, fox, dingo, jackal and hyena	Yes	CE
	Tasmanian sheep/G2	Sheep (cattle?)	Dog, fox	Yes	CE
	Buffalo/G3	Buffalo (cattle?)	Dog, fox?	Yes	CE
Echinococcus equines	Horse/G4	Horses and other equines	Dog	Probably not	CE?
Echinococcus ortleppi	Cattle/G5	Cattle	Dog	Yes	CE
Echinococcus Canadensis	Cervids/G8,G10	Cervids	Wolves, dog	Yes	CE

Adapted from Thompson, 2017 and Romig et al., 2016. CE = Cystic Echinococcosis, AE = Alveolar Echinococcosis, PE = Polysystic Echinococcosis.? Not known.

Table 2.5: Current taxonomy of *Echinococcus.*

Species	**Known Intermediate Hosts**	**Known Definitive Hosts**	**Infectivity to Humans**	**Disease**
Echinococcus intermedius	Camels, pigs, sheep	Dog	Yes	CE
Echinococcus felidis	Warthog, (zebra, wildebeest, bush pig, buffalo, various antelope, giraffe Hippopotamus?)	Lion	?	-
Echinococcus multilocularis	Some isolate variation	Rodents, domestic and wild pig, dog, monkey, (horse?) Fox, dog, cat, wolf, racoon-dog, coyote	Yes	AE
Echinococcus shiquicus	?	Pika and rodents Tibetan fox and?	?	?
Echinococcus vogeli	None reported	Rodents Bush dog	Yes	PE
Echinococcus oligarthra	None reported	Rodents Wild felids	Yes	PE

Adapted from Thompson, 2017 and Romig et al., 2016. CE = Cystic Echinococcosis, AE = Alveolar Echinococcosis, PE = Polysystic Echinococcosis, ? = Not known.

2.10. Diagnosis of Echinococcosis infections

The diagnosis of echinococcosis can be divided into two parts, parasitological, and immunological:

2.10.1. Parasitological methods

Parasitological diagnosis is based on the direct identification of tapeworms. Purgation with arecoline salts and inspection of the small intestine during necropsy are the two most used parasitological procedures (Eckert, 2003). Classical parasitological techniques include microscopic examination of intestinal scrapings, counting processes, and faecal sedimentation and flotation (Eckert, 2003). Because faeces are the major source of infective taeniid eggs, data derived from faecal samples may serve as a proxy for characterizing parasite-induced environmental contamination. Nonetheless, traditional routine diagnostic methods have a limited sensitivity for detecting taeniid eggs in faeces and it does not differentiate from other taeniid eggs. The enrichment of taeniid eggs and subsequent analysis by Polymerase Chain Reaction (PCR) using the flotation and sieving method described by Mathis et al. (1996) has overcome the low sensitivity observed in conventional routine diagnosis, opening up new diagnostic strategies (Conraths and Deplazes, 2015).

2.10.2. Immunological methods

Immunological diagnosis of echinococcosis involves usage of assays designed for the detection of antibodies, antigens, circulating immune complexes, and delayed hypersensitivity and lymph proliferative assays. Some of these methods are the; Casonis test which involves skin/intradermal injection of irradiated cystic fluid. When antibodies are present, the burning swelling will be formed on the skin. There are also tests such as Indirect Hemagglutination (IHA) The IHA is a simple serological test that can be used to detect antibodies and is currently the most common test used worldwide to quantify the antibody response. The IHA is used as a routine clinical test in veterinary hospitals because of its level of sensitivity and ease of use (Gourlay, 1965). The test is based on the property of *T. gondii* immunoglobulins to produce agglutination in the presence of cytoplasmic antigen-sensitized red blood cells and the parasite's membrane. It is considered a reliable method for the determination of specific immunoglobulins with values of sensitivity of 89% to 92%, specificity 60% to 100% and efficiency 94.80% (Hossain et al., 2023).

Another diagnostic method is Counter-Current Immunoelectrophoresis (CIEP), a technique that involves the electrophoresis of a specific antigen mixture within an agarose gel. This process facilitates the separation of distinct antigens along the gel slide, followed by the lateral diffusion of antibodies into the gel, enabling the detection of antigen-antibody interactions (Refaie and Dulake, 1975). Enzyme-Linked Immuno sorbent Assay (ELISA) is another test of an immunological assay commonly used to measure antibodies, antigens, proteins and glycoproteins in biological samples, some examples include diagnosis of HIV infection, pregnancy tests, and measurement of cytokines or soluble receptors in cell supernatant or serum (Engvall, 1972). Enzyme Immuno electro transfer Blot (EITB) is a synergistic union of three independently powerful techniques. Complex mixtures of biological molecules are first separated by sodium dodecyl sulfate Polyacrylamide gel electrophoresis (SDS-PAGE), and then electrophoretically transferred to a solid matrix. Molecules thus “blotted” are visualized directly or indirectly with enzyme-labeled antibodies by enzyme-linked immunosorbent assays (ELISAs) or with isotope-labeled antibodies by radio immune assays (RIAs) and radiography. Variations within the 3 basic techniques are numerous; however, the end results are generally good in terms of resolution, sensitivity, fidelity, and specificity (Eckert et al., 2001).

2.10.3. Molecular diagnosis

Molecular diagnosis of *Echinococcus* species is essential for species identification, epidemiological studies, and clinical diagnosis. Several molecular techniques have been developed to detect and differentiate *Echinococcus* species.

I. Polymerase Chain Reaction (PCR)

The PCR-based methods target specific genetic markers for *Echinococcus* species identification. Studies have demonstrated the use of this method in species identification (Zhang et al., 2020). Conventional PCR amplifies genes such as *cox1* (cytochrome c oxidase subunit 1), *nad1* (NADH dehydrogenase subunit 1), and *ITS1* (internal transcribed spacer 1) (Zhang et al., 2020). Nested PCR increases sensitivity by using two sets of primers in a two-step reaction (M'Rad et al., 2021). Multiplex PCR allows simultaneous detection of multiple *Echinococcus* species in a single reaction (Moghaddas et al., 2019).

II. Real-Time PCR (qPCR)

The qPCR provides a more sensitive and quantitative approach than conventional PCR by using fluorescent probes such as TaqMan or SYBR Green (Wassermann, Mackenstedt & Romig, 2014). Common targets include mitochondrial genes (*cox1*, *nad1*) and ribosomal DNA (rDNA), which help distinguish *Echinococcus* species with high specificity (Ahmad et al., 2022). This approach has been validated for distinguishing *Echinococcus* species in clinical samples (Wasserman, Mackenstedt and Roming, 2014; Ahmad et al., 2022).

III. *Loop-Mediated Isothermal Amplification (LAMP)*

The LAMP is a rapid and highly sensitive method that does not require a thermal cycler, making it suitable for field applications (Salant, Abbasi and Spira, 2012). It amplifies *Echinococcus* DNA within a short time and is particularly useful in resource-limited settings (Siles-Lucas et al., 2017).

IV. Restriction Fragment Length Polymorphism (RFLP)

The PCR-RFLP amplifies a specific DNA fragment and digests it with restriction enzymes, generating unique fragment patterns for different species (Šnábel et al., 2016). This method is effective for genotyping *Echinococcus* species.

V. Sequencing (Sanger/Next-Generation Sequencing, NGS)

Sequencing provides high-precision species identification and genotyping. The mitochondrial genes *cox1* and *nad1* are frequently used for species determination (Alves et al., 2021). Whole-genome sequencing (WGS) offers deeper insights into the genetic diversity and evolution of *Echinococcus* species (Huttner et al., 2018).

VI. Hybridization-Based Methods

The DNA microarray technology detects multiple *Echinococcus* species simultaneously using species-specific probes (Knapp et al., 2016). Fluorescence in situ hybridization (FISH) is another technique that allows the identification of *Echinococcus* DNA directly in tissue samples (Gottstein, Eckert and Vuitton, 2017).

VII. Metagenomic Approaches

Metagenomic sequencing enables the detection of *Echinococcus* DNA in clinical and environmental samples, such as blood, faeces, and water, by analysing microbial communities (Tamarozzi et al., 2021). This method is particularly useful for non-invasive diagnosis and surveillance as demonstrated by Tamarozzi et al. (2021).

2.11. Treatment of echinococcosis

2.11.1. Treatment of echinococcosis in humans

In the past, surgery was the primary and often the only treatment option for cystic echinococcal cysts (CDC, 2012). However, advancements in medical approaches have introduced alternatives such as chemotherapy, cyst puncture, and percutaneous aspiration, injection of chemicals, and re-aspiration (PAIR), which have proven effective in treating cystic echinococcosis. Despite these alternatives, surgery remains the most definitive treatment for complete cyst removal and achieving a full cure. In some cases, cysts may be inactive and asymptomatic, often resolving without any intervention. For active infections, medical treatment involving deworming medications is a cost-effective option and is particularly useful when surgical removal is risky due to the cyst's anatomical location (CDC, 2012). Additionally, medical treatment is often combined with surgery as preoperative chemotherapy to minimize the risk of cyst recurrence and reduce the viability of scolices (CDC, 2012).

2.11.2. Treatment of echinococcosis in animals

Several antihelminthic medications can be used to treat definitive hosts. Praziquantel, the most often utilized drug, works against both immature and adult tapeworms. There is limited expertise treating animal intermediate hosts, particularly those infected with *E. multilocularis* or *E. vogeli*. Cysts that can be entirely removed are frequently treated with surgery. Long-term treatment with benzimidazoles (e.g., albendazole or mebendazole) may suppress some cysts and/or extend the animal's life in non-surgical situations. Drugs are also utilized as a complement to surgery, particularly for *E. multilocularis* (CDC, 2019).

2.12. Prevention and control of echinococcosis

2.12.1. Prevention and control of echinococcosis in animals

The most effective strategy for preventing and controlling echinococcosis involves interrupting the life cycle of the parasite. According to the Centres for Disease Control and Prevention (CDC) (2012), surveillance of cystic echinococcosis is essential to prevent parasite transmission. Key preventive measures include restricting areas where dogs can feed to prevent them from consuming meat infected with cysts; preventing dogs from accessing infected sheep carcasses; implementing population control measures for stray dogs; prohibiting home slaughter of sheep and other livestock to reduce exposure risks; ensuring that dogs do not consume food or water contaminated with faecal matter. Additionally, keeping wild animals as pets is strongly discouraged due to the increased risk of parasite transmission (CDC, 2012).

Vaccination of animals can help prevent the development of the larval stages of Echinococcus granulosus (Eckert et al., 2001; Bech-Nielsen, 2002). In several European Union (EU) countries, such as Denmark and Belgium, E. granulosus infection in animals is a notifiable disease, meaning cases must be reported to health authorities (European Commission, 2020). Additionally, some countries have implemented targeted control programs. For example, Greece has enforced a dog control program since 1985, which includes mandatory dog registration and stray dog collection; preventive treatment of all owned dogs with anthelmintics; regular testing and treatment of sheep-herding dogs with praziquantel (Lightowlers et al., 2000; Zhang et al., 2003). These measures have contributed to reducing the transmission of echinococcosis in endemic regions.

Echinococcus vaccine is therefore ideal to prevent the development of oncosphere to hydatid cysts in sheep and other animals and thus to prevent the development in adult gravid tapeworms in dogs that are the ultimate hosts (Lightowlers et al., 2000; Zhang et al., 2003). The Marshall Lightowler and David Heath in Australia and New Zealand groups developed a defined recombinant vaccine for ovine CE (called EG95) in 1996 (Lightowlers et al., 2000). Lightowler et al. (1996) reported that field trials conducted over the following 8 to 10 years demonstrated over 95% protection in sheep for 12 months, achieved through colostral transfer of immunity following two injections, in countries such as Australia, New Zealand, Argentina, Italy, and China. While the EG95 ovine hydatidosis vaccine has become a reality and now requires innovative delivery strategies, there is currently no effective canine *echinococcus* vaccine available. This is not because there is a lack of application potential, because a dog's vaccine would have an enormous benefit in further reducing the infective period (now more than 10 years) needed to prevent *E. granulosus* transmission to people and their animals (Herd, 1977). In China studies on recombinant protein vaccinated dogs were carried out on mature adult *E. granulosus* worms, which resulted in substantial worm growth repression and egg production suppression (Zhang et al., 2006). CE is an eradicable disease, but many factors contribute to maintaining the transmission cycle, including comportmental and cultural factors that are often difficult to regulate or alter (Dakkak, 2010).

2.12.2. Prevention and control of echinococcosis in humans

To minimize the risk of Echinococcus transmission, contact with wild animals such as foxes, coyotes, and stray dogs should be avoided. Proper hygiene practices, including thorough handwashing with soap and clean water after handling domestic pets and before eating, are essential preventive measures (Hegglin et al., 2008). Public health education should emphasize these practices, particularly among children, to instil early awareness of infection prevention.

Veterinary interventions, including meat inspection to identify hydatid cysts and the enforcement of proper slaughter hygiene, play a crucial role in reducing transmission (Hegglin et al., 2008). Additionally, community-based public health initiatives should be integrated into primary healthcare systems, particularly in farming and rural populations where zoonotic risks are elevated.

Effective risk communication strategies must consider not only scientific evidence on transmission factors but also public perceptions, which may vary across regions and demographic groups (Hegglin et al., 2008). Tailored educational programs are necessary to address these differences and promote sustainable behavioural changes.

Public perceptions of zoonoses are critical to the successful implementation of prevention, control, and management measures. The development of public information strategies on risk and zoonosis prevention must be based not only on research results on risk factors but also on public analysis of the problem's perception. This can vary depending on the region and population (Hegglin et al., 2008). Most of sub-Saharan Africa lacks data on hydatidosis control. This could be explained by governments' poor priority for arid pastoral areas where the disease is prevalent (Mbaya et al., 2014). Turkana District of Kenya is one of the few areas with extensive data, and the African Medical Research Foundation has been running a hydatitis control program since 1983 (Magambo et al., 2006). Hydatid control in this region includes the components of human treatment (operative, puncture, aspiration, injection, and re-aspiration, PAIR and chemotherapy) and community education.

CHAPTER THREE: MATERIALS AND METHODS

3.1. Study Design

This cross-sectional study was conducted to detect the presence of *Echinococcus* species in the wildlife-livestock interface areas in South Luangwa National Park of Mfuwe district which is in the Eastern Province of Zambia between June 2019 and January 2021.

3.2. Study Site

This study was conducted in South Luangwa National Park, located in the Mfuwe District of Eastern Zambia, approximately 591 km northeast of Lusaka along the Great East Road. Established in 1972, the park spans 9,050 km² and hosts over 60 wildlife species, including significant populations of elephants, lions, and endemic Thornicroft's giraffes (Department of National Parks and Wildlife [DNPW], 2020).

South Luangwa National Park exhibits a tropical savanna climate (Köppen: Aw), characterized by distinct wet and dry seasons. During the dry season (April to October), average daytime temperatures range between 25-35°C, peaking in October, while nighttime temperatures drop to a chilly 10-15°C, particularly during the winter months from May to August. Precipitation during this period is minimal, with less than 10 mm of rainfall per month. The wet season (November to March) brings warmer conditions with average temperatures of 28-32°C accompanied by high humidity and substantial seasonal precipitation ranging from 800-1,000 mm, with December through February being the rainiest months. This seasonal transformation turns the arid landscape into lush greenery, attracting migratory birds and stimulating breeding activities among wildlife populations (Zambia Meteorological Department, 2021).

South Luangwa National Park was selected for the collection of wildlife carnivore/feline faecal samples. The selection of the park was based on the availability of wild carnivores and cats and the availability of intermediate hosts (wildlife ruminants, equines, and warthogs).

3.3. Sample Size

Sample size was calculated using the formula by Bech-Nielsen (2002) presented as:

$$n = Z^2PQ/L^2$$

Where:

- n = total sample size,
- Z= Z score (1.96 at a 95% confidence level),
- P = estimated proportion of prevalence (set at 50% since the actual prevalence was unknown),
- $Q = 1-P$,
- L= margin of error (set at 0.05).

Therefore, the maximum sample size was n = 1.96 (2) x 0.5 x 0.5/0.052 = 384. Further, using proportions, the number of stool samples collected for each kind was estimated as:

Proportion of wild carnivores = (number of estimated stool samples for wild carnivores/total sample size) × 100

Proportion of domestic carnivores = (number of estimated stool samples for domestic carnivores/total sample size) × 100

Thus, based on the total sample size collected of 965, proportion of wild carnivores was 65.39% while domestic carnivores were 34.61%.

3.4. Inclusion Criteria

Stool sample of carnivore animals in the park and domestic dogs in the game management area.

3.5. Exclusion Criteria

- Faecal samples >72 hours old (to avoid DNA degradation in tropical climates).
- Unidentified scat (cannot confirm species origin).
- Samples mixed with urine or excessive soil, which may inhibit lab analysis.
- Domestic dogs with recorded anthelmintic treatment (praziquantel) within 4 weeks prior to sampling (to prevent false-negative results).

3.6. Stool sample collection

The wild carnivore path and their specific range (territory) were followed with the help of the wildlife officers. Fresh faecal samples of wildlife carnivores were collected from environment in the park and GPS coordinates were taken from all collection sites. Faecal samples were mainly identified according to field signs as described by (Verster, 1965; Laís et al., 2019), and as guided by the wildlife officers. Collected faecal samples were preserved in 70% ethanol and were transported to the Parasitology laboratory at the School of Veterinary Medicine (UNZA) for taeniid egg examination and isolation.

Dog faecal samples were collected from households with dogs in communities in the game management area. Dog owners restrained the dogs by using muzzle on each dog. Veterinary assistants helped to collect the faecal sample direct from the dog's rectum and the sample was preserved in 70% ethanol. Thereafter, samples were transported to the Parasitology laboratory at the School of Veterinary Medicine (UNZA) for examination of taeniid eggs.

3.8. Microscopic isolation of taeniid eggs

After sieving out of the ethanol, 3g of dog and wild carnivore faecal materials were analysed by a Zinc Chloride ($ZnCl_2$) flotation (specific gravity [SG] = 1.25)–sieving technique (Mathis et al., 1996) and microscopically examined for the presence of taeniid egg. Faecal samples drained off ethanol were transferred to 50mls falcon tubes and rinsed with 10mls of distilled water. Four parts of $ZnCl_2$ (SG= 1.25) were mixed with one part of the faecal pellet, and after floatation, the top layer containing floating eggs was filtered through two sequential sieves of 50µm and 22µm (Franz Eckert GmbH, Germany) using distilled water. In the second sieve (22µm) retained eggs were washed off with distilled water via a funnel into a 15ml falcon tube. The contents in 15ml tubes were then centrifuged and the supernatant decanted to retain the pellet (sediment) containing eggs and stored in 2ml micro centrifuge tubes containing 70% ethanol. PCR was used on selected eggs from positive samples to identify taeniid eggs to species level.

3.9. DNA extraction from taeniid eggs

The contents from the 2ml micro centrifuge tubes were poured onto a petri-dish. Individual taeniid eggs were picked using a micro-pipette under the compound microscope (objective lens ×4 or x10) and picked eggs between minimum of 13 to maximum of 60 eggs from each positive sample with a mean of 22. Samples were transferred into 0.2ml thin wall PCR tubes containing 10µl of 0.02M NaOH. The taeniid eggs were lysed at 99 °C for 10 min and lysate used as DNA template in PCR (Nakao et al., 2003).

3.10. Polymerase chain reaction (PCR)

A nested PCR targeting the nad1 gene was performed. The first set of primers (Table 3.1) (Hüttner et al., 2008) were used in the primary PCR reaction mixture in a 25µl reaction consisting of 1×DreamTaq Green Buffer (20mM Tris-HCl (pH 8.0), 1mM DTT, 0.1mM EDTA, 100mM KCl, 0.5% (v/v) Nonidet P40, 0.5% (v/v) Tween 20) (Thermo Scientific, GENETEX Corporation, Tokyo, Japan), 0.2mM dNTPs, 0.25µM of each forward and reverse primer, 2mM MgCl2 and 0.625 U of Dream Taq Green DNA Polymerase (Thermo Scientific) and 2µl of the taeniid lysate as DNA template. The secondary PCR was performed under similar conditions and using primers set as shown in Table 3.1 (Hüttner et al., 2008). The primer used in the amplification of the targeted parasite and sequencing of PCR products are presented in Table 3.1**.**

Table 3.1: Primer used in the amplification of the targeted parasite and sequencing of PCR products.

Pathogen	**Primer sequences (5' - 3')**	**Number of cycles**	**References**
Echinococcus spp Primary	TGT TTT TGA GAT CAG TTC GGT GTG ATA TCA AAG TAA CCT GCT ATG CAG	40	(Hüttner et al., 2008)
Echinococcus spp Secondary	CAG TTC GGT GTG CTT TTG GGT CTG * TCT TGA AGT TAA CAG CAT CAC GAT * Amplicon size -545 – 552bp	40	(Hüttner et al., 2008).

*Primers used for sequencing

In both PCRs the following thermal cycler conditions were used 5min of initial denaturation at 94°C, 40 cycles of 94°C for 30s, 55°C for 30s and 72°C for 1min, a final extension at 72°C for 5min.

3.10.1 Agarose GelRed Electrophoresis

The PCR products were separated by electrophoresis on a 2% agarose gel stained with GelRed™ (Biotium, USA) and visualized under UV illumination using a Bio-Rad Gel Doc™ XR+ imaging system (USA). For accurate size determination of amplified DNA fragments, a 100 bp DNA ladder (Thermo Scientific™ GeneRuler 100 bp Plus, USA) was included as a molecular weight marker, providing reference bands ranging from 100 to 1000 base pairs to facilitate precise estimation of PCR product sizes. The gel was run at 100V for approximately 45 minutes in 1× TAE buffer until adequate separation of bands was achieved, after which the amplified fragments were analyzed by comparison with the reference ladder.

3.10.2. Restriction Fragment Length Polymorphism

The restriction digestion was performed in a 20 µl reaction volume containing 10 µl of secondary PCR product, 0.5 µl (5 units) of HphI restriction enzyme (Thermo Scientific), 2 µl of 10× reaction buffer (final concentration 1×), and 7.5 µl of nuclease-free water. The digestion was carried out overnight at 37°C, after which the digested products along with controls (undigested PCR product as negative control and known *Echinococcus spp.* samples as positive controls) were separated on a 3% agarose gel stained with GelRed. The gel electrophoresis was performed in 1× TAE buffer at 100V for 90 minutes, and the resulting banding patterns were visualized using a Bio-Rad Gel Doc XR+ system. *Echinococcus granulosus sensu lato* was identified based on characteristic restriction patterns according to Mulinge et al. (2018), while samples that could not be conclusively identified by PCR-RFLP were subsequently sequenced for definitive species determination.

3.10.3. DNA Purification and Sequencing of *PCR* product

The VP7 PCR products were purified using the Wizard® SV Gel and PCR Clean-Up System (Promega, Madison, WI, USA) following the manufacturer's protocol, which involved binding DNA to a silica membrane in the presence of a high-salt buffer, washing with 70% ethanol, and eluting the purified DNA in nuclease-free water. The purified DNA was then subjected to Sanger sequencing using the BigDye™ Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA) in a 10 µL reaction volume containing 1 µL of BigDye Terminator premix, 1.5 µL of sequencing buffer (5×), 1 µL (10 µM) of primer, and 20–50 ng of purified PCR product. The cycling conditions consisted of an initial denaturation at 96°C for 1 min, followed by 25 cycles of 96°C for 10 sec, 50°C for 5 sec, and 60°C for 4 min. The sequencing products were purified using ethanol/EDTA precipitation to remove unincorporated dye terminators, resuspended in Hi-Di™ formamide (Applied Biosystems), and electrophoresed on a 3500 Genetic Analyzer (Applied Biosystems) under standard run conditions (injection voltage: 1.2 kV, run voltage: 8.5 kV, run time: 20 min). The raw sequence data were assembled, base-called, and edited using GENETYX version 12 (GENETYX Corporation, Tokyo, Japan) to generate consensus sequences for downstream analysis.

3.11. Data analysis

DNA sequences were viewed and manually edited using GENtle v. 1.9.4 (http://gentle.magnusmanske.de). The sequences were compared with those on NCBI database using nucleotide the BLAST algorithm (http://www.ncbi.nlm.nih.gov/BLAST/) to determine the species.

3.12. Phylogenetic Analysis

Echinococcus species identification of amplified *nad1* gene was done by comparison with available sequences in the NCBI GenBank database using the BLAST nucleotide algorithm (http://www.ncbi.nlm.nih.gov/Blast.cgi). Multiple sequence analysis was performed using MEGA software using ClustalW aligner. The phylogenetic analysis was constructed according to the neighbour-joining method using MEGA. Finally, we used the mitochondrial gene of *taenia* as an out-group to construct inference tree of the *E. felidis* and *E. granulosus* as sister cluster to analyze the phylogenetic relationship between *E. felidis* isolated in this study and those reported worldwide.

3.13. Ethical approval

This study was approved by the UNZA biomedical research ethics committee and permission was obtained from ZAWA Mfuwe and from owners of the dogs.

CHAPTER FOUR: RESULTS

4.1. Examination of stool sample

Five (5) of the 297 lion samples that were tested were found to have Taeniid eggs (1.7%). Further, taeniid eggs were discovered in three out of 156 samples of hyena that were analysed (1.9%). For the civet samples, there was no microscopic detection of taeniid eggs (0/44=0%). In contrast to civet stool samples, 1 out of 134 samples from leopards were found to contain taeniid eggs (0.74%). There were no egg-containing dog samples among the 334 investigated dogs. The proportion of samples collected from domestic and wild carnivores positive for taeniid eggs on microscopy are presented in Table 4.1**.**

Table 4.1: Distribution of positive taeniid eggs in samples collected from domestic and wild carnivores after microscopic examination.

# faecal samples	Animal species	No. of faecal samples positive on microscopy	Total % (n)
334	Dog	0	0
297	Lion	5	1.7
156	Hyena	3	1.9
134	Leopard	1	0.74
44	Civet	0	0

4.2. Parasite identification

Positive PCR amplification of the nad-1 gene was picked from 179 eggs extracted from seven of the eight microscopically taeniid-positive samples, with each sample having between 1 and 60 retrieved eggs. The PCR amplification of the nad-1 gene generated DNA fragments ranging from 210 bp to approximately 550 bp when using reference DNA material from confirmed *Echinococcus felidis* samples. This size range corresponds to the expected amplicon lengths for this target gene across the analysed *E. felidis* specimens. No evidence of taeniid genera other than *Echinococcus* was detected in any amplicons. Three stool samples produced the nad-1 RFLP-PCR results shown in Table 4.2**.** These eggs' banding patterns (n = 17) were typical for *E. felidis* or *Taenia spp.* and therefore needed sequencing to confirm the species. There were no abnormal patterns that might be attributed to other *E. granulosus spp*. Using 17 banding patterns, secondary PCR products were sequenced. All the sequences were

99-100% identical to *E. felidis* sequence accession number EF558357 (Hüttner et al., 2008). Each sequence enabled a clear identification of the *Ehinococcus* species.

Table 4.2: Individual taeniid eggs identified.

Host	Area	No. of taeniid eggs isolated	PCR % positive (n)	Species identified
Lion	South Luangwa National Park	118	14.4 (17/118)	*E. filidis*
Hyena	South Luangwa National Park	41	0 (0/41) *	-
Leopard	South Luangwa National Park	20	0 (0/20) *	-

(*) = need for further serological test (ELISA)

The PCR analysis of the nad-1 gene revealed all DNA fragments 200-500 bp band derived from different samples as shown in Figure 4.1.

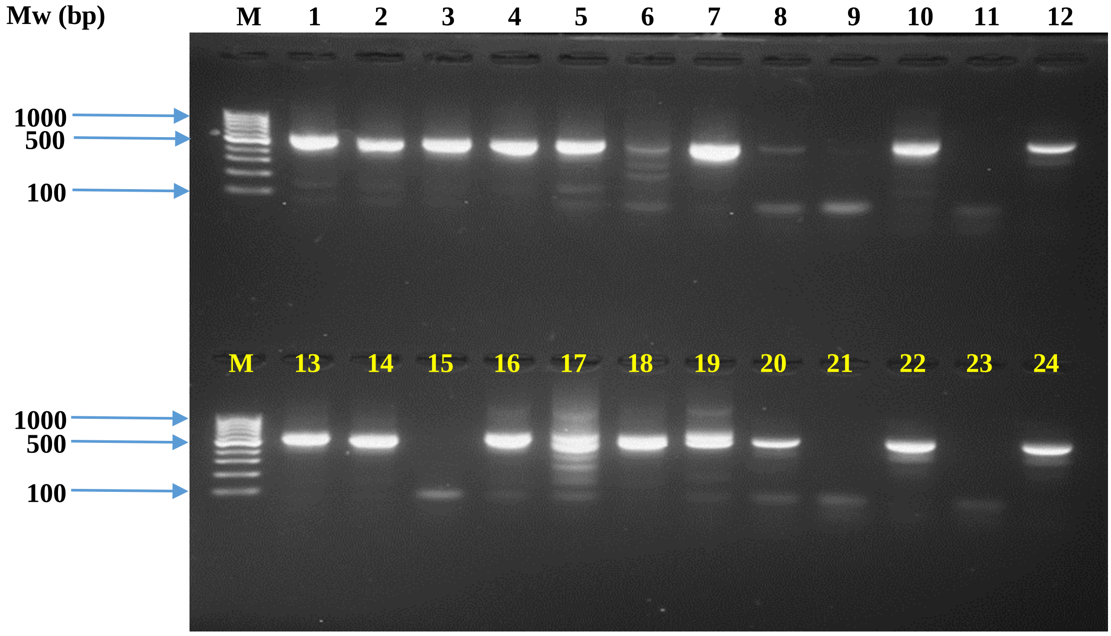

Figure 4.1: Agarose gel (1.0%) electrophoretogram with 100-bp DNA ladder. PCR analysis of the *nad1* gene revealed all DNA fragments band derived from different samples.

The PCR products were separated on a 2% agarose gel and stained in Gelred. The lanes M: 100 bp step DNA ladder; lanes 1-10, 13-14, 16-20 and 22 were the PCR positive taeniid eggs of *E. felidis* while lanes 15 and 21 were PCR negative taeniid eggs. The lanes 11 and 23 were negative controls and lanes12 and 24 were the positive controls of *E. felidis*.

4.3. Phylogenetic tree

In the phylogenetic tree of *Echinococcus* which was constructed from the mitochondrial genes, *E. felidis* is positioned as a sister taxon of *E. granulosus* sensu stricto. The obtained sequences (LC727787.1 *Echinococcus felidis* lion Zambia) of the mitochondrial nad-1 gene showed 98% identity to previously published sequence of *E felidis* (accession number MG 271924 (Mulinge et al., 2018) in Kenya. All DNA fragments (<200bp) that can be amplified from adult worms showed 98% similarity with *E. felidis* sequences from Kenyan dog's accession number MG271925 (Mulinge et al., 2018) and lions in Uganda EF558357 (Hüttner et al, 2008). In the phylogenetic tree of *Echinococcus* which was constructed from the mitochondrial genes, *E. felidis* is positioned as closely related to a taxon of *E. granulosus* sensu stricto (accession number MN886282 (Alvi et al., 2020). Overall, the results grouped 5 out of the 9 samples in a clade with genotypes (accession numbers KY794644, MG271924, MG271923, EF367326, MG271925 and EF558357), with bootstrap support of 98% of Sample Zambia.

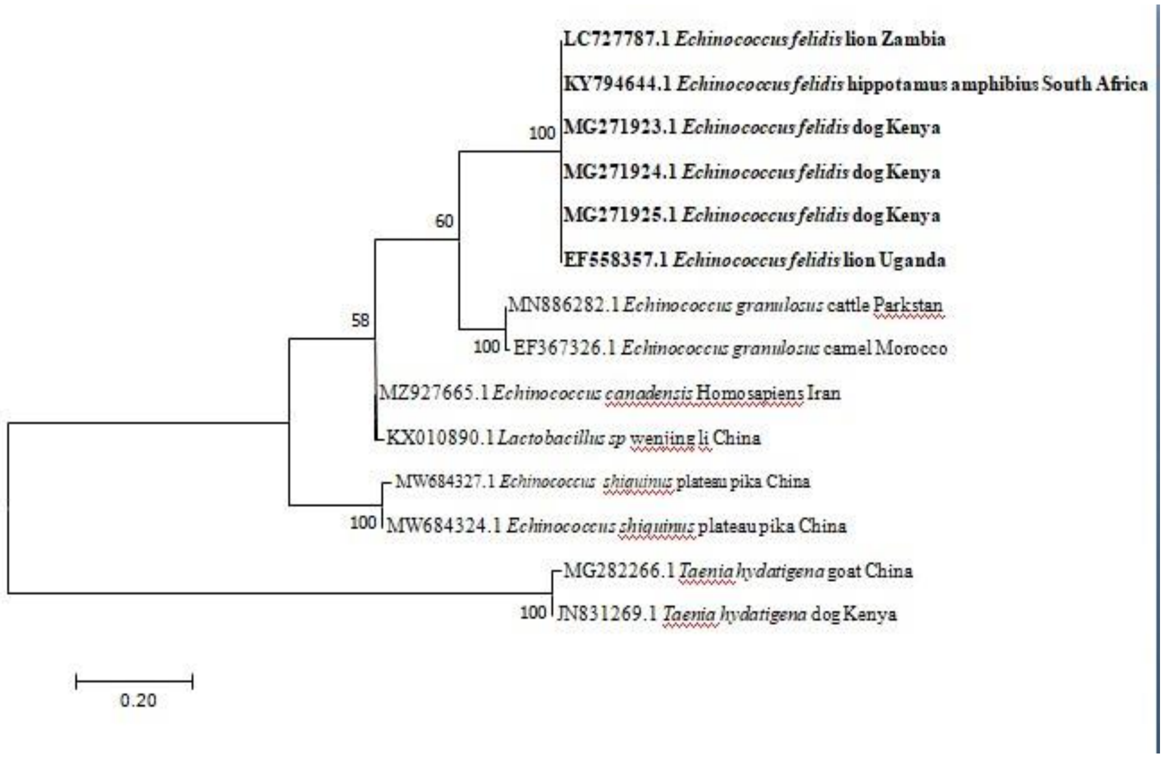

Figure 4.2: Phylogenetic tree of *Echinococcus spp*. and *Taenia spp*. inferred from nuclear-coding genes. *E. Felidis* is positioned as sister taxon of *E. Granulosus.*

CHAPTER FIVE: DISCUSSION

5.1. Occurrence of *Echinococcus. spp.*

This study aimed at investigating whether the wild carnivores of the South Luangwa national park could be harbouring, the tapeworm *Echinococcus*. It also investigated the possibility of dogs living in wildlife-livestock interface areas, also called Game Management Areas (GMAs) in Mfuwe area of Mambwe district being infected with *Echinococcus*. Regarding dog sampling, there were no egg-containing dog samples among those investigated dogs. Given that taeniid egg detection in carnivore faeces has a very low diagnostic sensitivity (uneven shedding of eggs, no identification of pre-existing infections), and since none of the eggs were detected in dogs our investigation, may not reflect a zero prevalence. However, faecal examination is currently the only method that can be used to characterize the molecular makeup of the *Echinococcus spp* in living dogs. Failure to find any taeniid positive dog faecal sample was not surprising because our sampling of dogs happened just a few weeks after dogs were dewormed in the communities. However, Banda et al. (2020) in their study in Western province, found that 3.5% (10/289) of dogs were positive for taeniid eggs of which two were identified to be those of *T. hydatigena* and two as *E. canadensis*. In another study in Kenya, 11% (178/1621) of dogs were found to have taeniid eggs with 4.4% being identified as those of *Echinococcus spp* (Mulinge et al., 2018).

Only lions' stool among the wild canid animals tested were positive for *Echinococcus spp* which was later identified to be *E. felidis.* Given that *E. felidis* is present in lions in South Langwa National Parks, the absence of this species in dogs in the area is somewhat surprising given that most dogs in the study area are utilized for hunting.

Our research shows that *E. felidis* is highly prevalent in lions across Zambia as has been observed by Banda, (unpublished data) that *E. felidis* is also highly prevalent in lions of the Kafue national park. Lions have been known to host *E. felidis* which appears to be equally ubiquitous but less frequently in wild animals. The identification of 17 eggs of *E. felidis* supports the theory that lions serve as hosts for this parasite. According to Romig et al. (2011), *E. felidis* was first identified in South African lions. It has also been identified in dogs and spotted hyena thereby showing the increase in its host range (Romig et al., 2011; Mulinge et al., 2018; Huttner et al., 2009). For a while, it was used interchangeably with *E. granulosus*, and the term "lion strain" was frequently used (Romig et al., 2011; Hüttner et al., 2009). Based on preserved adult worms from South Africa and eggs from recent faecal samples from lions in Uganda's Queen Elizabeth National Park, it was recently molecularly identified and reinstated as a separate species (Hüttner et al., 2009). In southern, central, and eastern Africa, there has been several reports of lions contracting *Echinococcus* infections. However, the taxa responsible for these infections have never been identified (Mathis et al., 1996).

The *E. felidis* identified in this study showed similarity to those identified earlier in Africa indicating expansion of regional distribution. This research confirms that lions are infected with *E. felidis* which is also known to be the definitive host. These results are compatible with findings of Kagendo et al. (2014) in a study conducted in Kenya, where they described the infection of *E. felidis* and *E. granulosus* (n = 27 and n = 20, respectively). Although we were unable to identify any hyenas with *E. felidis* infection, *E. felidis* is frequently found in Kenya in both lions and hyenas. Since hyenas are frequently coprophagous and the eggs may have come from ingested lion faeces, other reports indicate that the previous characterization of nine *E. felidis* eggs in one of the five hyena faecal samples from Queen Elizabeth National Park in Uganda was not deemed sufficient evidence for a host role of that species (Kim et al., 1998). Additionally, this finding conflicts with a study by Wasserman et al. (2015) that found 67% (4/6) of the lion eggs that were assessed to be positive for *E. equinus*. Nevertheless, according to Hüttner et al. (2009) and Kagendo et al. (2014), lions and spotted hyenas from national parks and reserves in Uganda and Kenya were regularly found to have *E. felidis* and *E. granulosus s.s.* but not *E. equinus* infections. However, lions have long been regarded as ideal hosts for *E. felidis,* and for a while, its adaptability to serve as a reliable host was used to support the particular identity of *E. felidis* (as species, subspecies, or strain). Our findings are consistent with records of gravid infections with *E. granulosus s.s.* in Kenya demonstrating that lions are capable hosts for *Echinococcus species*.

We found a 98% similarity between the sequences of the mitochondrial nad1 gene derived from *E. felidis* (accession number MG 271924) with those previously reported sequences from Kenya (Mulinge et al., 2018). An analysis of all amplified DNA segments (less than 200 bp) from eggs showed a 98% similarity to *E. felidis* sequences from Kenyan dogs with accession number MG271925 (Mulinge et al., 2018) and Ugandan lions with EF558357 (Hüttner et al, 2008). The *E. felidis* is positioned as being closely related to a taxon of sensu stricto (accession number MN886282) in the phylogenetic tree of *Echinococcus* that was created using the mitochondrial DNA (Alvi et al., 2020). Nuclear DNA (Knapp et al., 2007) and mtDNA (Nakao et al., 2007) phylogenetic investigations demonstrated that *E. felidis* and *E. granulosus s.s.* are sister species with identical branch lengths in a particular clade and bootstrapping values of 99%. This research indicates that it may have some zoonotic potential and that lions may be ideal hosts for a variety of *Echinococcus spp*. The *E. granulosus* complex includes *E. felidis* (often known as the lion strain), which is closely related to *E. granulosus sensu stricto,* this species that was recently believed to be limited to canids, notably domestic dogs, as the only definitive hosts based on more extensive data comprising nuclear sequences and epidemiological characteristics (Rostami et al., 2015).

In this study, we collected taeniid eggs from lion excrement in Mambwe district's South Luangwa National Park and amplified DNA from individual eggs. Although mitochondrial and nuclear DNA sequences matched those of other *Echinococcus species,* considerable percentage divergence of mitochondrial genes indicated the presence of a unique species. All DNA fragments (200 bp) that could be amplified from eggs were 98% identical to Ugandan material (Hüttner et al, 2008). *Echinococcus felidis* is positioned as a sister taxon of *Echinococcus granulosus* sensu stricto in the phylogenetic tree of *Echinococcus* based on mitochondrial DNA.

Our findings support the assertion that lions are acceptable hosts for a wide variety of *Echinococcus* species. According to Banda et al. (2020), the low frequency of CE in cattle reflects the findings of an earlier investigation in the same research region, where 2.1% of 4061 animals were detected infected with nonspecific *Echinococcus* cysts (Banda et al., 2020).

This study represents the first documented investigation of *Echinococcus species* in both wild and domestic canines within the game management areas surrounding South Luangwa National Park, Zambia. Our findings provide crucial baseline data on the presence of these parasites in a previously unstudied region, marking an important initial step in characterizing echinococcosis transmission dynamics in Zambia's wildlife-livestock-human interface. We did not attempt to provide prevalence estimates due to some uncertainty regarding the host species of the faecal samples, the potential for repeated field sampling from the same individual animal (as we were getting faecal samples from the environment), and the low sensitivity of the egg detection method as a diagnostic technique (Huttner et al., 2014). Nevertheless, among the taeniid infected canids, lions have been known to shade more taeniid eggs. Further investigations are still needed to determine whether these observations are artifacts. It was obvious that the approach used in collecting samples was highly subjective and that it mainly relied on the skills of the game rangers who were helping to identify the wildlife canid faecal samples. Therefore, taeniid eggs need to be validated by molecular methods to get accurate results. For our amplicons, banding patterns or sequences were typical of *Echinococcus felidis* other than other taeniid genera. Other *Echinococcus spp* were found in earlier reports using the same methodology (Huttner et al., 2014, Romig et al., 2016). We, therefore, can safely say that lions of the south Luangwa national park are final hosts to *E. felidis* and that it would be interesting if, future studies could investigate the presence of larval stages, hydatid cysts in herbivores or omnivores, which are the intermediate hosts of the parasite.

CHAPTER SIX: CONCLUSION AND RECOMMENDATIONS

6.1. CONCLUSION

The study's major goal was to detect *echinococcus species* in wild and domestic canids in wildlife management areas to establish host ranges in Zambia.

The presence of *echinococcus* in lions, as reported in our work, has potential long-term benefits. This study documented that *Echinococcosus* infection, one of the most neglected zoonotic diseases, is prevalent in the lions of the South Luangwa National Park, despite the low infection prevalence. The isolation of *E. felidis* in lion excrement reveals a new geographical area of transmission foci. Though dogs in the study area were not found to be infected with any taeniid tapeworms, it does not rule out the possibility of them getting infected with *Echinococcus* tapeworms given their proximity to the national park.

According to the findings of this study, the phylogenetic tree revealed that *E. felidis* shares the same cluster as *granulosus*, which causes cystic echinococcosis in humans and thus possess a potential risk of zoonotic infection. As a result, more detailed research is needed to unravel the wildlife-domestic transmission overlap hypothesis in biodiversity-rich environments such as Zambia's game management regions. This work established an essential baseline for *Echinococcus spp*. infection in lions, which may be beneficial in developing preventative and control methods.

6.2. RECOMMENDATIONS

Given the occurrence of *E. felidis* in Zambia, it is advised that public health measures:

- Such as stray dog control and the establishment and strengthening of meat inspection services at abattoirs be promoted in the Mambwe area.
- Deworming of pets should be recommended. These approaches would undoubtedly help to effectively manage echinococcosis and limit the danger of transmission to people.
- With the present study conducted in wild canids and domestic dogs, more investigations, particularly in cattle, would be encouraged, and repeated studies in dogs would be critical to establishing a clear epidemiological picture.

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