

**PREVALENCE AND RISK FACTORS OF *GIARDIA* INFECTIONS  
IN DAIRY CATTLE HERDS IN LUSAKA AND CHILANGA  
DISTRICTS OF ZAMBIA**

**BY**

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## **DECLARATION**

I, **Cliff Kakandelwa**, do hereby declare that this dissertation represents my own work and that it has never been submitted before for the award of a degree or any other qualification at this university or any other university.

**Signature:** .....**Date:** .....

## **APPROVAL**

This dissertation of Cliff Kakandelwa has been approved as partial fulfilment of the requirements for the award of Master of Science in One Health Analytical Epidemiology by the University of Zambia.

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Examiner:.....Date.....

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Examiner:.....Date.....

## ABSTRACT

*Giardia* is an intestinal protozoan parasite of mammals including humans. The objectives of the cross sectional study conducted between May 2013 and April 2014 were to estimate the prevalence of *Giardia* infections in dairy herds on farms in Chilanga and Lusaka districts of Lusaka Province, Zambia, and to evaluate risk factors associated with the infections. Farms were grouped into smallholder and commercial farms. A total of 377 calves aged one day to one year were sampled from 34 farms. All the 377 faecal samples were analyzed for *Giardia* antigen using a commercially available monoclonal antibody-based Enzyme-linked immunosorbent assay kit. Chi square and Fisher's exact tests were used and binary logistic regression was used to evaluate factors associated with infections.

The overall prevalence of *Giardia* for the two districts was 34.5% (130/377; 95% CI= 29.7-39.3) while the overall farm prevalence was 79.4% (27/34). For individual smallholder farms, the animal level prevalence of *Giardia* ranged from zero to 100% and within commercial herds, it was 12.5% to 60.9%. The prevalence was significantly higher in calves up to three months old than in older ones ( $p = 0.010$ ). There was no significant difference in the prevalence of *Giardia* between smallholder and commercial dairy farms ( $p = 0.300$ ) and this was also the case between male and female calves ( $p = 0.633$ ). The present study did not find a clear association between presence of *Giardia* in faecal samples and occurrence of diarrhoea in calves ( $p = 0.205$ ).

Several management factors were evaluated, however, only husbandry system was found to be a significant risk factor for infection with the parasite. It was found that herds reared under intensive husbandry system were 2.083 (95% CI = 1.086-59.308,  $p = 0.041$ ) times more likely to be positive for *Giardia* than those under free range system.

The findings of the present study demonstrate that *Giardia* infections are common in dairy herds in Chilanga and Lusaka districts especially in calves up to three months of age which may play an important role in the epidemiology of giardiasis. Furthermore, husbandry system is an important factor in the perpetuation of the infections.

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## LIST OF SYMBOLS AND ABBREVIATIONS

%	Percent
<	Less than
≤	Less than or equal to
≥	Greater than or equal to
®	Registered trade mark
°	Degree
°C	Degree Celsius
CI	Confidence interval
CSO	Central Statistical Office
CWP	Cyst wall protein
DNA	Deoxyribonucleic acid
ELISA	Enzyme-Linked Immunsorbent Assay
GDH	Glutamate dehydrogenase
GLORF	<i>Giardia lamblia</i> open reading frame
HPLC	High performance liquid chromatography
IARC	International Agency for Research on Cancer
IFA	Immuno-Flourescence Assay
IFS	International Foundation for Science
LAMP	Loop mediated isothermal amplification
mg	Milligram
ml	Mililitre
nm	Nanometre

OD	Optical density
PCR	Polymerase Chain Reaction
pH	potential of Hydrogen
RNA	Ribonucleic acid
SACIDS	Southern African Centre for Infectious Disease Surveillance
SDS-PAGE	Sodium dodecyl sulphate polyacrylamide gel electrophoresis
SPSS	Statistical Package for the Social Sciences
tpi	Triose phosphate isomerise
USA	United States of America
WHO	World Health Organization
$\beta$	Beta
$\mu$ l	Microlitre

## CHAPTER 1

### 1.0 INTRODUCTION

*Giardia* is a ubiquitous intestinal flagellated protozoan parasite of mammals. The species *G. duodenalis* is found in humans and various mammalian species (Thompson *et al.*, 2000). *Giardia* infections are common in various hosts including humans, non-human primates, domestic and wild animals, with high frequency in dairy calves (O’Handley *et al.*, 2000a; Thompson, 2000; van Keulen *et al.*, 2002; Olson *et al.*, 2004; Thompson, 2004; Pereira *et al.*, 2007; Forondo *et al.*, 2008; Geurden *et al.*, 2008a; Lebbad *et al.*, 2009; Mircean *et al.*, 2012; Ankarklev *et al.*, 2012; Ignatius *et al.*, 2012; Júlio *et al.*, 2012; Laishram *et al.*, 2012; Minetti *et al.*, 2013). It is estimated that 280 million cases of human intestinal infections associated with *Giardia* occur every year worldwide (Thompson, 2004).

Molecular studies have demonstrated that *G. duodenalis* is a species complex comprising at least eight assemblages or genotypic groupings named A to H (Thompson, 2004; Smith *et al.*, 2007). Among these assemblages, A and B have zoonotic potential as they infect humans, wildlife, companion animals, and livestock (Caccío *et al.*, 2005). The other genotypic groupings appear to be host specific; assemblages C and D have been identified in dogs and cats, assemblage E in cattle, sheep, goats and pigs, assemblage F in cats, and assemblage G in rats (Thompson, 2004; Caccio *et al.*, 2005). Furthermore, a novel assemblage H has been detected in marine vertebrates (Lasek-Nesselquist *et al.*, 2010).

In order to enhance knowledge, especially through molecular techniques, on the epidemiology and host-parasite interactions, the World Health Organization (WHO) included *Giardia* in the 'Neglected Diseases Initiative' (Savioli *et al.*, 2006).

Infections are spread via the faecal-oral route by ingestion of cyst-contaminated food or water (Coklin *et al.*, 2007). Ingestion of as few as 10 *Giardia* cysts can cause giardiasis (Rendtorff, 1954; Wolfe, 1992). For its pathogenicity, and public health significance of zoonotic transmission, *Giardia* has emerged as an important parasite of dairy cattle (Olson *et al.*, 2004). Furthermore, it has been revealed that *Giardia* infections are very common in calves (O'Handley *et al.*, 2000a). Through a number of studies, it has been demonstrated that both dairy and beef calves may harbour more than one genotype of *G. duodenalis* which can be of zoonotic significance (Trout *et al.*, 2005; Mendonca *et al.*, 2007).

Calves have been reported to be infected with *G. duodenalis* as early as four days of age, and the highest intensity of cyst excretion ( $10^5$ - $10^6$  cysts/gram) between the ages of one month and three months has been documented (Ralston *et al.*, 2003). As calves infected with *Giardia* shed large numbers of cysts, they pose a potential zoonotic reservoir for human infections (O'Handley *et al.*, 1999). Farmers also incur economic losses due to treatment expenses on the animals (Xiao, 1994). The losses can be substantial in dairy herds in which it has been reported to be very common as compared to other domestic animals (O'Handley *et al.*, 1999; Olson *et al.*, 1995; Olson *et al.*, 2004).

In Zambia, dairy farming is common and categories of dairy producers include traditional, smallholder and commercial (Neven *et al.*, 2006; Pandey and Muliokela, 2006). For traditional producers, productivity ranges from one to three litres of milk per cow per day. Their animal husbandry practice is characterized by rearing of mostly traditional breeds in open range grazing whereby cattle move in search of water and pasture. Smallholder producers' productivity is above three litres of milk per day per cow. However, it is still lower than that of commercial producers which is about 15-24 litres of milk per cow per day. Most smallholder producers are organized under co-operatives. In Zambia, there are about 2,500 smallholder dairy farmers affiliated to dairy co-operatives in milk marketing (Pandey and Muliokela, 2006).

Previously, no study was carried out on the prevalence of *Giardia* in cattle in Zambia. In light of the lack of data on the occurrence of the parasite in cattle in Zambia, the clinical importance, causation of production losses and zoonotic potential of *Giardia* infections, more knowledge about the prevalence of the parasite was required. The present study was therefore aimed at determining the epidemiology of the *G. duodenalis* in selected districts of Lusaka province. It was also important to establish if the prevalence of giardiasis was associated with certain factors including calf age, season, production system and sex. The aim of the study was to describe the epidemiology of *Giardia duodenalis* infection in dairy cattle in Lusaka and Chilanga districts. Specific objectives of the study were:



- to estimate the prevalence of *Giardia* infections in dairy herds on farms in Chilanga and Lusaka districts of Lusaka Province, Zambia,
  - to evaluate risk factors associated with *Giardia* infections in dairy cattle on dairy farms and,
  - to recommend mitigation measures for possible prevention and control of *Giardia* infections on dairy cattle farms in Zambia.
- Information generated from this study was expected to be used as a basis for further extensive studies.

## CHAPTER 2

### 2.0 LITERATURE REVIEW

#### 2.1 Background

It has been suggested that the protozoan parasite *Giardia* has colonized the human intestines since pre-historic times as evidenced by its isolation from 1800 to 2400 year-old human faecal material in Tennessee, United States of America (Faulkner *et al.*, 1989). However, the parasite was first observed by the Dutch microscopist Antony van Leeuwenhoek in his own stool in 1681 (Dobell, 1920; Flanagan, 1992). Furthermore, it was not until 1859 that Lambl described the morphology of *Giardia* (Flanagan, 1992).

A lot of controversy has surrounded the taxonomy of *Giardia* for many years so much that different names have been used for the same species (Thompson, 2004). However, with the development of molecular techniques such as polymerase chain reaction (PCR) based tools, *Giardia* has been identified in a number of mammalian species and as a result, the taxonomy of the parasite has been revised (Thompson, 2004; Caccio *et al.*, 2008; Monis *et al.*, 2009). Despite the advances of molecular tools, further research is required as different markers have at times assigned the same isolate to different *Giardia* assemblages (Smith *et al.*, 2007; Caccio *et al.*, 2008; Caccio and Ryan, 2008).

## 2.2 Taxonomy and nomenclature of *Giardia*

*Giardia* is a protozoan parasite belonging to the family *Hexamitidae* of the order *Diplomonadida* in the *Trepomonadea* class of the phylum *Metamonada* (Cavalier-Smith, 1993; Adam, 2001). The family *Hexamitidae* is characterized by flagellated protozoa with diploid nuclei, a unique attachment organelle called the ventral disc, and absence of mitochondria and peroxisomes (Morrison *et al.*, 2007).

Based on morphological features and host range, six species of *Giardia* are recognized: *G. duodenalis* in mammals, *G. muris* and *G. microti* in rodents, *G. psittaci* and *G. ardeae* in birds, and *G. agilis* in amphibians (Table 2.1) (Adam, 2001; Monis *et al.*, 2009). Furthermore, molecular studies have demonstrated that *G. duodenalis* is a species complex comprising at least eight assemblages or genotypic groupings (Monis *et al.*, 2003; Thompson, 2004). Two of the assemblages, A and B, have zoonotic potential (Trout *et al.*, 2006; Uehlinger *et al.*, 2006; Coklin *et al.*, 2007; Mendonca *et al.*, 2007; Geurden *et al.*, 2008a; Liu *et al.*, 2012). Each of the assemblages A and B has two sub-genotypes with zoonotic potential. Sub-genotypes AI and AII constitute two distinct subgroups in assemblage A while for assemblage B it is BIII and BIV (Monis *et al.*, 2003). While AII consists of human isolates of *Giardia*, AI consists of both animal and human isolates (Thompson, *et al.*, 2000). However, assemblages C to G appear to be host-specific. This is because assemblages C and D have been identified in dogs and cats, assemblage E in cattle, sheep, goats and pigs, assemblage F in cats, and

assemblage G in rats (Becher *et al.*, 2004; Thompson, 2004; Cassio *et al.*, 2005; van der Giessen *et al.*, 2006; Muhid *et al.*, 2011).

There is a proposed novel nomenclature based on large genetic distances that have been observed to separate assemblages (Jerlstrom-Hultqvist *et al.*, 2010). However, the proposed new species names have not yet been validated by the International Code for Zoological Nomenclature (Ryan and Caccio, 2013).

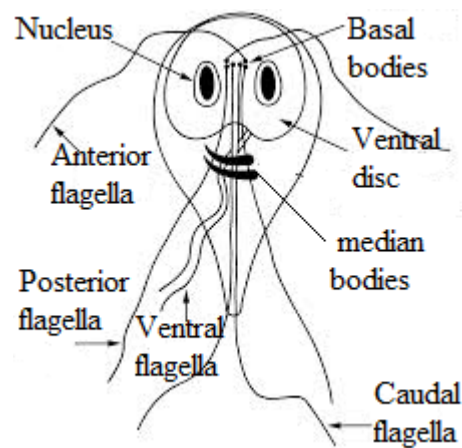
**Table 2.1:** Recognized *Giardia* species, assemblages and host range (adapted from Monis *et al.*, 2009).

Species	Proposed species	Host(s)
<i>G. duodenalis</i>		Most mammals including humans, livestock, wildlife and pets
Assemblages:		
A (Group I)	<i>G. duodenalis</i>	Humans and other mammals
A (Group II)		Mainly humans
B (Group III)	<i>G. enterica</i>	Humans and other mammals
B (Group IV)		Humans
C/D	<i>G. canis</i>	Dogs, other canids
E	<i>G. bovis</i>	Cattle and other hoofed livestock
F	<i>G. cati</i>	Cats
G	<i>G. simondi</i>	Marine vertebrates/rats
H	*	Pinnipeds
<i>G. muris</i>	<i>G. muris</i>	Rodents
<i>G. microti</i>	<i>G. microti</i>	Rodents
<i>G. psittaci</i>	<i>G. psittaci</i>	Birds
<i>G. ardeae</i>	<i>G. ardeae</i>	Birds
<i>G. agilis</i>	<i>G. agilis</i>	Amphibians

\* Not yet formally described

### 2.3 Morphology of *Giardia duodenalis*

The morphology of *Giardia* includes some atypical characteristics including presence of two diploid nuclei, a unique attachment organelle called the ventral sucking disc, and absence of mitochondria and peroxisomes (Fig 2.1) (Adam, 2001, Marti *et al.*, 2003; Souza *et al.*, 2004; Thompson, 2004; Hehl *et al.*, 2007; Morrison *et al.*, 2007; Plutzer *et al.*, 2010). The parasite is dependent on fermentative metabolism for energy (Adam, 2001).



**Figure 2.1:** Trophozoite of *G. duodenalis* revealing a pear-shaped body, nuclei, ventral disc and four pairs of flagella (Adapted from Faubert, 2000).

Based on phylogenetic studies, it has been suggested that *Giardia* is either a primitive early-branching eukaryote or comprises many divergent eukaryotic lineages that have adapted to a microaerophilic environment (Marti *et al.*, 2003; Thompson, 2004; Yang *et al.*, 2005; Hehl *et al.*, 2007; Morrison *et al.*, 2007; Chen *et al.*, 2008; Feng *et al.*, 2012; Nino *et al.*, 2013).

The trophozoites are binucleate with four pairs of flagella (anterior, caudal, posterior-lateral and ventral), the ventral disc, median body and funis (Fig

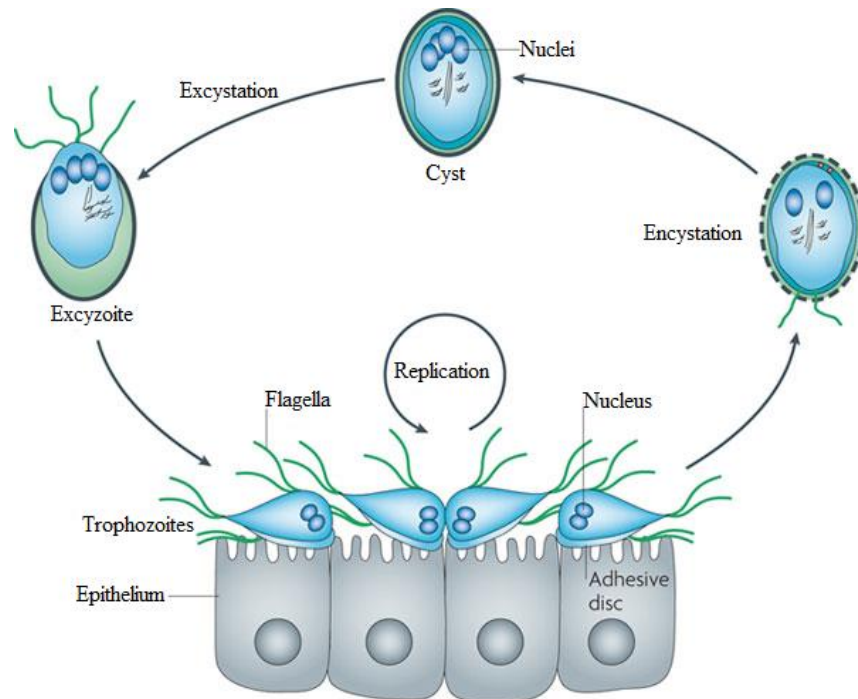
2.1) (Faubert, 2000; Campanati *et al.*, 2002; Thompson, 2004). Furthermore, the trophozoite has a pear-shaped body, about 12-15  $\mu\text{m}$  long and 5-9  $\mu\text{m}$  wide, with the posterior end being pointed (Thompson, 2004). Trophozoites have flagella which are important for motility (Campanati *et al.*, 2002). The anterior portion of the ventral surface of the organism forms a sucking disc which serves to attach the organism to the intestinal mucosa of the host (Morrison *et al.*, 2007). The ventral disc is composed of cytoskeletal proteins mostly tubulin and giardins (Ankarklev *et al.*, 2010). The median body forms the giardial 'smile' and is located on the dorsal aspect of the trophozoite posterior to the ventral disc (Elmendorf *et al.*, 2003). The functional role of the median body is not well known. However, it has been suggested that the median body is an important site for microtubule nucleation (Holberton and Wald, 1981). The funis is composed of sheets of microtubules, and its functional role is associated with movement of the caudal region of the trophozoite (Campanati *et al.*, 2002).

Variant Surface Proteins (VSPs) thought to play a role in evading host immune defences are present on the surface coat of the trophozoites (Prucca and Lujan, 2009; Jerlstrom-Hultqvist *et al.*, 2010). The cysts may be either round or oval, and they contain four nuclei. Its rigid outer wall protects the parasite against adverse environmental conditions (Olson *et al.*, 2004; Thompson, 2004).

## **2.4 Life cycle and transmission**

The life cycle of *Giardia* is direct (Fig 2.2) and comprises two developmental stages; the trophozoite and the cyst which is the infective stage (Midlej and Benchimol, 2008; Kahn and Line, 2010). Following

ingestion of a cyst, trophozoites are released by excystation in the small intestines (Alvarado and Wasserman, 2009). Factors such as stomach acidity and pancreatic enzymes trigger the excystation process (Bingham and Meyer, 1979; Boucher and Gillin, 1990; Nino *et al.*, 2013).



**Figure 2.2:** Life cycle of *G. duodenalis* depicting excystation, replication and encystation (Source: Ankarklev *et al.*, 2010).

The most common location of the trophozoite stage is in the crypts within the small intestine of the vertebrate host (Thompson, 2004). Trophozoites live closely attached, through the ventral adhesive disc, to the mucosa and remain extracellular and absorb nutrients from the brush border. As a result of facilitating attachment, the ventral disc is crucial in the virulence of *Giardia*. Trophozoites multiply by asexual binary fission on the surface of

the mucosa and consequently the parasite establishes itself (Olson *et al.*, 2004).

As trophozoites, together with the faecal mass, pass through the small intestine, they encyst and are excreted with the faeces. Encystation may be initiated by the presence of bile salts and depletion of cholesterol (Kaul *et al.*, 2001; Arguello-Garcia *et al.*, 2009; Faso *et al.*, 2013). The implication of encystation is a reduction in adhesion, metabolism and multiplication of the trophozoite which results in disassembly of the ventral disc, internalization of flagella, and encasement in a protective cyst wall (Arguello-Garcia *et al.*, 2009). Encystation is crucial to the survival of the parasite in adverse environmental conditions, and its transmission to susceptible hosts (Arguello-Garcia *et al.*, 2009; Ortega-Pierres *et al.*, 2009). The prepatent period, before cysts appear in the faeces, is generally three to 10 days (Olson *et al.*, 1995; Kahn and Line, 2010). Cyst shedding may be continuous over several days and weeks although it is often intermittent, particularly in the chronic phase of infection (Kahn and Line, 2010).

The cyst is the infective stage and represents the resting stage of the organism. Its rigid outer wall protects the parasite against changes in environmental temperature (Olson *et al.*, 2004; Thompson, 2004), dehydration and chlorination, all of which would destroy the trophozoite. Therefore, encystation is a major virulent factor which is important for transmission and progression of giardiasis (Thompson and Monis, 2012).

Although there is some evidence from epidemiological and molecular genetic studies that *Giardia* is capable of sexual reproduction, the frequency



of recombination and its impact on the epidemiology of infection with the parasite is unknown (Meloni *et al.*, 1995; Cooper *et al.*, 2007; Birky, 2009; Lasek-Nesselquist *et al.*, 2009; Monis *et al.*, 2009; Ortega-Pierres *et al.*, 2009; Siripattanapipong *et al.*, 2011). Some of the implications of sexual reproduction in the parasite would be adaptation of the parasite to host immune defences, and new hosts, and a revision of its taxonomy and epidemiology (Birky, 2009; Caccio and Sprong, 2009; Lasek-Nesselquist *et al.*, 2009).

Transmission occurs by the faecal-oral route, either by direct contact with an infected host, or through contaminated food or water (Gow and Waldner, 2006; Hamnes *et al.*, 2006a). There is a suggestion of mechanical transmission of the parasite through insect vectors (Graczyk *et al.*, 2003). In a study conducted in North Carolina, it was revealed that synanthropic flies carried potentially viable zoonotic species of *Giardia* (Graczyk *et al.*, 2003).

Factors that facilitate infection include overcrowding, the high excretion of cysts by infected animals and the low dose needed for infection. The minimum dose to initiate an infection is thought to be between 10 and 25 cysts (Rendtorff, 1954; De Cameri *et al.*, 1977). *Giardia* cysts are infectious upon excretion and very resistant to environmental effects (Olson *et al.*, 2004; Thompson, 2004). However, some studies have revealed that some cysts undergo a maturation period of up to seven days before becoming infective (Grant and Woo, 1978; Thompson and Smith, 2011).

There are four main cycles of transmission that have been proposed to maintain host-specific and zoonotic assemblages of *Giardia* in mammalian

hosts: human, livestock, dog/cat and wildlife cycles (Thompson, 2004; Appelbee *et al.*, 2005; Thompson R *et al.*, 2008). Human to human transmission of *Giardia* can occur either directly in settings where hygiene levels may be compromised like in day care centres or indirectly through the accidental ingestion of cysts in contaminated water or food (Pereira *et al.*, 2007; Ignatius *et al.*, 2012; Júlio *et al.*, 2012). Through this transmission, assemblages A and B can be maintained within the human cycle. However, some studies have shown that zoonanthroponotic (reverse zoonotic) transmission is occurring, and is a vital factor in understanding the epidemiology of *Giardia* infections (Graczyk *et al.*, 2002; Thompson and Smith, 2011). Following a study in Uganda, humans were considered to be the source of infection of *Giardia* in habitats of free-ranging mountain gorillas, and possibly some cattle which had access to the habitats through habitat disturbance and human encroachment (Graczyk *et al.*, 2002).

For the livestock cycle, *Giardia* has been reported in both dairy and beef cattle (Xiao and Herd, 1994a; O’Handley *et al.*, 1999; Ralston *et al.*, 2003; Becher *et al.*, 2004; Muhid *et al.*, 2011). Within the livestock cycle, transmission is common among infected calves and chronically infected adults. However, transmission of the parasite is particularly high amongst dairy calves (Xiao and Herd, 1994a; Becher *et al.*, 2004). While the livestock cycle ensures maintenance of assemblage E, studies have revealed that a small proportion of cattle in a herd may harbour genetic groupings of *Giardia* with zoonotic potential (O’Handley *et al.*, 2000a).

The dog and cat cycles ensure maintenance of assemblages C/D and F respectively (McGlade *et al.*, 2002; Mircean *et al.*, 2012). However,

zoonotic transmission of *Giardia* between humans and dogs in the same household has been reported from communities in tea growing areas of India (Traub *et al.*, 2004). In a Brazilian study, zoonotic assemblage A1 was isolated from dogs and children in the same locality suggesting the existence of a zoonotic cycle of the parasite in that community (Volotao *et al.*, 2007).

Furthermore, a study in Thailand revealed that dogs were a potential source of *Giardia* infections for humans, and also responsible for maintenance of the parasite within the canine cycle of transmission (Traub *et al.*, 2009). Intensive contact between large numbers of dogs sharing the same shelter can favour the transmission of dog specific assemblages of *Giardia* as they are able to out-compete other assemblages (Thompson, 2004). It has also been suggested that household dogs are likely to harbour zoonotic assemblage A as the dog to dog transmission of *Giardia* is less frequent in such settings (Thompson and Monis, 2004).

For the wildlife cycle, animals like beavers and deer have been reported to harbour infections with zoonotic genotypes of *Giardia* (Deng and Cliver, 1999; Sulaiman *et al.*, 2003). In Italy, water buffaloes (*Bubalus bubalis*) were found to harbour both host-specific and zoonotic genotypes of *Giardia* (Caccio *et al.*, 2007). However, the parasite was found to be rare in indigenous wildlife species in Australia (Thompson *et al.*, 2010). Transmission of *Giardia* cysts between wildlife and domestic animals may occur where there is common sharing of pastures especially in such situations as human encroachment of wildlife habitats (Graczyk *et al.*, 2002; Thompson *et al.*, 2009; Solarczyk *et al.*, 2012).

Further, it has been suggested that *Giardia* likely spills from domestic cycles into wildlife hosts, and following infection, the wildlife populations serve as reservoir hosts for subsequent infection of domestic animals and humans (Thompson *et al.*, 2009).

While assemblage A and to some extent, B can infect livestock, companion animals and wildlife, there is lack of clear evidence on the frequency of transmission of these assemblages between different hosts (Thompson, 2004; Hunter and Thompson, 2005; Souza *et al.*, 2007). This has left a gap in full understanding of the epidemiology of *Giardia* infections.

## **2.5 Pathogenesis and clinical features**

Although the pathogenesis of *Giardia* is not completely understood, an intricate pathophysiological process is initiated by infection with the parasite resulting in variable clinical signs of abdominal pain, diarrhoea and weight loss (Buret, 2008). Studies have also revealed that the pathogenesis of *Giardia* infections in cattle is similar to that in laboratory animals and humans (O’Handley *et al.*, 2001; Scott *et al.*, 2002).

A rise in numbers of intraepithelial lymphocytes, increases in epithelial permeability and activation of T lymphocytes has been observed in *Giardia* infections (Geurden *et al.*, 2010; Koh *et al.*, 2013). Trophozoite toxins and T-cell activation initiate a diffuse shortening of brush border microvilli and decreased activity of the small intestinal brush border enzymes, particularly lipase, proteases and disaccharidases (McDonnell *et al.*, 2002; Scott *et al.*, 2002; Buret, 2007).

The diffuse microvillus shortening leads to a decrease in overall absorptive area in the small intestine and an impaired uptake of water, electrolytes, and nutrients (O’Handley *et al.*, 2001; McDonnell *et al.*, 2002). The combined effect of this decreased resorption and the brush border enzyme deficiencies results in malabsorptive diarrhoea (O’Handley *et al.*, 2001; McDonnell *et al.*, 2002). Steatorrhoea and mucous diarrhoea which has been described in *Giardia* infected hosts may be due to the reduced activity of lipase and increased production of mucin by goblet cells (Kahn and Line, 2010).

*Giardia* infections in domestic animals are often asymptomatic. However, in calves, giardiasis can cause diarrhoea that does not respond to treatment with antibiotic or anti-coccidia drugs (O’Handely *et al.*, 1999; Geurden *et al.*, 2006a; Kahn and Line, 2010). Infection may also result in numerous diarrhoea episodes which in turn adversely affects production and results in economic losses for farmers (Xiao, 1994). In young animals, especially below six months of age, the excretion of watery faeces with a mucoid appearance may be an indication of infection with the parasite. In lambs and goat kids, giardiasis was found to have a negative effect on feed efficiency, rate of weight gain and time to slaughter in both experimental and natural infections (Olson *et al.*, 1995; Olson *et al.*, 2004; Aloisio *et al.*, 2006). In companion animals like dogs and cats, especially in the young animals (puppies and kittens), the major complication of persistent infections is impairment of growth and development (Farthing, 1994).

Severity of the disease is dependent on factors like the developmental, nutritional and immunity of the host as well as virulence factors of the

parasite (Chin *et al.*, 2002; Scott *et al.*, 2002). Although gross intestinal lesions are rarely observed, microscopic lesions consisting of villous atrophy and cuboidal enterocytes may be reported (Kahn and Line, 2010).

In humans, clinical manifestations vary, ranging from absence of symptoms to acute or chronic infection depending on factors such as age and health of the infected host as well as infective dose and genetic background of the parasite (Eckmann, 2003). The acute phase may be characterised by nausea, vomiting, flatulence in addition to persistent fatty and yellowish diarrhoea, abdominal pain, weakness and rapid weight loss (10-20%), while chronic infection presents with intermittent or recurrent diarrhoea which is foul-smelling, frothy or yellowish in appearance (Thompson *et al.*, 1993; Farthing, 1994).

## **2.6 Epidemiology**

*Giardia duodenalis* has a world-wide distribution (Wade *et al.*, 2000; Degerli and Ozcelik, 2003; Bomfim *et al.*, 2005; Thompson J *et al.*, 2008; Winkworth *et al.*, 2008; Muhid *et al.*, 2011; Geurden *et al.*, 2012; Di Cristanziano *et al.*, 2013; Wang *et al.*, 2014). Various factors such as sensitivity and specificity of a diagnostic test used, number of animals tested, the area studied, number of farms, the health status of the animal, and whether only a one-off faecal sample was examined, considering the intermittent nature of cyst excretion, have contributed to differences in reported prevalence of *Giardia* globally (McGlade *et al.*, 2002; Van Gool *et al.*, 2003; Thompson J *et al.*, 2008; Geurden *et al.*, 2004). Furthermore, the

factors make it difficult to make comparisons of different prevalence studies.

### **2.6.1 *Giardia* in the environment of animals**

In a study conducted in south-eastern New York state, *Giardia* was detected in 4% (n = 782) of soil from 37 dairy farms by microscopy, and soil moisture content was significantly associated with presence of the parasite in soil (Barwick *et al.*, 2003). In another similar study aimed at determining level of contamination by *Giardia* in a river basin in a livestock farming area in Spain, revealed that there was an association between a rise in cysts in river water in spring and summer, and increased shedding of cysts by cattle in the same seasons (Castro-Hermida *et al.*, 2009).

### **2.6.2 *Giardia* in wildlife species**

#### **2.6.2.1 *Giardia* in terrestrial wildlife**

*Giardia* has also been reported in deer and wild boar in Spain and Poland (Castro-Hermida *et al.*, 2011; Solarczyk *et al.*, 2012), in cervine (elk and deer) in California (Deng and Cliver, 1999), and in buffaloes in Pakistan and Sri Lanka (Goraya *et al.*, 2004; Abeywardena *et al.*, 2014). Occurrence of the parasite with a prevalence of 4.8% (n = 351) was also reported in indigenous wildlife species in Australia (Thompson *et al.*, 2010). The prevalence of *Giardia* among asymptomatic cervine animals in the Californian study using an improved immunofluorescence assay was 3.7% (n = 82) (Deng and Cliver, 1999). *Giardia* was also found in free-ranging cervids in Norway (Hamnes *et al.*, 2006b).

Furthermore, a study conducted in irrigation catchment areas in Australia involving analysis by PCR of 445 faecal samples from various wildlife (Kangaroo, fox, wild bird) and livestock (sheep, dairy and beef cattle) species revealed an overall prevalence of *Giardia* of 1.1% in wildlife and 6.7% in livestock, and zoonotic assemblage A was isolated in some of the samples (McCarthy *et al.*, 2007). *Giardia* has also been reported in non-human primates in Belgium and the Netherlands using PCR technique (Levecke *et al.*, 2009).

Furthermore, *Giardia* has been reported in an endangered wild carnivore, the African painted dog (*Lycaon pictus*), in Zambia and Namibia, and in captive dogs in Australia with prevalence of 26% (n = 71) in wild populations and 62% (n = 16) in captive ones based on microscopy techniques (Ash *et al.*, 2010). The same study revealed that there was a significant difference in prevalence between the animals in the wild and in captivity probably due to confinement in the same area which allows accumulation of cysts in the environment of captive animals. Another interesting finding that emerged from the study was the occurrence of zoonotic *Giardia* assemblages A and B in higher proportions than host-specific assemblages C and D in both wild and captive populations.

#### **2.6.2.2 *Giardia* in marine wildlife**

The parasite has also been detected in faeces of marine vertebrates including seals in the east and west coast of the USA by PCR (Lasek-Nesselquist *et al.*, 2010). In that study, it was revealed that *Giardia* isolates belonged to zoonotic assemblages A and B including a novel genotype which was designated as assemblage H.



Furthermore, a low prevalence (3.8%, n = 709) of *Giardia* was detected by PCR analysis of stomach and intestinal epithelial cell scrapings of eight species of cultured fingerlings, eight species of wild freshwater fish, and five species of marine fish in Australia (Yang *et al.*, 2010). In the same study, *G. duodenalis* assemblages A, B and E as well as *G. microti* were identified in fish.

### **2.6.3 *Giardia* in domestic animals**

#### **2.6.3.1 *Giardia* in cattle**

Studies in North America and Europe have revealed that *Giardia* is highly prevalent in dairy calves, and farm prevalences of between 45% and 100% have been reported (Xiao, 1994; Olson *et al.*, 1995; Ruest *et al.*, 1998; de Graaf *et al.*, 1999; O’Handley *et al.*, 1999; Wade *et al.*, 2000; O’Handley *et al.*, 2001; Huetink *et al.*, 2001; Olson *et al.*, 2004; Trout *et al.*, 2004; Maddox-Hyttel *et al.*, 2006; Trout *et al.*, 2006; Geurden *et al.*, 2008a). These studies have also demonstrated an association between *Giardia* infection and diarrhoea and significant production losses. In the United States of America (USA), Canada, Germany and the Netherlands, *Giardia* has been implicated as an aetiological agent either alone or in combination with other enteric pathogens like virulent *Escherichia coli*, *Salmonella*, rotavirus and coronavirus in calf diarrhoea (O’Handley *et al.*, 1999; Huetink *et al.*, 2001; Bjorkman *et al.*, 2003; Barigye *et al.*, 2008; Gulliksen *et al.*, 2009; Gillhuber *et al.*, 2014).

A multicentre prevalence study in Europe covering some regions of Italy, France, Germany and United Kingdom on *Giardia* in dairy calves aged two

to 16 weeks using a monoclonal antibody-based enzyme-linked immunosorbent assay (ELISA) demonstrated that infection with the parasite is particularly higher in younger animals less than two months of age than in older calves (Geurden *et al.*, 2012). In the same study, the overall animal prevalence of *Giardia* for the four countries was 45.4% (n= 2072), and the overall herd prevalence was 89.9% (n= 207). In another study in Southern Germany, the prevalence of *Giardia* by microscopy among 1,564 diarrhoeic calves aged from one to twelve months old was 7.2%, and highest cyst shedding of 28.4% (n= 95) was in those aged two to three months old (Gillhuber *et al.*, 2014).

The multicentre study also showed that although host-specific *G. duodenalis* assemblage E was most prevalent, 43% of the isolates were typed as zoonotic assemblage A. Mixed assemblages A and E infection were also isolated in 32% of the samples. Similarly, an earlier study conducted in Belgium revealed that calves were often infected with the zoonotic *Giardia* assemblage A either as a mixed or single infection (Geurden *et al.*, 2008a). Furthermore, a study in Southern Germany which involved 152 calves up to 130 days old revealed that there was a potential risk for animal handlers as some of the calves were infected with zoonotic assemblage A of the parasite (Gillhuber *et al.*, 2013). Similar findings were revealed in a study conducted in Egypt where zoonotic assemblage A was present among beef calves (Sabry *et al.*, 2009). However, a study in Malaysia involving dairy calves revealed that the animals were infected with *Giardia* belonging to the non-zoonotic assemblage E (Muhid *et al.*, 2011). Furthermore, in a similar study in Sri Lanka, it was found out that the majority of *Giardia* isolates among

calves of upto three months of age were due to assemblage E (97.2%, n=141) and some zoonotic assemblage A (2.8%) (Abeywardena *et al.*, 2014). In the study in Sri Lanka, faecal samples were collected from the calves weekly for six weeks and analysed by PCR with a resulting overall prevalence of *Giardia* of 41.5% (n= 340).

The reported prevalence of *Giardia* in Argentina in a study that included 620 calves less than seven weeks old from 43 dairy farms by microscopy was 34.5% (n= 620, CI = 30.7% - 38.3%) (Tiranti *et al.*, 2011). In the same study, it was demonstrated that shedding of *Giardia* cysts was higher in calves younger than two weeks old than in older ones, and high levels of infection with the parasite were recorded on farms located in areas with poorly drained soils.

The parasite has also been reported in dairy and beef cattle in China (Liu *et al.*, 2012; Wang *et al.*, 2014) and among dairy cattle in Pakistan (Ayaz *et al.*, 2012). The prevalence of *Giardia* reported in both dairy and beef calves in Canada and the USA has been as high as 100% in some herds (Xiao and Herd, 1994a; Olson *et al.*, 1997a; O’Handley *et al.*, 1999; Fayer *et al.*, 2000; Ralston *et al.*, 2003; Coklin *et al.*, 2010). In these studies, most calves continued to excrete low numbers of *Giardia* cysts after weaning. It has also been revealed that the infections are common toward the end of the neonatal period (O’Handley and Olson, 2006).

A Canadian study that was conducted to determine the prevalence and pattern of infection of *Giardia* in ranch raised beef calves and their dams from birth to weaning by immunofluorescence microscopy revealed

infection rates of 85% at five weeks of age among calves (Ralston *et al.*, 2003). However, another Canadian study in British Columbia showed a lower prevalence of 36% (n= 193) among beef calves based on immunofluorescence microscopy (McAllister *et al.*, 2005). In the USA, *Giardia* infections were detected in 819 weaned beef calves aged six to 18 months with an overall prevalence by PCR of 33.5%, and herd prevalences of up to 100% (Santin *et al.*, 2011).

A study in Ontario, Canada, reported, by immunofluorescence microscopy, a *Giardia* farm prevalence of 96% (n= 45) in dairy cattle and 97% (n= 30) in beef cattle with calves being more infected than older heifers and cows, and infection levels were similar for both types of farms (Dixon *et al.*, 2010). While all *Giardia* isolates from beef cattle in the Ontario study belonged to host-adapted assemblage E, some isolates from dairy cattle included zoonotic assemblage B. Similarly by immunofluorescence staining, Appelbee *et al.* (2003) had reported a *Giardia* prevalence of 34% (n= 495) in beef calves aged two to 10 weeks from nine farms across Alberta in Canada, and most calves were infected with the livestock-specific assemblage E (97.6%, n= 42). It was suggested by the same study that beef calves infrequently harboured zoonotic assemblage A.

On Prince Edward Island in Canada, a prevalence study involving dairy and beef cattle showed that *Giardia* was more prevalent in calves (less than six months of age) than adult cattle (Uehlinger *et al.*, 2011). In the study, the prevalence of *Giardia* in calves was 51% (n= 183), and 38% (n= 892) in adult cattle by direct immunofluorescence antibody microscopy. Based on

molecular characterization of PCR positive samples from calves in the same study, zoonotic assemblage A, besides the livestock-specific assemblage E, was isolated (Uehlinger *et al.*, 2011). However, in adult cattle, only assemblage E was isolated, and it has been suggested that immunologically competent adult cattle are able to resist infection, and competition between genotypes in a host tends to favour the host-adapted assemblage (Thompson, 2004; Uehlinger *et al.*, 2011).

Another study that involved pre-weaned calves upto four months old from two dairy and four beef farms in Western Australia and New South Wales reported an overall *Giardia* prevalence of 26.9% by PCR (n= 364, CI: 22.4% - 31.5%) and 94.7% (n= 75) of the isolates belonged to the livestock-specific genotype (Ng *et al.*, 2010).

In Africa, the parasite has been reported in cattle in some countries including Uganda, Egypt, Nigeria, Ethiopia and Algeria (Nizeyi *et al.*, 2002; Sabry *et al.*, 2009; Magaji *et al.*, 2013; Wegayehu *et al.*, 2013; Ouchene *et al.*, 2014). The prevalence of *Giardia* by direct microscopy (direct wet mount and formalin ether concentration) in cattle of different ages in the study in Ethiopia was 2.3% (n= 384). The Nigerian study also employed microscopy using formalin ether concentration of faecal samples (Magaji *et al.*, 2013). However, all the samples were of adult cattle slaughtered at an abattoir, and the overall prevalence of infection with *Giardia* was 27.7% (n= 224). Assemblages A and E of *Giardia* were reported in waste water from a slaughter house for cattle, sheep and pigs in France (Bertrand and Schwartzbrod, 2007).

A study in Egypt revealed a *Giardia* prevalence of 30.8% (56/182) by microscopy among beef calves (Sabry *et al.*, 2009). In the same study, 20% of *Giardia* isolates belonged to the zoonotic assemblage A. The parasite has also been reported in cattle in Tanzania at 21.1% (n= 19), and isolates belonged to assemblages A and B (Di Cristanziano *et al.*, 2013).

#### **2.6.3.2 *Giardia* in sheep and goats**

*Giardia* has been reported in sheep in many countries including Australia, Canada, England and USA (Buret *et al.*, 1990; Taylor *et al.*, 1993; Olson *et al.*, 1997b; Ryan *et al.*, 2005; Santín *et al.*, 2007), and in sheep and goats in China (Zhang *et al.*, 2012a).

While cumulative incidence of *Giardia* in sheep has been reported to be nearly 100%, in goats, incidence of 100% has been recorded (Castro-Hermida *et al.*, 2005; Castro-Hermida *et al.*, 2006). The parasite was reported among dairy goats in Brazil at 14.3% by microscopy, 42.2% in Spain and about 20% among asymptomatic adult goats in France by immunofluorescence microscopy (Bomfim *et al.*, 2005; Castro-Hermida *et al.*, 2005; Ruiz *et al.*, 2008). In the Brazilian study, infections were more in kids from one to three months of age than they were in adult goats (Bomfim *et al.*, 2005). Another study in France estimated the prevalence of *Giardia* in goat kids aged between five and 12 months at 38% (n= 200) (Castro-Hermida *et al.*, 2004). On the Canary Islands in Spain, the study included 315 goat kids 2-6 months of age from 40 farms, and the farm prevalence of *Giardia* was 95.5% (Ruiz *et al.*, 2008). Using nested PCR for identification of genotypes of *G. duodenalis*, only livestock-specific assemblage E was identified in the Spanish study.

In another study conducted in France, it was revealed that all adult periparturient goats shed *Giardia* cysts at some point around kidding posing as a source of infection for the neonates (Castro-Hermida *et al.*, 2005). The *Giardia* prevalence in a study conducted in Belgium was 25.5% and 35.8% in lambs and goat kids, respectively (Geurden *et al.*, 2008b). The Belgian study revealed that although the host specific assemblage E was commonest identified, zoonotic assemblage A was also present in some lambs and goat kids. Furthermore, zoonotic assemblages A and B were identified by PCR from 310 faecal samples from goats aged three months to seven years from eight farms in Malaysia (Lim *et al.*, 2013). The Malaysian study revealed that the prevalence of *G. duodenalis* was 6.8% (n= 310).

In East Africa, occurrence of *Giardia* among goats with a prevalence of 21.9% (n= 41) has been reported in Tanzania, and the majority of parasite isolates belonged to livestock-specific assemblage E and a few were zoonotic assemblages A and B (Di Cristanziano *et al.*, 2013).

Similar findings were reported in a study conducted in North America in that zoonotic assemblage A was found among ewes (Santin *et al.*, 2007). In that study the prevalence of *Giardia* in ewes was 12% (n= 32) and 4% in lambs (n= 31). A *Giardia* prevalence of 11.1% (n= 477, CI= 8.3% - 13.9%) was determined by PCR in lambs aged up to two months old in Australia (Yang *et al.*, 2009).

The parasite has also been reported in lambs in Norway according to a longitudinal study which found a prevalence of 23% (n= 550) and 31% at the first and second sampling, respectively (Robertson *et al.*, 2010). In

another study in Spain that was determined by immunofluorescence assay occurrence of shedding of *Giardia* cysts by lambs aged 1-3 months old on 16 farms revealed a prevalence of 42% (n= 386), and prevalence by farm was 100% (Gomez-Munoz *et al.*, 2009).

A higher prevalence of *Giardia* infections in neonatal lambs than in adult sheep has been reported in Spain (Castro-Hermida *et al.*, 2011). Another study in Australia also revealed that infections with the parasite were higher in lambs aged below twelve months than in adult sheep (Ryan *et al.*, 2005). It has been suggested through experimental studies that lambs do not rapidly develop high antibody titers against *Giardia* (Yanke *et al.*, 1998). Furthermore, it has been suggested that ewes on positive farms are potential source of infection to lambs especially around the peri-parturient period when ewes excrete an increased number of cysts (Xiao *et al.*, 1994).

Therefore, it is possible that *G. duodenalis* infects all ruminants (O'Handley and Olson, 2006; Minetti *et al.*, 2013).

#### **2.6.3.3 *Giardia* in pigs**

*Giardia* has been reported in pigs in Denmark, Slovenia and Canada (Olson *et al.*, 1997b; Maddox-Hyttel *et al.*, 2006; Langkjær *et al.*, 2007; Stukelj *et al.*, 2011). The age-specific herd prevalence of *Giardia* in the Danish study which included 50 herds by immunofluorescence microscopy was 18% for sows, 22% for piglets and 84% for weaners. It was speculated that reduced immunity in weaners during the transition from dependence on maternally conferred immunity to the time pigs started building up their own immunity contributed to the age-related infection levels observed in the study. Another



possible explanation for higher *Giardia* infections in weaners than piglets could be that piglets may be infected in the farrowing unit and start shedding cysts when introduced to weaner pens (Maddox-Hyttel *et al.*, 2006; Hamnes *et al.*, 2007). In the same study, it was observed that *Giardia* infections were higher among pigs raised on solid floors (porous concrete) than those on slatted floors, and increased age of weaners was a risk factor for infection.

In Norway, prevalence of *Giardia* in pooled faecal samples of piglets aged four to 33 days from 684 litters by immunofluorescence was 1.5% (Hamnes *et al.*, 2007). A study conducted in Western Australia, revealed an overall prevalence of *Giardia* in domestic pigs by PCR of 31.1% (n= 289) (Armson *et al.*, 2009). Although assemblage E was the most abundant of the isolates in the study, zoonotic assemblage A was also identified in both pre and post weaned pigs. However, it was revealed that infection with the parasite was higher in post-weaned (four weeks to six months, n= 156) than in pre-weaned piglets (11 days to three weeks, n= 123). It is possible that weaners might be more susceptible than pre-weaned piglets to infection with the parasite as a result of stress associated with weaning (Hamnes *et al.*, 2007).

In Canada, *Giardia* infections in pigs were low with reported herd prevalence of 14% (n= 21) and animal level prevalence of 1% (n= 633, CI: 0.4% - 2%) by direct immunofluorescence microscopy (Budu-Amoaka *et al.*, 2011).

#### **2.6.3.4 *Giardia* in horses**

There is wide variation in the prevalence of *Giardia* reported in horses (0–25%) depending on such factors as geographical location, age and health status of the animal (Xiao and Herd, 1994b; Olson *et al.*, 1997b; Atwill *et al.*, 2000). A study in the USA revealed that *Giardia* infections were common in horses (Xiao and Herd, 1994). The study revealed that *Giardia* infection rates were higher for foals (17–35%) than other age groups. Foals started to excrete *Giardia* cysts between two and 22 weeks of age. However, in another USA study, *Giardia* cysts were not detected in foals less than six months of age by immunofluorescence microscopy (Olson *et al.*, 1997b). Similarly, a Brazilian study showed a low *Giardia* prevalence in horses of 0.5% (n= 396) by microscopy, and age was found not to be a risk factor for infection with the parasite (Souza *et al.*, 2008). However, among 107 horses aged four to 10 years in Iraq, a slightly higher occurrence of *Giardia* was estimated at 19.6% using Giemsa stain (Butty, 2011). Furthermore, zoonotic *Giardia* assemblages A and B were isolated in a survey of horses from USA and Australia (Traub *et al.*, 2005).

#### **2.6.3.5 *Giardia* in domestic dogs and cats**

*Giardia* has been reported in dogs and cats worldwide (Kirkpatrick, 1988; Covacin *et al.*, 2011; McDowall *et al.*, 2011; Wang *et al.*, 2011). A survey on the prevalence of *Giardia* in puppies conducted in the USA reported a prevalence of 36–50% (Hahn *et al.*, 1988). However, another study in the United States, reported national prevalence of *Giardia* infection in dogs and cats at 15.6% (n= 16,114) and 10.8% (n= 4,978) respectively by ELISA (Carlin *et al.*, 2006).

*Giardia duodenalis* was found to be the commonest enteric parasite of domestic dogs and cats in Australia and Romania (McGlade *et al.*, 2002; Mircean *et al.*, 2012). The overall prevalence of *Giardia* infection in dogs by ELISA was 34.6% (n= 416) in a study conducted in Romania (Mircean *et al.*, 2012). *Giardia* has been reported in healthy household dogs (15.2%, n= 152) and cats (13.6%, n= 60) in the Netherlands (Overgaauw *et al.*, 2009). In Japan, the prevalence of *Giardia* infections in puppies of up to three months old from nine petshops was reported to be 23.4% (n= 1,794) by ELISA, and all isolates belonged to dog specific assemblages ( Itoh *et al.*, 2010). However, another Japanese study reported a low prevalence of 2.6% (n= 77) in domestic dogs and 1.8% (n= 55) in cats both aged three months to 15 years by sucrose flotation and immunofluorescence staining methods (Yoshiuchi *et al.*, 2010).

A study aimed at determining the prevalence and associated risk factors for *Giardia* in dog and human communities in Thailand, revealed that the parasite is common in dogs (56.8%, n= 229) and humans (20.3%, n= 204) by zinc sulphate floatation and microscopy, immunofluorescence and PCR (Traub *et al.*, 2009). The role of the dog as a possible source of *Giardia* infections for humans was also highlighted in the same study in that majority of dogs (78%) harboured at least one zoonotic assemblage A or B of *Giardia*. *Giardia* occurrence has also been reported in dogs at a rescue shelter in central London (21.0%) (Upjohn *et al.*, 2010). Most of the *Giardia* isolates in the same study were dog specific assemblages C and D with a few being zoonotic assemblage A.

However, in urban areas of southern Germany it was reported that assemblage A (60%, n= 33) of *Giardia* was more prevalent than assemblages C and D in individual and group housed asymptomatic dogs visiting veterinary clinics based on microscopy and ELISA (Leonhard *et al.*, 2007). The same study also revealed that zoonotic assemblage A was more prevalent in individual than group housed dogs. It has been suggested that where the frequency of transmission is high, the dog-specific genotype is likely to out-compete and become the dominant genotype (Thompson, 2004).

*Giardia* was the most prevalent parasite (9.3%) in a study which analysed 1,159 faecal samples by immunofluorescence assay on occurrence of intestinal parasites in different dog populations in northern Belgium (Claerebout *et al.*, 2008). The estimated prevalence of *Giardia* in 1,400 kennel dogs in a study in Italy was 20.5% based on microscopy and PCR (Scaramozzino *et al.*, 2008). In another study in central Italy, the reported prevalence of *Giardia* in cats was 4.4% (n = 181) by immunofluorescence antibody test, and three cats harboured zoonotic assemblage A while other positive cats were infected with host specific assemblage F (Paoletti *et al.*, 2010). In a study in the USA, *Giardia* was detected in 44.4% (n = 18) of three to six months old cats by immunofluorescence microscopy, and cat-specific assemblage F was isolated (Fayer *et al.*, 2006). Similarly, in Colombia, *Giardia* was reported in cats with a prevalence of 6.5% (n = 46) by PCR, and only the host specific assemblage F was isolated (Santin *et al.*, 2006).

#### **2.6.3.6 *Giardia* in rabbits**

*Giardia* has been reported to infect other animal species like rabbits in China (Zhang *et al.*, 2012b; Liu *et al.*, 2014). The prevalence of the parasite in 378 rabbits aged four to six months in China was 7.4% by microscopy, and zoonotic assemblage B of *Giardia* was isolated from all positive animals.

#### **2.6.4 Risk factors**

Factors that may be associated with risk of infection with *Giardia* can be narrowed down to demographic and management factors (Xiao, 1994; O’Handley *et al.*, 1999). Demographic factors may include age distribution of animals sampled, size of the farm, geographic location, herd size, and other species of animals present on the farm.

Calves aged over nine days were found to be more likely to be infected with *Giardia* when compared with those less than four days (Gow and Waldner, 2006). One study in North America revealed that dairy calves as young as two days of age were harbouring the parasite (Mark-Carew *et al.*, 2010). However, the burden of infection has been reported to be low in dairy cattle above six months of age (Buret *et al.*, 1990, Becher *et al.*, 2004). The high occurrence of *Giardia* in young animals could be due to the slow development of specific immunity by the host against the parasite (O’Handley *et al.*, 2003). As a result, young animals can be considered to be a source of infection for susceptible hosts.

Adult animals are also a potential source of *Giardia* especially for neonates as a peri-parturient rise in cyst excretion has been reported in cattle, sheep,

goats and pigs (Xiao and Herd, 1994; Xiao *et al.*, 1994; Wade *et al.*, 2000; Castro-Hermida *et al.*, 2005). High levels of infection of calves with the parasite have been recorded on farms located in areas with poorly drained soils (Tiranti *et al.*, 2011). Poorly drained soils may increase the retention of moisture which in turn prolongs survival of the cysts in the environment (Barwick *et al.*, 2003).

Management factors include general management (type of flooring, calf housing, and frequency and method of cleaning) (Maddox-Hyttel *et al.*, 2006), separation of the dam from the calf and administration of colostrum, and direct contact with infected animals. Generally, intensive management has been found to favour transmission of *Giardia* cysts (Hamnes *et al.*, 2006a). Previous studies revealed that animals reared indoors especially under group housing were more likely to be infected with the parasite than those housed outside (Quigley *et al.*, 1994; Reust *et al.*, 1998).

Through risk factor analysis, a study conducted in Europe demonstrated that calves in contact with the dam had higher odds of infection with the parasite than those that were separated from the dam (Geurden *et al.*, 2012). Furthermore, it has been suggested that cows on positive farms are potential source of infection to calves especially around the peri-parturient period when cows excrete an increased number of cysts (Xiao and Herd, 1994a; Wade *et al.*, 2000).

The periparturient rise in *Giardia* cyst excretion has been attributed to the reduced immunity as a result of hormonal alterations during late gestation and early lactation (Xiao, 1994). In view of the important role played by

older cattle as a source of infection, it has been recommended that prudent management of manure can aid in reducing environmental contamination, and infection of calves (Fayer *et al.*, 2000).

Some management practices that reduce direct contact between animals such as separation of a calf from the dam immediately after birth may aid in reducing the transmission of the cysts to calves (Wade *et al.*, 2000). Some studies revealed that cattle housed on a concrete floor were more at risk for *Giardia duodenalis* infection than those housed on slatted ones (Xiao *et al.*, 2004; Maddox-Hyttel *et al.*, 2006).

Furthermore, a Malaysian study indicated that keeping calves in pens on sand floors was associated with an increased risk of infection with *Giardia* (Muhid *et al.*, 2011). Concomitant infections with other pathogens such as rotavirus and *Cryptosporidium* are also a risk factor for infection of calves with *Giardia* (Huetink *et al.*, 2001; Mark-Carew *et al.*, 2010).

Seasonality through its influence on management practices of dairy farms such as calving has also been shown to contribute to the possibility of cattle becoming infected with the parasite (Huetink *et al.*, 2001; Mark-Carew *et al.*, 2010; Wang *et al.*, 2014). A study in America indicated that cattle were at higher risk of infection with *Giardia* in summer than in winter (Wade *et al.*, 2000). Another study showed that cattle sampled in winter (November through March) and spring (April through June) shed fewer cysts than those sampled in summer (July through October) (Mark-Carew *et al.*, 2010). It was speculated that *Giardia* infections could have been propagated within herds by close contact of cattle due to confinement in winter, and access to

contaminated water and pasture in summer while spring was characterized by a low number of calves as the calving season began in late summer. However, another study among feedlot cattle in the United States showed that increased animal density was associated with a low prevalence of *Giardia* (Hoar *et al.*, 2009). In the same study, it was revealed that use of coccidiostats as feed additives was associated with a high prevalence of *Giardia*.

A number of studies have revealed that early administration of colostrum seemed to decrease the risk of infection in calves (Wade *et al.*, 2000; O'Handley *et al.*, 2003; Duranti *et al.*, 2009).

#### **2.6.5 *Giardia* in Zambia**

In Zambia, the parasite has been reported in pigs (Siwila and Mwape, 2012) and in humans (Siwila *et al.*, 2011). The reported prevalence of *Giardia* in those studies was 12% (n = 217) by immunofluorescence assay for pigs and 29% (n = 786) in humans based on immunofluorescence microscopy. Despite the few studies on prevalence of *Giardia* in humans in Zambia, the parasite has been reported in humans in many parts of the world (Khan *et al.*, 2011; Ankarklev *et al.*, 2012; Ignatius *et al.*, 2012; Júlio *et al.*, 2012).

#### **2.7 Public health significance**

The zoonotic transmission of *G. duodenalis* has attracted much debate. A number of animal species have been implicated as potential reservoirs, including livestock, pets and aquatic animals (Caccio`and Ryan, 2008).



Although the World Health Organization has considered *G. duodenalis* to have a zoonotic potential (WHO, 1979; Thompson, 2004), direct evidence has been lacking. However, two distinct genotypes of *G. duodenalis*, A and B, found in cattle which are also isolated from humans and other animals are proposed to be zoonotic (Thompson *et al.*, 2000; Thompson J *et al.*, 2008).

Although molecular evidence in some studies have revealed that cattle are normally infected with the non-zoonotic livestock assemblage E, molecular evidence of the zoonotic transmission of *G. duodenalis* has been demonstrated among dairy farm workers in India (Khan *et al.*, 2011). In the study conducted in India, it was revealed that besides the livestock-specific assemblage E zoonotic assemblage A was identified in a number of dairy calves (Khan *et al.*, 2011). In that study, the overall prevalence of *Giardia* by both microscopy and ELISA in cattle was 12.2% (n = 180) and 27.4% (n = 51) among dairy farm workers. Further, assemblage A1 found in humans and cattle showed a 100% homology with each other. As a result of such studies, the zoonotic potential of *Giardia* is no longer in doubt. However, there is limited data on the frequency of zoonotic transmission (Hunter and Thompson, 2005). This requires further and extensive exploration especially through molecular epidemiological studies.

## **2.8 Diagnostic tools**

### **2.8.1 Microscopic examination**

The motile, piriform trophozoites may be seen in saline smears of watery faeces. They can be distinguished from trichomonads which have a single rather than double nucleus, an undulating membrane, and no concave ventral surface. Light microscopy remains the most feasible and cheap method for the diagnosis of *Giardia* in routine work (Zajac *et al.*, 2002). However, compared to immunological assays microscopy has a low sensitivity, and require a skilled microscopist (Geurden *et al.*, 2004).

As sodium chloride, sucrose, and sodium nitrate flotation media are very hypertonic and distort the cysts, zinc sulphate (specific gravity 1.18) is most appropriate for flotation of cysts in faecal specimens. To aid identification, the cysts can be stained with iodine or trichrome (Addiss *et al.*, 1991; Khan *et al.*, 2011; Wegayehu *et al.*, 2013). As *Giardia* cysts are excreted intermittently, it is recommended that several faecal examinations (e.g. three samples collected over three to five days) should be performed if giardiasis is suspected (O'Handley *et al.*, 1999; Van Gool *et al.*, 2003).

### **2.8.2 Serology**

#### **2.8.2.1 Immunofluorescence and Enzyme-linked immunosorbent assays**

Several immunofluorescence assay (IFA) and enzyme-linked immunosorbent assay (ELISA) test kits are commercially available for the detection of *Giardia* antigen in faecal specimens (Zajac *et al.*, 2002; Geurden *et al.*, 2004; Trout *et al.*, 2005; Cardona *et al.*, 2011). However,

most of the test kits were developed and evaluated for use in human stool samples. The direct fluorescent antibody assay with fluorescein isothiocyanate-conjugated anti-*Giardia* monoclonal antibody recognizes epitopes on cyst surface (O'Handley *et al.*, 2000a). One of the highly conserved cyst antigens detected by most ELISA is the cyst wall protein 1 (CWP-1) which is normally secreted in large amounts by encysting trophozoites (Boone *et al.*, 1999; Hehl *et al.*, 2007; Amazonas *et al.*, 2008; Chen *et al.*, 2008; Pinheiro *et al.*, 2008; Bae *et al.*, 2009). Some copro-antigen detection ELISA has a higher diagnostic sensitivity than IFA because ELISA is capable of detecting both encysting trophozoites and cysts compared to IFA which only detects cysts (Boone *et al.*, 1999; Geurden *et al.*, 2004). However, IFA can also be used as a quantitative test (Xiao and Herd, 1993). In calves, both ELISA and IFA have been found to be more sensitive and specific than microscopic examination for the diagnosis of infection (Traub *et al.*, 2009; Feng and Xiao, 2011). However, phase contrast and scanning electron microscopy were found to be reliable in differentiating various types of *Giardia* cysts (Erlandsen *et al.*, 1990; Karanis *et al.*, 1996).

Freezing and thawing of specimens containing *Giardia* cysts does not appear to prevent the detection of the parasite by immunofluorescent (Erlandsen *et al.*, 1990). However, detection of the parasite by bright field microscopy following freezing and thawing of specimens has been reported to be almost impossible. Erlandsen *et al.* (1990) further demonstrated that morphological characteristics of trophozoite contained within the cysts and appearance of cyst wall disappeared after two cycles of freezing and

thawing. However, the antigenicity of the cyst wall was retained after at least three cycles of freezing and thawing, and could be detected by immunofluorescence.

Although these assays work well, they are relatively expensive. Due to the high cost, indirect immunofluorescence is usually restricted to research and epidemiological studies (Zajac *et al.*, 2002). Rapid solid-phase qualitative immunochromatography assays which can help with diagnosis of giardiasis on-site are available (Cardona *et al.*, 2011).

Immunochromatography uses monoclonal antibodies directed against cyst wall proteins, and a commercial test for use in dogs is available (Geurden *et al.*, 2008c). However, the laboratory based immunofluorescence and ELISA assays are still reported to be more sensitive than the immunochromatographic assays for the clinical diagnosis of *Giardia*.

### **2.8.3 Molecular techniques**

#### **2.8.3.1 Loop mediated isothermal amplification (LAMP) and polymerase chain reaction (PCR)**

An emerging diagnostic technique, loop mediated isothermal amplification (LAMP) has been used to detect *Giardia* in faecal and environmental samples (Plutzer and Karanis, 2009). It has been revealed that LAMP is highly selective, does not require a thermocycler and is not affected by presence of inhibitors (Karanis and Ongerth, 2009). In PCR, inhibition of PCR is common where DNA is extracted from faecal specimens (Amar *et al.*, 2002). However, PCR is predominantly used.

The PCR assays are based on the amplification of a gene fragment with primers that bind to deoxyribonucleic acid (DNA) sequences that are conserved in all *G. duodenalis* assemblages (Molina *et al.*, 2007; Gomez-Munoz *et al.*, 2011; Vanni *et al.*, 2012). Polymerase Chain Reaction enables identification of *Giardia* to species level and hence assessing the zoonotic potential of the parasite (Sulaiman *et al.*, 2003; Itagaki *et al.*, 2005; Caccio and Ryan, 2008; Traub *et al.*, 2009).

Several genetic markers which differ in their information content and the nature of the DNA fragment selected for detecting and characterizing *Giardia* are used (Caccío *et al.*, 2005; Caccío *et al.*, 2008) including triosephosphate isomerase (tpi) gene, glutamate dehydrogenase (GDH), beta-Giardin ( $\beta$ -Giardin), elongation factor 1 $\alpha$  (EF-1 $\alpha$ ) and *G. lamblia* open reading frame C4 (GLORF-C4) and 16S ribosomal DNA (Caccío *et al.*, 2005; Khan *et al.*, 2011; Geurden *et al.*, 2012).

There is high genetic heterogeneity displayed by *Giardia* at the triosephosphate isomerase (tpi) gene (Monis *et al.*, 1999). Therefore, through phylogenetic analysis on the nucleotide sequences of the tpi gene, confirmation of the formation of distinct groups can be made. The tpi gene codes for a protein with 257 amino acid residues that functions in the cytosol of *Giardia* (Amar *et al.*, 2003).

Giardin is a family of structural proteins (alpha, beta and gamma) found in microribbons attached to microtubules on the disc of cytoskeleton of *Giardia* (Adam, 2001). As rRNA sequences are highly conserved across

life, the small subunit ribosomal RNA (SS rRNA) is very useful for molecular comparisons on *Giardia* (Adam, 2001; Berrilli *et al.*, 2004).

Although PCR assays are very costly for diagnostic laboratory use, they are more sensitive and specific than microscopy and immunological assays (Savioli *et al.*, 2006; Caccio and Ryan, 2008).

Furthermore, techniques such as proteomics have been used to further evaluate protein variation, cellular structure and host-*Giardia* interactions (Steuart, 2009). Proteomics is concerned with the study of the proteins within an organism under a given set of conditions (Barrett *et al.*, 2000). Separation of proteins and identification is the principle underlying proteomics, and separation is done in gel based systems including sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) or high performance liquid chromatography (HPLC). While SDS-PAGE separates proteins on the basis of size, HPLC employs columns that retain proteins based on physical or chemical property (Wang and Hanash, 2003). Through proteomics, assemblage and host specific proteins, structural proteins and proteins released by trophozoites have been identified resulting in enhanced understanding of the pathogenesis, and possible development of drugs and vaccine against *Giardia* (Nash, 2002; Steuart, 2009).

To address the limitations of molecular methods in determining the viability and infectivity of *Giardia* cysts, a new integrated culture assay (Electrophysiological analysis of cell culture- reverse transcription – polymerase chain reaction) was evaluated and found to be a rapid and cost-

effective method for assessing infectivity of *Giardia* cysts from environmental samples (Alum *et al.*, 2012).

## **2.9 Treatment and prevention/control**

### **2.9.1 Treatment**

At the moment, there is no drug permitted for the treatment of *Giardia* infections in ruminants (O'Handley and Olson, 2006). Through previous studies, benzimidazoles like albendazole, mebendazole and fenbendazole have been found to be effective in reducing the excretion of *Giardia* cysts by infected calves (Xiao *et al.*, 1995). In vitro studies revealed that these anthelmintics were much more effective than metronidazole, tinidazole and quinacrine against *Giardia* trophozoites (Edlind *et al.*, 1990; Morgan *et al.*, 1993; Xiao *et al.*, 1995). However, other studies showed that the dose of 20mg/kg daily for three days in calves for anti-giardial treatment was much higher than that for anthelmintic treatment (10mg/kg once) (Barr *et al.*, 1993; 1994).

It has been suggested that benzimidazoles inhibit the polymerization of tubulin to microtubules, which are a major component of flagella, median body and ventral disc, resulting in distortion of trophozoite morphology and inhibition of its attachment to the intestinal mucosa (Morgan *et al.*, 1993; Arias *et al.*, 2008). The benzimidazoles were found to have a wide safety margin compared to the nitro-imidazoles which were mutagenic and teratogenic in food animals (Edlind *et al.*, 1990; Xiao *et al.*, 1995; Arias *et al.*, 2008). Furthermore, fenbendazole and albendazole (5-20 mg/kg/day for

3 days) have been reported to significantly lower the peak and duration of cyst excretion (Geurden *et al.*, 2006a; Geurden *et al.*, 2010).

In an experimental study involving treatment of dairy calves which had been infected with *Giardia* with oral fenbendazole at 5mg/kg body weight once daily for three days in Canada, it was found that the anthelmintic had a clinical benefit as it reduced the number of days calves had diarrhoea (O’Handley *et al.*, 2000b). Furthermore, there was an environmental benefit as the number of cysts shed by calves significantly reduced due to treatment of infected calves with fenbendazole (O’Handley *et al.*, 2000b). Paromomycin (50-75 mg/kg *per os*, for 5 days) was also found to be highly efficacious and safe against an experimental *Giardia* infection in calves (Geurden *et al.*, 2006b). However, re-infection is common where frequency of transmission is high and cattle are exposed to a contaminated environment (O’Handley *et al.*, 2000b; O’Handley *et al.*, 2001).

Daily administration of drugs like paromomycin and fenbendazole may be a long term solution to the control of giardiasis in ruminants (Geurden *et al.*, 2006a; Uehlinger *et al.*, 2007). However, this kind of treatment regimen is impractical as it does not make economic sense, and there is a risk for the parasite to develop resistance to the drugs. As calves are unable to mount an effective immune response against *Giardia*, infections may be reduced by access to colostrum (O’Handley *et al.*, 2003).

There is scanty data available on the effectiveness of treatment against giardiasis in pigs, sheep and goats (Geurden *et al.*, 2009; Stukelj *et al.*, 2011).



For treatment of giardiasis in humans, metronidazole together with other nitroimidazoles like tinidazole, ornidazole and secnidazole is the most widely used drug (Gardner and Hill, 2001; Solaymani-Mohammadi *et al.*, 2010). It is usually administered in doses of 5mg for children and 250mg in adults for three times a day for five to seven days. Other drugs effective against the parasite include quinacrine, furazolidone, paromomycin, albendazole and nitazoxanide (Nash *et al.*, 2001; Rossignol, 2009). Albendazole given as a single dose of 400mg/kg for five days has been found to be safe and as effective as metronidazole in treatment of giardiasis. Metronidazole was suspected to be genotoxic to human cells (Elizondo *et al.*, 1996; Gardner and Hill, 2001). Therefore, paromomycin compared to metronidazole is recommended for use in patients during the first trimester of pregnancy (Gardner and Hill, 2001). However, there are no studies that have demonstrated an increased risk for cancer or teratogenicity with metronidazole use (IARC, 1977; Rossignol, 2009). There is no defined vaccine available for use in humans (Jenikova *et al.*, 2011).

### **2.9.2 Prevention/control**

As *Giardia* cysts are a source of infection for animals on most farms, discarding faeces promptly from pens, frequent change of bedding, regular cleaning and disinfection of pens can help reduce environmental contamination (Wade *et al.*, 200; Bomfim *et al.*, 2005; Maddox-Hyttel *et al.*, 2006). Furthermore, it has been revealed that storage of cattle slurry for three months greatly reduces the number and viability of *Giardia* cysts and this can reduce contamination of surface water by run-off from manure contaminated fields (Grit *et al.*, 2011). It has been speculated that ammonia

present in urine within the slurry may reduce cyst survival and infectivity. Strips of vegetation have also been suggested to be an effective buffer against cyst contamination of surface water by run-off from manure contaminated dairy calf areas (Miller *et al.*, 2007).

Some chemical disinfectants have been found to be effective in inactivating *Giardia* cysts in water although their effectiveness is dependent on such factors as the chemical and its concentration, temperature, pH, and the contact time (Jarroll *et al.*, 1980; Jarroll *et al.*, 1981; Ongerth *et al.*, 1989; Gerba *et al.*, 1997; Geurden *et al.*, 2006a). A study conducted in North America revealed that iodine-based disinfectants were more active than chlorine-based compounds against *Giardia* cysts at both 30 minutes and eight hours of contact time (Ongerth *et al.*, 1989). The study also demonstrated that *Giardia* cysts were completely inactivated by heating water to 70°C for 10 minutes. Furthermore, the cysts can be inactivated by use of quaternary ammonium compounds and using boiling water in cleaning of the pens (Geurden *et al.*, 2006a; Kahn and Line, 2010).

Despite attempts to come up with an efficacious vaccine, there is no *Giardia* vaccine available for use in ruminants (Uehlinger *et al.*, 2007; Thompson R *et al.*, 2008). A study conducted in Canada to evaluate the efficacy of a *G. duodenalis* vaccine in preventing giardiasis in two weeks old calves revealed that the number of trophozoites in the small intestines was not different between vaccinated and non-vaccinated calves (Uehlinger *et al.*, 2007). The study also indicated that changes consistent with enteritis were similar in a vaccinated and non-vaccinated calf. Paradoxically, vaccinated

calves excreted more *Giardia* cysts in their faeces than did non-vaccinated ones. Furthermore, serological immune response in vaccinated calves did not help reduce cyst shedding. However, a *G. duodenalis* vaccine derived from trophozoites isolated from sheep is available for dogs and cats in North America (Olson *et al.*, 2000).

## **CHAPTER 3**

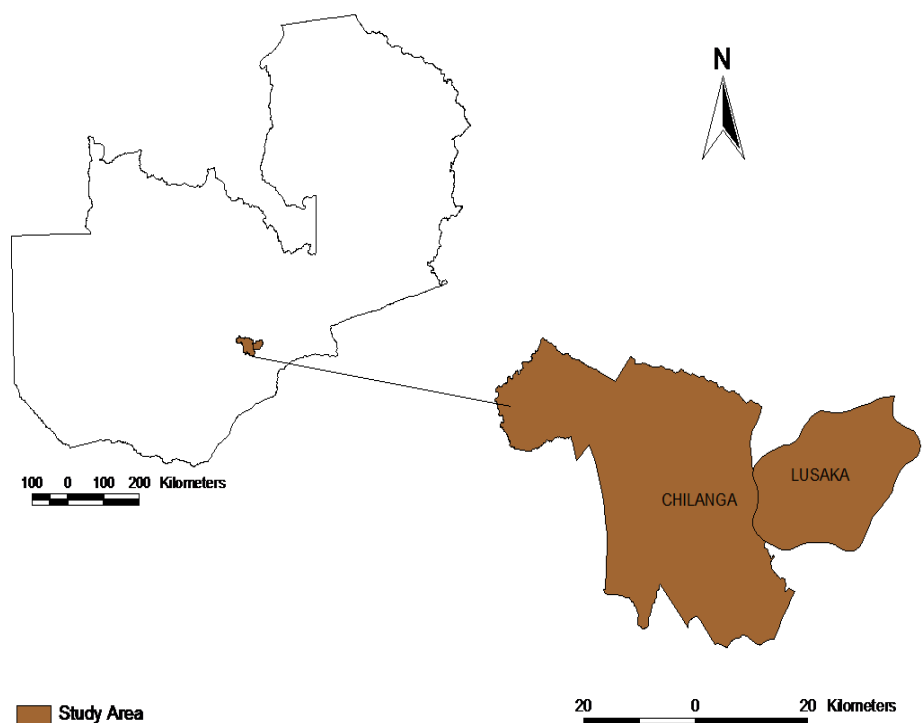
### **3.0 MATERIALS AND METHODS**

#### **3.1 Study area**

Zambia is a landlocked Southern African country located between latitudes 8° and 18° south and longitudes 22° and 34° east. It has a land area of 752,612 Km<sup>2</sup> and a human population of 13,092,666 people with 60.5% residing in rural areas where agriculture is the main source of livelihood. Administratively, the country consists of the following ten provinces: Central, Copperbelt, Eastern, Luapula, Lusaka, Muchinga, Northern, North-Western, Southern and Western. The provinces are subdivided into districts. Most of the total national population is in Lusaka province (2,191,225). Lusaka province consists of Lusaka (Capital city), Chongwe, Kafue, Luangwa and Chilanga districts. Chilanga was formerly under Kafue district but it was created into a district in 2012 and has a population of 107,051 people (CSO, 2012).

This study was conducted in Chilanga and Lusaka districts of Lusaka province which lies in the central part of Zambia (Fig 3.1). The total land area of Lusaka district is 360 Km<sup>2</sup> with a human population of about 1,747,152 representing 79.7% of the total provincial population (CSO, 2012). The district lies between longitude 10 and 28 degrees east of the prime meridian and between latitude 15 and 30 degrees south of the equator. The selection of Lusaka and Chilanga districts was based on dairy cattle population and the existence of different husbandry systems in Lusaka

province. Lusaka has some smallholder and a number of commercial dairy farms while there is a thriving smallholder dairy enterprise in Chilanga.



**Figure 3.1:** A topographic map of Zambia showing the location of Chilanga and Lusaka districts where sampling from selected dairy farms was conducted.

### 3.2 Study design and sampling

A cross-sectional study was conducted between May 2013 and April 2014. The sampling unit was the dairy herd. There were 37 smallholder and seven commercial dairy farms in Chilanga and Lusaka districts.

The sample size was estimated using the simple random formula (Martin *et al.*, 1987). To get the maximum sample size, prevalence was estimated at 50% with a margin of error of 0.05 and a confidence level of 95%.

The minimum sample size after adjusting for a finite population was 34 smallholder farms. A total of 27 smallholder farms were randomly selected from the total 37 dairy herds. Since there were very few commercial dairy farms in the two districts ( $n = 7$ ), all of them were included in the study. Out of the 34 herds, a total of 377 units of analysis were selected.

Faecal samples were collected from all calves in small herds ( $n \leq 10$ ) while for large herds, sampling was done proportionally at 10% sampling fraction during each study visit. Faecal samples were collected from calves between one day and one year of age in the selected herds.

A single faecal sample was collected per rectum from a calf (Fig 3.2) at each farm using an individual disposable latex glove (Uehlinger *et al.*, 2011). The glove was tied off and marked with the calf's identity number or name, age, sex and faecal consistency (normal, mucoid or diarrhoeic), and placed in a cool box. The samples were immediately transported to the laboratory at the School of Veterinary Medicine, University of Zambia in the cool boxes. Samples were kept at  $-20^{\circ}\text{C}$  until analysis.



**Figure 3.2:** Collection of faecal sample *per rectum* from a dairy calf at one of the farms in Lusaka which was visited during the study.

### 3.3 Questionnaire survey

At the time of sample collection, farm owners were interviewed using a semi-structured questionnaire (Appendix B) to collect data on demographic information and farm management practices that are hypothesized to be associated with the risk of *G. duodenalis* infection. Information regarding other animals kept on the farm, type of husbandry, housing type, floor type, bedding type, frequency of cleaning, type of feeding, water source, other diseases seen, whether deworming was done or not and how often and type of dewormer used among other things was also obtained.

### 3.4 Laboratory analysis

A monoclonal antibody-based ELISA for detecting *G. duodenalis* antigen in faecal specimens was used to test and determine the presence of the parasite in the faecal samples (Techlab<sup>®</sup>, Inc., Blacksburg, Virginia). The test uses

monoclonal and polyclonal antibodies specific to a cell-surface antigen of the organism (Uehlinger *et al.*, 2011), and was performed according to the manufacturer's instructions.

Briefly, the wash buffer was prepared by diluting the wash buffer concentrate (50 ml) with 950 ml distilled water. A sample was thoroughly mixed with 400 µl diluent in a ratio of 1:5 dilutions in a microcentrifuge tube. One hundred microlitres of diluent were transferred to each test well of the microassay plate and using a pipette a drop, 50 µl of the sample was then added to a microassay plate, already containing diluent.

The wells were gently tapped and the plate sealed with a plate sealer and incubated for one hour at room temperature. After that, the contents of assay wells were shaken out and discarded. Each well was then washed with wash solution (wash buffer) by directing the fine-tipped nozzle of the squirt bottle to the bottom of the well, the plate shaken and the wash solution discarded. Washing was repeated three times. The plate was struck onto a dry paper towel to remove any residue liquid in the wells, and the paper towel discarded.

Thereafter, 50 µl of conjugate was added to each well and the plate gently tapped and sealed after which it was incubated for 30 minutes at room temperature. Washing procedure was repeated after which 100 µl of Substrate was added to each well and the wells gently tapped to mix. Wells were further incubated at room temperature for ten minutes after which 50 µl of Stop Solution (0.6N sulphuric acid) was added to each well, and the wells gently tapped. After two minutes, absorbance was read at 450 nm on a



microplate ELISA reader (Tecan<sup>®</sup>, Austria GmbH). A positive and negative control samples were included for each analysis.

For spectrophotometric interpretation, the absorbance value of the negative control reading was supposed to be  $< 0.150$  OD<sub>450</sub> and that for the positive control was  $\geq 0.500$ . Samples with absorbance values  $< 0.150$  at 450 nm were considered negative while positive ones had absorbance values  $\geq 0.150$ .

### **3.5 Data analysis**

Data were entered using a Microsoft Excel<sup>®</sup> (version 2010) spreadsheet and verified by checking against responses in questionnaires and results of sample analysis forms. The data were exported to Statistical Package for the Social Sciences (SPSS version 16.0). Proportions of positives, with 95% confidence intervals were estimated. The relationships between disease/parasite presence and hypothesised risk factors was investigated using Chi-square when all observed cell sizes were larger than five or Fisher's exact test when any observed cell size was less than or equal to five in univariate analyses. Multiple effects of predictor variables were investigated using binary logistic regression. A significance level of 5% was used for all tests.

## CHAPTER 4

### 4.0 RESULTS

#### 4.1 Prevalence of *Giardia* infections

##### 4.1.1 Overall prevalence

Faecal samples were collected from 377 calves from 34 dairy farms of Chilanga and Lusaka districts. Of the 377 samples, 117 were collected from 27 smallholder dairy farms while 260 were from seven commercial farms. A total of 130 samples were found positive for *Giardia* infection on ELISA giving an overall prevalence of 34.5% (130/377; CI = 29.7 – 39.3). A total of 27 herds were positive for *Giardia* resulting in an overall herd prevalence of 79.4% (CI= 64.2 – 94.7). The farm prevalence was calculated as the number of farms with at least one positive calf compared with the total number of farms in the study. For individual smallholder farms, the animal level prevalence of *Giardia* ranged from zero to 100% and within commercial herds, it was 12.5% to 60.9%. The farm prevalence for Chilanga was 76.7% (CI= 61.6 – 91.8) and 100% for Lusaka.

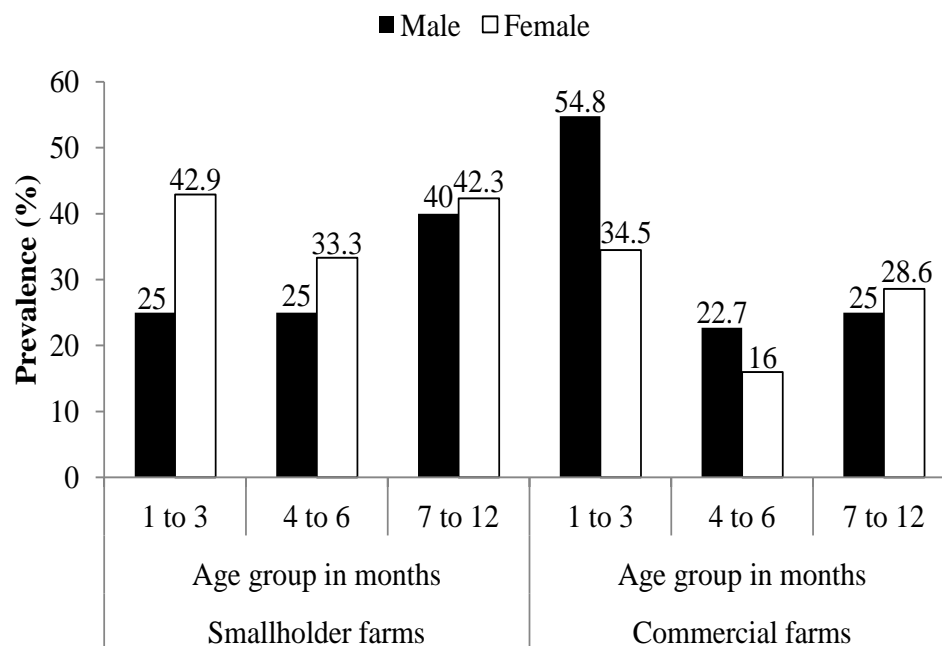
##### 4.1.2 Prevalence by farm type

A total of 34 herds were tested, 27 smallholders and 7 commercial dairy farms from the two study areas. A herd was considered positive if at least one calf tested positive for *Giardia*. Out of the 27 herds tested from smallholder farms, 20 were positive for *Giardia* giving a herd level prevalence of 74.1% (95% CI = 57.6 – 90.6).

All the herds on commercial farms tested positive for *Giardia* giving a prevalence of 100%. There was no significant difference in the prevalence of *Giardia* between smallholder and commercial dairy farms ( $p = 0.300$ ). Out of the 117 faecal samples collected from smallholder farmers, 42 were positive for *Giardia* antigen giving a prevalence of 35.9% (95% CI = 27.2 – 44.6) while that for calves from commercial farms was 33.8% (88/260; 95% CI = 28.1 – 39.6); giving a total of 130 positive animals.

#### **4.1.3 Prevalence by sex**

There were a total of 202 and 175 female and male calves, respectively (Fig 4.1). The prevalence of *Giardia* among females was 33.3% (66/202; CI = 26.2 – 39.2) and 36.6% (64/175; CI = 29.5 – 43.7) for males. Of the 42 *Giardia* positive calves from smallholder herds, there were 27 females and 15 males. Out of the 27 female calves, 42.9% were aged one to three months while among males this age group represented 25% (Figure 4.1). Among commercial herds, the distribution of female and male calves positive for *Giardia* was 39 and 49 respectively. There was no significant difference in the prevalence of *Giardia* between the sexes ( $p = 0.448$ ). The prevalence among males and females in the two farming systems was not statistically different ( $p = 0.633$ ).



**Figure 4.1:** Prevalence of *Giardia* in male and female calves in three different age groups from smallholder and commercial dairy farms.

#### 4.1.4 Prevalence by age

Ages of the sampled calves ranged from one day old to 360 days old. There were a total of 169, 71 and 137 calves in the age groups of one to three months, four to six months and seven to twelve months, respectively. The overall prevalence of *Giardia* in calves less than three months, four to six months and those above seven months of age was 42.0% (71/169; CI = 34.6 – 49.4), 22.5% (16/71; CI = 12.8 – 32.3) and 31.4% (40/132; CI = 23.6 – 39.2), respectively.

For smallholder herds, the prevalence of *Giardia* in calves below three months, four to six months and above seven months old was 34.6% (18/52), 29.2% (7/24) and 41.5% (17/41), respectively. Among commercial farms the prevalence for calves below three months, four to six months and above

seven months old was 45.3% (53/117), 19.1% (9/47) and 27.1% (26/91), respectively. There was a statistically significant difference in the overall prevalence among the three age groups ( $p = 0.010$ ). The prevalence of *Giardia* in smallholder and commercial farms from both sexes of calves among the different age groups is depicted in Fig. 4.1.

#### **4.1.5 Prevalence by faecal consistency**

Most of the faecal samples collected were of normal consistency 89.1% (336/377). However, some of the faecal samples were diarrhoeic (10.6%) and mucoid (0.3%). Of the 336 faecal samples with normal consistency, 112 (33.3%) were positive for *Giardia* antigen.

For diarrhoeic and mucoid faecal samples, prevalence of *Giardia* was 45.0% (18/40) and zero, respectively. Based on type of farm, the prevalence of *Giardia* in calves with diarrhoea was 63.6% (7/11) and 37.9% (11/29) for smallholder and commercial herds, respectively. Statistically, there was no difference in prevalence of *Giardia* among the different categories of faecal consistency ( $p = 0.205$ ).

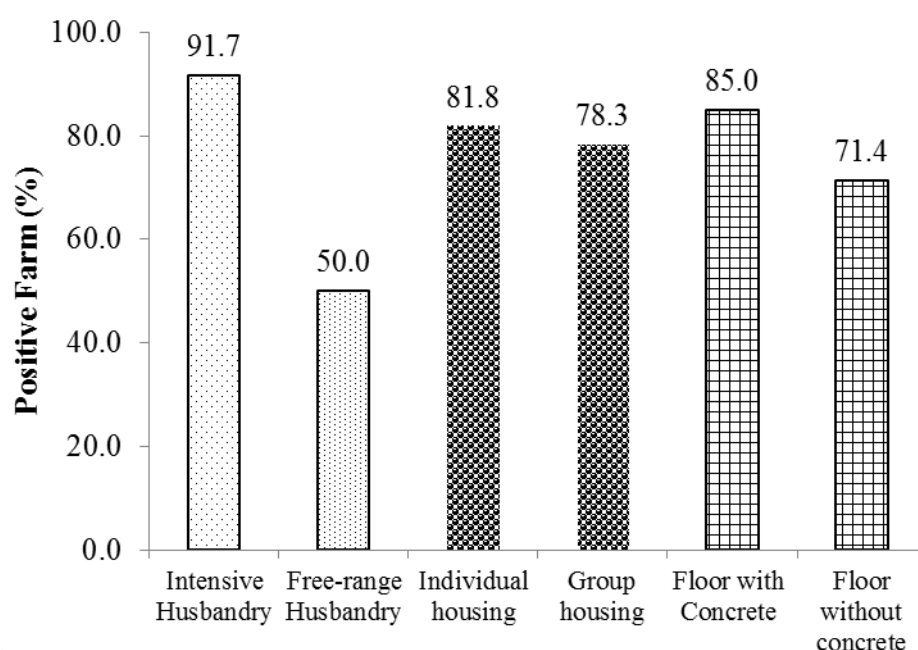
#### **4.2 Questionnaire survey**

The farm questionnaire was administered to the 34 farms included in the study. Table 4.1 summarises some of the factors that were evaluated.

Most herds practiced intensive husbandry for calves up to three months of age. Of the 34 herds that were tested, 24 practiced intensive animal husbandry system while 10 practiced free range. The prevalence of *Giardia*

was 91.7% (22/24; 95% CI = 80.66 – 102.74) for intensive husbandry (Fig 4.2).

For farms that practiced free range, 50% (5/10; 95% CI = 19.01 – 80.99) had at least one positive calf for the parasite. There was a significant difference in the prevalence for the two husbandry systems ( $p = 0.014$ ).



**Figure 4.2:** Prevalence of *Giardia* on the dairy farms taking into account husbandry system, housing and floor types.

For each herd, calves were housed either in individual pens (11/34) or as a group (23/34) (Fig 4.3 A- B). Based on type of housing, the herd prevalence of *Giardia* was 81.8% (9/11; CI = 59.0% - 104.6%) for individual and 78.3% (18/23; CI = 61.5% - 95.2%) for group housing.

**Table 4.1:** Evaluation of the association of type of bedding, type of feeding and source of water with the occurrence of *Giardia* infection in dairy calves.

Factor	Levels	Total	No +ve (%)	P value
Bedding type	Straw-hay	14	11	1.000
	Saw-dust	1	1	
	None	19	15	
Frequency of bedding removal	Daily	3	3	0.926
	Weekly	16	13	
	Monthly	4	3	
	None	11	8	
Type of feeding	Bottle	19	14	0.442
	Bucket	6	6	
	Suckle	9	7	
Water source	Borehole	28	22	0.780
	Borehole, dam	1	1	
	Borehole, stream	2	2	
	Dam	2	1	
	Stream	1	1	

There were two types of floor for calf pens at the farms: concrete and without concrete (soil) (Fig 4.3 A- B). A total of 20 herds were raised on concrete floors while 14 had no concrete floors. The percentage of positive herds for concrete floors was 83.3% (17/20; CI = 69.4% - 100.7%) and 71.4% (10/14; CI = 47.7% - 95.1%) for soil floors. There was no statistical difference in prevalence of *Giardia* between calves in pens with concrete floor and those without concrete floor ( $p = 0.295$ ).

While 15 out of 34 farms provided bedding for calf pens, 19 did not. Out of a total of 14 herds on straw/hay bedding (Figure 4B; Table 4.1), 11 were positive for *Giardia* and each of the herds on saw-dust and sand bedding was positive. The prevalence of *Giardia* on herds without any bedding was 78.9% (15/19).



**Figure 4.3:** Group calf housing on floor without concrete and bedding (A), and Individual calf housing on concrete floor with hay bedding, and bucket feeding system (B).



Frequency of bedding removal from calf pens ranged from daily (3/34), weekly (16/34) to monthly (4/16).

In herds where sand was used as bedding, it was rarely removed. All three herds where bedding from calf pens was changed daily were positive for *Giardia*. While herds that changed bedding weekly had a prevalence of 81.2% (13/16), it was 75% (3/4) among those that employed a monthly interval. Furthermore, among herds where bedding was rarely changed, prevalence of *Giardia* was 72.7% (8/11). There was no significant difference in the level of infection of herds with the parasite based on frequency of bedding removal from calf pens ( $p = 0.926$ ).

Calves were fed on milk through either a bottle, bucket or allowed to suckle (Table 4.1). There was no significant difference in prevalence of *Giardia* among herds based on method of feeding calves ( $p = 0.442$ ).

Source of water for calves on majority of the farms was borehole (82.4%,  $n = 34$ ). Of the 28 farms whose water source for the animals was borehole, 78.6% (CI = 63.4% – 93.8%) were positive for *Giardia*. Sources of water for the rest of the farms were borehole and stream, borehole and dam, dam, and stream (Table 4.1). Based on source of water for the calves, there was no significant difference in levels of infection with *Giardia* among the farms ( $p = 0.780$ ).

Out of a total of 34 herds, 19 used disinfectants for cleaning animal quarters while none was applied among the remaining herds. The prevalence of *Giardia* among herds that used disinfectants and those that did not was

84.2% (16/19; CI = 67.8% – 100.6%) and 73.3% (11/15; CI = 50.9% – 95.7%), respectively. There was no significant difference in prevalence of *Giardia* between herds that used disinfectants to clean calf pens and those that did not ( $p = 0.672$ ).

Various disinfectants were used for cleaning calf pens and most of the farms used quaternary ammonium (benzalkonium chloride) and iodine (povidone-iodine) based compounds. Based on type of disinfectant used for animal quarters, all herds that used common salt, quaternary ammonium, phenol (cresol) and lime (calcium oxide) based compounds were positive for *Giardia*. The prevalence of *Giardia* among herds that used iodine and peroxygen (hydrogen peroxide e.g. Virkon®) based compounds was 80% (4/5) and 33.3% (1/3) respectively. There was no significant difference in prevalence of *Giardia* based on type of disinfectant used to clean calf pens ( $p = 0.439$ ).

During the questionnaire survey, information on whether other disease conditions occurred on the farm was also collected. The main disease conditions reported among the herds were gastrointestinal (21/34), lumpy skin (3/34), ophthalmic (3/34) and respiratory infections (4/34). Three farms did not report presence of any disease conditions. Of the farms that reported occurrence of diarrhoea, 85.7% (18/21) were positive for *Giardia*. Furthermore, the farms reported, lumpy skin disease, ocular and respiratory infections. Additionally, they reported problems of lack of a veterinarian, poor management and negligence (where a farm worker forgot to feed calves).

Among three herds that reported problems of lumpy skin and ophthalmic infections, two of them were positive for *Giardia*. The rest of herds with respiratory infections and those that did not report any disease conditions also had at least a calf positive for the parasite. Diarrhoea (21/34), coughing (4/34) and lumpy skin (2/34) were the major clinical signs reported in most cases of calf mortality among the herds. Other conditions mentioned in cases of calf mortality were injuries and poor management (3/34).

Farmers were also interviewed on whether they dewormed their animals and the frequency. While all the herds were routinely dewormed, the frequency of deworming varied. Deworming of the animals was done once (2/34), twice (24/34), thrice (7/34) and four times (1/34) per year (Table 4.2). For herds dewormed once, twice and thrice per year, the proportions of those that tested positive for *Giardia* were 1/2, 19/24 and 6/7 respectively. Common dewormers used were albendazole (11/34), ivermectin (7/34) and levamisole (8/34). For other herds, two or three of the preceding dewormers were used. The prevalence of *Giardia* among herds that used albendazole, ivermectin and levamisole was 72.7% (8/11), 57.1% (4/7) and 87.5% (7/8), respectively.

Besides cattle, some farmers kept sheep (3/34), goats (5/34), pigs (5/34), donkeys (1/34), poultry (2/34), and one commercial farm had wildlife/impala (1/34). There were ten farms which did not rear other types of livestock. Each of the other seven farms kept multiple species of livestock (Appendix A). There was no association between prevalence of *Giardia* and presence of other types of animals kept on each farm type ( $p = 0.829$ ).

**Table 4.2:** Deworming frequency, dewormer types used and proportions of dairy herds positive for *Giardia* in the study area.

Variable	Levels	No. of herds	positive herds	Proportion (%)
Deworming frequency	Once per year	2	1	50.0
	Twice per year	24	19	79.2
	Thrice per year	7	6	85.7
	Four times per year	1	1	100.0
Dewormers	Albendazole	11	8	72.7
	Ivermectin	7	4	57.1
	Levamisole	8	7	87.5
	<sup>1</sup> Albe, <sup>2</sup> Iver	5	5	100.0
	<sup>3</sup> Leva, Albe	1	1	100.0
	Leva, Iver	1	1	100.0
	Leva, Albe, Iver	1	1	100.0

### 4.3 Risk factors associated with *Giardia* infection

A step-wise binary logistic regression model was employed to investigate and estimate risk factors associated with *Giardia*. The model fitted the data as the Hosmer and Lemeshow test was insignificant ( $p = 0.225$ ) and the Omnibus test of model coefficients was significant ( $p = 0.032$ ). The results revealed that husbandry type was the only predictor for *Giardia*. It was found out that herds reared under intensive husbandry system were 2.083

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<sup>1</sup> Albendazole

<sup>2</sup> Ivermectin

<sup>3</sup> Levamisole

(95% CI = 1.086 – 59.308,  $p = 0.041$ ) times more likely to be positive for *Giardia* than those under free range system. Other variables under investigation were insignificant predictors of being positive for *Giardia*.

Based on the logistic regression model, the mean predicted probability of a herd being positive for *Giardia* under a specific animal husbandry system was calculated. It was revealed that the mean predicted probability of a herd being positive for *Giardia* was 0.917 (95% CI = 0.889 – 0.925) and 0.500 (95% CI= 0.367 – 0.633) for intensive and free-range husbandry system, respectively.

## CHAPTER 5

### 5.0 DISCUSSION

The present study was aimed at obtaining data on occurrence of *G. duodenalis* on dairy farms in Chilanga and Lusaka districts, and the results demonstrate that the parasite is present in calves especially up to three months of age on dairy farms in the two districts. The overall prevalence of *Giardia* in dairy calves in the two districts was 34.5% and this was within the range of findings from some studies in other countries where prevalences ranged from 14% to 100% (Geurden *et al.*, 2004; O’Handley and Olson, 2006; Caccio and Ryan, 2008). In the present study, the herd prevalence was 79.4%, similar to the prevalences reported in previous studies (53% - 100%) (Geurden *et al.*, 2004; O’Handley and Olson, 2006).

The highest prevalence of *Giardia* infection was in calves  $\leq 3$  months of age. The high occurrence of *Giardia* in young animals could be due to the reported slow development of specific immunity by dairy calves against the parasite (O’Handley *et al.*, 2003). This finding is consistent with that of a previous multicentre study in Europe where it was found that the risk of infection with *Giardia* was high in calves up to two months of age (Geurden *et al.*, 2012). These results also complement the findings of a study in Belgium which revealed that the prevalence of *Giardia* was highest among calves aged one and two months (Geurden *et al.*, 2004; Trout *et al.*, 2004). In a study in Algeria, it was discovered that *Giardia* mostly infected dairy calves aged four to 12 months (Ouchene *et al.*, 2014). It was also revealed by a study in Uganda that pre-weaned cattle were more infected with

*Giardia* (10% , n= 30) than post-weaned cattle (2% , n=20) (Nizeyi *et al.*, 2002).

A study in Australia revealed that *Giardia* infection was significantly higher in calves 4-7 weeks of age (52.3%) than in those  $\leq 3$  (17.8%) or  $\geq 8$  weeks of age (29%) (Becher *et al.*, 2004). In the Australian study, the overall prevalence was 88.9% (n=54). Furthermore, a study conducted in the Northeast of China, revealed that the highest prevalence of *Giardia* was in calves less than two months old (16.1%, n=137) (Liu *et al.*, 2012). An American study found that the peak prevalence of *Giardia* was in calves aged three months, and this then declined through eight months (Trout *et al.*, 2005). In addition, Olson *et al.* (1997a) and Trout *et al.* (2005) found the highest prevalence of *Giardia* in calves aged three months. However, in the study by Olson *et al.* (1997a) the authors speculated that giardiasis was not age dependant as 80% of the calves (n= 386) aged seven to 24 weeks were infected. A study in the United Kingdom (UK) revealed that the highest risk of infection was seen in calves in the age group six to 10 weeks (43.6%, n=78) (Minetti *et al.*, 2013) similar to a study in West Bengal, India which indicated that the prevalence of *Giardia* infections was more in calves than in adult cattle (Khan *et al.*, 2011). In the Indian study, the overall prevalence of *Giardia* by both microscopy and ELISA in cattle was 12.2% (n=180), and the prevalence among the age groups 0-2 months, 3-12 months and above 12 months was 27.5% (n=40), 12.5% (n=72), and 2.9% (2/68), respectively. Other studies indicated that the peak prevalence of *Giardia* varied between four weeks and 4-5 months of age (O'Handley *et al.*, 1999; Huetink *et al.*, 2001; Becher *et al.*, 2004; Muhid *et al.*, 2011; Tiranti *et al.*,

2011). This finding was largely attributed to calf management as revealed in the study by Huetink *et al.* (2001) whereby calves in individual hutches were unable to make contact with each other hence reducing chance of transmission of *Giardia*. High prevalence was observed in calves after two months of age when they were moved to group housing. The findings of the present study are consistent with the above mentioned studies reporting high prevalence in young calves.

In the present study, the prevalence of *Giardia* between male and female calves was similar. In contrast, a study carried out in Bangladesh using sandwich-ELISA revealed that the prevalence of *Giardia* was higher in male (14.3%) than female (12.5%) calves (Suman *et al.*, 2011). In that study, the prevalence of *Giardia* among calves was 13.3% (n=15). Possible explanation for difference in the results between the study by Suman *et al.* (2011) and the current one could be due to differences in the number of calves examined (15 vs 377). In the Bangladesh study, only one calf tested positive for *Giardia* for each of seven and eight male and female calves, respectively. However, in Pakistan a study among adult dairy cattle showed that more females (31.7%, n=640) were infected with *Giardia* than males (26.3%, n= 80) (Ayaz *et al.*, 2012).

The present study did not find an association between presence of *Giardia* in faecal samples and occurrence of diarrhoea in calves. This could be due to the chronic intermittent diarrhoea episodes that are associated with *Giardia* infections so much that one time sampling may be unable to detect an association (Xiao, 1994; O'Handley *et al.*, 1999; O'Handley *et al.*, 2003). This finding is in agreement with that obtained in a study among dairy



calves of up to six months of age in Canada (Ruest *et al.*, 1998). This finding also echoed that of a study in the Netherlands which did not find an association between presence of *Giardia* cysts in faecal specimens and occurrence of diarrhoea in individual hutch calves or adult cattle (Huetink *et al.*, 2001). Similarly, a Danish study that involved dairy calves aged one to twelve months found no association between occurrence of diarrhoea and presence of the parasite in the animals (Maddox-Hyttel *et al.*, 2006). In addition, a study in Australia did not establish any relationship between faecal consistency and infection with *Giardia* (Becher *et al.*, 2004). Another study in China did not find obvious correlation between *Giardia* and diarrhoea in cattle (Liu *et al.*, 2012). Furthermore, a multicentre study in Europe found no correlation between infection and reduced faecal consistency (Geurden *et al.*, 2012). Although there was lack of association between presence of *Giardia* in faecal samples and occurrence of diarrhoea in calves in the current study, a previous study revealed that there was a potential risk for animal handlers as some of the calves could be infected with zoonotic assemblage of the parasite (Gillhuber *et al.*, 2013)

However, some studies have suggested that there is a connection between presence of the parasite and occurrence of diarrhoea in cattle (Xiao *et al.*, 1993; O’Handley *et al.*, 1999; Barigye *et al.*, 2008; Gillhuber *et al.*, 2014). The discrepancies in results between the current study and the cited studies could be due to differences in the sampling technique. In the studies conducted by Xiao *et al.* (1993), Barigye *et al.* (2008) and Gillhuber *et al.* (2014), only diarrhoeic calves were included in the study. As *Giardia* was the only pathogen found in some of the diarrhoeic faecal samples, the

authors suggested that the parasite contributed to diarrhoea. Furthermore, in the study by Xiao *et al.* (1993) outbreaks of diarrhoea in calves were attributed to *Giardia*, despite presence of *Cryptosporidium* in some samples, due to a wide age range (11 to 164 days) of calves affected and their good response to metronidazole treatment.

Occurrence of infection with *Giardia* was similar between smallholder and commercial dairy farms. This could have been due to common management practices in the farms which was intensive, regardless of the size of the enterprise.

There was no significant difference in the prevalence of *Giardia* between groups of calves housed individually or in groups in the current study. These results are similar to those that were obtained in studies in Germany, UK, France, Italy, Western Australia and Canada where there was no difference in *Giardia* prevalence between calves housed individually, group penned or pastured (O’Handley *et al.*, 2000a; Geurden *et al.*, 2012). Furthermore, other studies in Denmark revealed that different type of calf housing including individual calf hutches, solid-wall stalls or grouped calves had no tangible influence on the prevalence of *Giardia* infections in young calves (Maddox-Hyttel *et al.*, 2006). However, a study in Canada revealed that *Giardia* was highly prevalent in calves housed in individual hutches, and these did not have contact with other animals (Coklin *et al.*, 2010). This finding in the Canadian study was attributed to a number of factors such as spread of cysts from one hutch to another during run-off following heavy rains in the summer when the study was conducted. In addition, the hutch area where the study was conducted is often populated with different groups

of calves in spring and summer, and has been used for several years. Furthermore, cow-to-calf- transmission of *Giardia* could have occurred at birth (Huetink *et al.*, 2001). Despite the differences in housing in the present study, there was no significant difference probably due to similar management practices.

It was also revealed in the present study that the prevalence of *Giardia* was similar among calves raised on floors with concrete and without concrete. This could also be attributed to similar management and hygiene practices on many farms. However, in a Canadian study a higher prevalence of *Giardia* was reported at a farm where calves were housed on the ground than those on cement floors (Coklin *et al.*, 2007). In the Canadian study, it was discovered that the individual hutches which were used to house calves on the ground had been used for many years. Therefore, cysts could still have been in the environment, and transmitted through shedding to other calves, or mechanically by personnel working with the calves. This finding was similar to that of a Malaysian study where dairy calves of up to four and half months of age kept in pens with concrete floors reported a low prevalence of the parasite (Muhid *et al.*, 2011). In the Malaysian study, it was discovered that concrete floors were thoroughly washed daily with water compared to sand floors where cleaning was occasional (Muhid *et al.*, 2011).

In the current study, the levels of infection with *Giardia* among animals that received albendazole and other anthelmintics were similar. This could be due to failure of anthelmintic treatment as a result of calves being re-infected from an environment highly contaminated with *Giardia* cysts

(O’Handley *et al.*, 1999; O’Handley *et al.*, 2000b; O’Handley *et al.*, 2001; O’Handley *et al.*, 2003). Another possible explanation could be that the anthelmintic dose (10 mg/kg once) used by farmers in the current study was not effective against *Giardia*. However, previous studies have revealed that fenbendazole and albendazole (5-20 mg/kg/day for 3 days) significantly lower the peak and duration of cyst excretion (Geurden *et al.*, 2006a; Geurden *et al.*, 2010). Other studies have demonstrated that combination of fenbendazole treatment of the animals, environmental cleaning and ammonia on the third day of treatment resulted in total suppression of cyst excretion for only about two weeks (Geurden *et al.*, 2006a).

Levels of infection with the parasite among pens that were cleaned with quaternary ammonium compounds and other disinfectants were alike in the current study. Although some chemical disinfectants like quaternary ammonium compounds have been found to be effective in inactivating *Giardia* cysts, their effectiveness is dependent on such factors as the chemical and its concentration, and the contact time and cleaning procedures of the calf pens (Ongerth *et al.*, 1989, McDonnell and Russell, 1999; Geurden *et al.*, 2006a). Furthermore, studies have revealed that the parasite has the propensity to occur among calves despite the extensive application of disinfectant to the surroundings (O’Handley *et al.*, 1997). These factors could account for the finding in the current study. However, in one study, disinfection of calf housing was significantly correlated with odds for infection (Geurden *et al.*, 2012).

The present study also showed that type of bedding, frequency of bedding removal from calf pens, type of feeding, presence of other animal species,

and source of water were not significantly associated with infection with *Giardia*. However, one study revealed that prevalence of *Giardia* was higher in calves that were allowed to nurse from the dam for the first three days following calving than in those that were immediately separated from the dam and fed colostrum through a bottle (Quigley *et al.*, 1994). The differences in the findings could be due to differences in the risk factor data collection. The current study collected the data at farm/herd level while the study by Quigley *et al.* (1994) looked at individual animal level.

Based on the present study, herds reared under intensive husbandry system were more likely to be positive for *Giardia* than those under free range system. One possibility for this finding could be that cysts shed outside are subject to adverse environmental conditions and do not survive for a long period (Bingham *et al.*, 1979). Another possible explanation is that intensive husbandry tends to favour accumulation of a large number of infectious cysts in the calves' environment and infections can occur even in individual pens where disinfection is inadequate (Maddox-Hyttel *et al.*, 2006). This finding in the current study concurs with that of a Canadian study which revealed that the prevalence of *Giardia* was higher in calves housed indoors than in those outdoors (Ruest *et al.*, 1988). However, a study that included a comparison of levels of infection with the parasite between herds on intensive and semi-intensive systems did not find any significant difference between them (Muhid *et al.*, 2011). The difference in the finding between the study by Muhid *et al.* (2011) and the current study could be due to the small sample size used in the previous study. In that study, only thirteen calves (n= 120) from eight farms under intensive management tested

positive to *Giardia* compared to 123 (n=352) from 24 farms in the current study. A small sample size may not permit detection of small risk differences.

## CHAPTER 6

### 6.0 CONCLUSION AND RECOMMENDATIONS

#### 6.1 Conclusion

Based on the present study, it can be concluded that *Giardia* is present on most dairy farms in Chilanga and Lusaka districts, particularly in younger calves. Risk factors influencing presence of the parasite are management related.

Frequency of deworming of the animals was not adhered to by most farmers, and other conditions of ill-health reported among the herds were diarrhoea, lumpy skin, ophthalmic and respiratory infections.

#### 6.2 Recommendations

To prevent transmission of *Giardia*, it is recommended that an integrated approach involving discarding faeces promptly from calf pens, frequent change of bedding, regular cleaning using boiling water and subsequent disinfection of calf pens with quaternary ammonium compounds should be used on dairy farms. Farmers are encouraged to alternate between use of fenbendazole and albendazole when deworming the calves as the drugs tend to significantly lower the peak and duration of cyst excretion.

Future studies by animal and public health experts should focus on genetic characterization of the parasite so as to elucidate the zoonotic importance of *Giardia* on dairy farms in Lusaka province and identify the species and/or assemblages present in Zambia.

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## LIST OF APPENDICES

### Appendix A: Number of farms keeping multiple species of livestock

Livestock type kept	Number of farms	No. of positive farms
Sheep and goats	1	1
Sheep and pigs	1	1
Goats and pigs	1	0
Goats and poultry	1	1
Pigs and poultry	1	1
Sheep, goats and pigs	1	1
Sheep, poultry and donkeys	1	1

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<sup>4</sup>Each of the other seven farms had either sheep and goats, sheep and pigs, goats and pigs, goats and poultry, pigs and poultry, sheep, goats and pigs or sheep, poultry and donkeys.

## Appendix B: Questionnaire

Serial No.....

A survey to determine the prevalence and risk factors of *Giardia* infections in dairy cattle herds in Lusaka and Chilanga districts of Zambia.

### GENERAL FARM INFORMATION AND MANAGEMENT PRACTICES

#### A. OWNER AND FARM IDENTITY

1. Farm owner/manager: .....
2. Contact address/phone number.....
3. Gender of farm owner: a. Female [   ]    b. Male    [   ]
4. Farm name: .....
5. Farm size: .....
6. Village: .....7. District:.....
8. Province: .....9. Location (GPS reading):.....
10. No. of milking cows: .....11. Date of interview: .....

#### B. HERD COMPOSITION

1. What types of animals are kept at the farm? (circle letter)  
a. Cattle      b. Sheep      c. Goats      d. Other  
(specify).....
2. What is the total number of animals?  
a. Cattle.....  
b. Sheep.....  
c. Goats.....  
d. Others (specify).....
3. What is the total number of:

- a. Calves.....
- b. Lambs.....
- c. Kids.....
- d. Others (specify).....

## C. MANAGEMENT PRACTICES

### 1. Type of husbandry?

- a. Intensive [   ]
- b. Semi-intensive [   ]
- c. Extensive [   ]
- d. Free range
- e. Other
- (specify).....

### 2. Type of housing

Type of housing	Calves	Lambs	Kids
a) Individual			
b) Group housing			
c) Others, specify			

### 3. Type of flooring

	Calves	Lambs	Kids
a) Concrete floor			
b) Slotted floor			
c) Soil			
d) Other (specify)			

### 4. Type of bedding

Type of bedding	Calves	Lambs	Kids
a) None			
b) Straw/hey			
c) Sand			
d) Saw dust			
e) Other (specify)			

### 5. How often is the bedding removed or cleaned?

	Calves	Lambs	Kids
a) Daily			

- b) Several
- c) Weekly
- d) Less than
- e) Monthly

6. Do you use disinfectants when cleaning animal quarters?

- a. No.....b Yes.....

If yes, specify type of disinfectants.....

7. How are the neonates fed?

	Calves	Lambs	Kids
a) Suckle			
b) Bottle fed			
c) Other (specify)			

8. What is the drinking water source?

- a) Borehole.....
- b) Stream water.....
- c) Pool/dam water.....
- d) Other, specify.....

9. On average, how many litres of milk does each cow produce per day?

- a) Dry season.....
- b) Wet season.....

10. What is the total milk production per day?

- a) Dry season.....
- b) Wet season.....

#### D. DISEASES

1. What are the common disease conditions experienced in the listed animals? (list diseases for each animal species)

Calves.....

Lambs.....

Kids.....

Others.....

2. What is the major cause of mortality in the animal species below? (list causes for each animal species)

Calves.....

Lambs.....

Kids.....

Others (specify).....

3. Do you have a veterinarian who regularly attends to the animals on this farm?

Yes. ....

No.....

If yes, provide details of the attending veterinarian.....

4. Do you deworm your animals?

Yes.....

No.....

5. If yes to 4 above, how often do you deworm them?

Once per year.....

Twice per year.....

Three times per year.....

Other, specify.....

6. What dewormers do you use?

Levamisole.....

Albendazole.....

Ivermectin.....

Others, specify.....

7. When was the last time you dewormed the animals?

Last week.....

Two weeks ago.....

Three weeks ago.....

A month ago.....

Other, specify.....

8. Do you have a TB problem on the farm?

Yes.....

No.....

If yes, what measures have you put in place?.....

9. Do you have a brucellosis problem at the farm?

Yes.....

No.....

If yes, what measures have you put in place?.....

END OF QUESTIONNAIRE INTERVIEW

Duration of the interview.....