DISSERTATION

MOLECULAR EPIDEMIOLOGY OF *GIARDIA* AND *CRYPTOSPORIDIUM* IN DOGS AND CATS IN CHIANG MAI, THAILAND

Submitted by

Sahatchai Tangtrongsup

Department of Clinical Sciences

In partial fulfillment of the requirements

For the Degree of Doctor of Philosophy

Colorado State University

Fort Collins, Colorado

Summer 2013

Doctoral Committee:

Advisor: Mo Salman
Co-Advisor: Michael Lappin

Lora Ballweber
John Reif

ABSTRACT

MOLECULAR EPIDEMIOLOGY OF *GIARDIA* AND *CRYPTOSPORIDIUM* IN DOGS AND CATS IN CHIANG MAI, THAILAND

Giardia duodenalis and *Cryptosporidium* spp. are the common causes of diarrhea in humans and animals, including domestic and wildlife, throughout the world. The species complex *G. duodenalis* and the genus *Cryptosporidium* consist of host-adapted and zoonotic genotypes/species. Companion animals, especially dogs and cats, can be infected by the host-adapted as well as the zoonotic genotype/species of these organisms. Therefore, these animals have been questioned regarding their potential to serve as reservoirs for human transmission. In this dissertation, an epidemiological study of *Giardia* and *Cryptosporidium* as well as the molecular characterization of these organisms in dogs and cats in Chiang Mai, Thailand was completed. A greater understanding of the prevalence and risk factors associated with *Giardia* and *Cryptosporidium* infection can aid veterinarians in the control and prevention of these important diseases. Furthermore, the potential for zoonotic transmission will be reduced.

In Chapter 1, *Giardia* and *Cryptosporidium* and its epidemiology in dogs and cats are reviewed as well as an update on the situation regarding giardiasis and cryptosporidiosis in Thailand.

In Chapter 2, a preliminary study to determine the prevalence of *Giardia* and *Cryptosporidium* infection in dogs and cats in Chiang Mai, Thailand is described. Fecal samples were collected for two months (July and August, 2008). The genotype/species of these two organisms were determined as well as the risks associated with infection such as age, sex, diarrhea status, housing type and the presence of co-infection of *Cryptosporidium* (for *Giardia*

infection) or the presence of *Giardia* in the case of *Cryptosporidium* infection. It was shown that *Giardia* and *Cryptosporidium* infections were common in dogs in Chiang Mai and that dogs could be a potential reservoir for zoonotic transmission to humans.

In Chapter 3, the larger cross-sectional study is described. Samples were collected a year later from August 2009 to February 2010. The objectives were to determine the effect of seasonality (wet months or rainy vs. dry months or winter), to determine the potential risk factors associated with *Giardia* and *Cryptosporidium* infections, as well as to determine the genotype/species of these organisms. The results suggested that *Giardia* infection in dogs was prevalent in the rainy season, whereas seasonality was not significantly associated with *Cryptosporidium* infection. Young dogs, dogs living in crowded settings, dogs having diarrhea or chronic diarrhea, and dogs shedding *Cryptosporidium* oocysts had a high risk for *Giardia* infection. Risk factors associated with *Cryptosporidium* infection in dogs were age less than one year and dogs having diarrhea. *Giardia duodenalis* assemblage A and *C. parvum* were identified in this study; however, the potential role in zoonotic transmission could not be determined.

Chapter 4 presents a brief report on the comparison of sugar and sedimentation concentration techniques prior to immunofluorescent assay to detect *Giardia* cysts and *Cryptosporidium* oocysts. Concentration of fecal samples may enhance the detection of cysts and oocysts. However, in frozen samples the spherical structure of *Giardia* cysts or *Cryptosporidium* oocysts may be affected by the freeze-and-thaw process; therefore, the use of sugar concentration technique may not appropriate for frozen fecal samples.

Chapter 5 compares the PCR assays using different target genes in detecting *Giardia* and *Cryptosporidium* in dogs and cats from Chiang Mai, Thailand. Three PCR assays for *Giardia* were compared, including the PCR targeting to glutamate dehydrogenase (gdh), triose phosphate

isomerase (tpi), and β-giardin gene. Three PCR assays for *Cryptosporidium*, a heat shock protein targeting PCR and two PCR assays to detect SSU-rRNA (one step PCR vs nested PCR assays), were compared. *Giardia gdh* and *Cryptosporidium* one-step SSU-rRNA PCR assays had the highest amplification rates. Using a multilocus analysis approach, most of the *Giardia* isolates were dog genotypes, whereas 30%-40% of *Cryptosporidium* species were *C. parvum*. This finding may suggest a potential role of zoonotic transmission of *Cryptosporidium* from dogs and cats in this region of Thailand.

The research described in this dissertation raises the knowledge in the field of canine and feline giardiasis and cryptosporidiosis. The results provide additional prevalence and risk analysis results for dogs and cats in Chiang Mai, Thailand. The molecular analyses suggest that the use of multilocus analysis is superior to using only one locus. In addition, the results also suggest that sugar flotation was not appropriate as a concentrating method for frozen fecal material and that sedimentation should be used when freezing of the sample is necessary.

ACKNOWLEDGMENT

This dissertation is an accumulation of my work in a graduate study that would not have been possible without the assistance and guidance of several people during this long journey.

First and foremost, I would like to thank my advisor Dr. Mo Salman and co-advisor Dr. Michael Lappin for their continued support and guidance, both academic and living, throughout my PhD program. Without them, I would not have gained the invaluable knowledge and experience vital to my career. In addition, I am thankful for the many opportunities these mentors gave me for learning, training, and attending conferences and workshops. I would also like to thank Drs. John Reif and Lora Ballweber for their invaluable advice and support as great committee members. I would like to thank my colleagues in Faculty of Veterinary Medicine, Chiang Mai University, Thailand, as without their help in covering my teaching duties I would not have been able to pursue my PhD degree.

I would like to thank the Higher Education Commission, Royal Thai Government for providing me a Higher Educational Strategic Scholarship for Frontier Research Network (SFR) for my PhD program, as well as the CSU-Program of Economically Important Infectious Animal Diseases through a special grant from USDA-NIFA, PVM student grant program and the Research Council of the College of Veterinary Medicine and Biomedical Sciences, Colorado State University for financial support for the *Giardia* and *Cryptosporidium* study in Thailand. My PhD study and dissertation would not have been possible without them.

Sincere thanks to Dr. Valeria Scorza, Mellisa Brewer, Jennifer Hawley and Arianne Morris; they made my life in the lab so wonderful by not only teaching and training me on the laboratory techniques, but also by being such great sister-like friends.

I would also like to thank the staff at the Small Animal Teaching Hospital, Faculty of Veterinary Medicine, Chiang Mai University, Chiang Mai, Thailand for their help in the collection of samples. I would like to acknowledge the owners and their animals that participated in my study. Without their support I would not have had the materials for this project.

Last but not least, I would like to thank my parents, Mr. Sumet and Mrs. Kimhong Tangtrongsup, my wife Suwimol and my son Sirawit Andy Tangtrongsup for their love, understanding, encouragement, and unfailing support through the good times as well as the bad.

TABLE OF CONTENTS

ABSTRACT ii

ACKNOWLEDGMENT v

CHAPTER 1: LITERATURE REVIEW 1

1.1 *Giardia duodenalis* and its epidemiology in dogs and cats 1

1.1.1 Taxonomy 1

1.1.2 *Giardia* species 1

1.1.3 Genetic characterization of *Giardia* using molecular techniques 3

1.1.4 Life cycle 8

1.1.5 Transmission 9

1.1.6 Pathogenesis 10

1.1.7 Canine and Feline giardiasis 12

1.1.8 Zoonotic Consideration 21

1.2 *Cryptosporidium* spp. and its epidemiology in dogs and cats 22

1.2.1 Taxonomy and Nomenclature 22

1.2.2 *Cryptosporidium* species 22

1.2.3 Host specificity 23

1.2.4 Genetic characterization of *Cryptosporidium* using molecular techniques 23

1.2.4 Life cycle 24

1.2.5 Transmission 25

1.2.6 Pathogenesis 25

1.2.7 Canine and feline cryptosporidiosis 26

1.2.8 Zoonotic considerations 32
1.3 Giardiasis and cryptosporidiosis in Thailand 33
1.3.1 Human giardiasis and cryptosporidiosis 33
1.3.2 *Giardia* and *Cryptosporidium* infections in livestock 34
1.3.3 *Giardia* and *Cryptosporidium* infections in dogs and cats in Thailand 35
1.4 Tables 37
1.5 Figures 60
1.6 References 62
CHAPTER 2: PREVALENCE AND MOLECULAR ANALYSIS OF *GIARDIA* AND *CRYPTOSPORIDIUM* SPP. INFECTION IN DOGS AND CATS IN CHIANG MAI, THAILAND: A PRELIMINARY FINDING 86
Summary 86
2.1 Introduction 87
2.2 Materials and Methods 90
2.2.1 Study location 90
2.2.2 Sample collection 91
2.2.3 Diagnostic methods 91
2.2.4 DNA sequence and phylogenetic analysis 92
2.2.5 Data analysis 93
2.3 Results 94
2.3.1 Detection of *Giardia* and *Cryptosporidium* spp. infection in dogs and cats in Chiang Mai, Thailand 94

2.3.2 Univariate and multivariate analysis for risk associated with *Giardia* and *Cryptosporidium* infection in dogs and cats in Chiang Mai, Thailand 95
2.3.3 Genotyping of *Giardia* and *Cryptosporidium* isolated from positive dogs and cats in Chiang Mai, Thailand 95
2.4 Discussion 96
2.5 Conclusion 100
2.6 Tables 101
2.7 Figures 109
2.8 References 112
CHAPTER 3: PREVALENCE OF AND RISK FACTORS FOR *GIARDIA* AND *CRYPTOSPORIDIUM* INFECTION IN DOGS AND CATS IN CHIANG MAI, THAILAND: 2009-2010 STUDY 117
Summary 117
3.1 Introduction 118
3.2 Materials and Methods 121
3.2.1 Study location 121
3.2.2 Sample collection 121
3.2.3 Diagnostic methods 122
3.3 Results 124
3.4 Discussion 128
3.5 Tables 134
3.6 Figures 151
3.7 References 154

CHAPTER 4: A COMPARISON OF IFA DETECTION OF *GIARDIA* AND *CRYPTOSPORIDIUM* INFECTION AFTER TWO FECAL CONCENTRATION TECHNIQUES IN FROZEN FECAL SAMPLES 157

Sources and manufacturers 165

Declaration of conflicting interests 165

References 169

CHAPTER 5: COMPARISON OF MOLECULAR GENOTYPING USING GLUTAMATE DEHYDROGENASE (GDH), β-GIARDIN, (BG) AND TRIOSE PHOSPHATE ISOMERASE (TPI) FOR *GIARDIA* ISOLATES AND HEAT SHOCK PROTEIN 70 KDA (HSP70) AND SMALL SUBUNIT RIBOSOMAL RNA (SSU-RRNA) FOR *CRYPTOSPORIDIUM* ISOLATES FROM DOGS AND CATS IN CHIANG MAI, THAILAND 172

Summary 172

5.1 Introduction 173

5.2 Materials and Methods 175

5.2.1 Source of fecal materials 175

5.2.2 Molecular analysis 176

5.3 Results 177

5.3.1 *PCR results and sequencing analyses* 177

5.3.2 Phylogenetic analyses 179

5.3.3. *Giardia duodenalis* sub-assemblages and risk factors (age, diarrhea and housing type) 180

5.4 Discussion 180

5.5 Tables 184

5.6 Figures 191

5.7 References 197

CHAPTER 6: CONCLUSIONS AND FUTURE DIRECTIONS 200

6.1 Conclusions 200

6.2 Future direction 201

6.2.1 National study 201

6.2.2 Determining the potential for *Giardia* and *Cryptosporidium* zoonotic transmission from dogs and cats to their owners. 202

APPENDIX: QUESTIONNAIRE 203

CHAPTER 1: LITERATURE REVIEW

1.1 *Giardia duodenalis* and its epidemiology in dogs and cats

1.1.1 Taxonomy

Giardia is a flagellate, binucleate enteric protozoan parasite. This organism has a pear- or teardrop-shaped body with four pairs of flagella, one pair of median bodies, and a ventral adhesive disc. This parasite has been placed in Phylum Metamonada, class Trepomonadea, order Diplomonadida, family Hexamitidae [1].

1.1.2 *Giardia* species

Giardia was discovered more than 300 years ago by Antony van Leeuwenhoek in 1681. It was described in detail by Lambl in 1859 and, as a result, human isolates have been named after him [2]. The taxonomy of *Giardia* has been an issue of debate since then due to the similarity of trophozoite and cyst morphology for some species described in various hosts [3]. Since the discovery of *Giardia* 51 species have been described but only six species are currently accepted as valid (Table 1.1).

1.1.2.1 Giardia species characterized by morphology

In 1952, Filice utilized morphologic criteria to divide *Giardia* into three species: *G. agilis*, *G. muris*, and *G. duodenalis*. Indeed, the morphologic characteristics of *G. agilis* and *G. muris* are clearly distinguishable from others; however, in *G. duodenalis* several described species have been grouped together awaiting more efficient techniques or criteria to differentiate them. Almost 30 years later three more species were identified: *G. psittaci* from parakeets, *G. microti* from voles, and *G. ardeae* from Great Blue Herons based on morphology seen using an electron microscope [4-6].

1.1.2.2 Giardia duodenalis, a species complex

Among the six members in this genus, *G. duodenalis* (syn. *G. intestinalis* or *G. lamblia*) is a common cause of gastrointestinal disease in humans, domestic animals and wildlife [7, 8]. Eight distinct assemblages (A-H) (Table 1.2) have been identified based on allozymes and PCR DNA sequencing analyses based on small subunit rRNA (SSU-rRNA), glutamate dehydrogenase (gdh), triose phosphate isomerase (tpi), and elongation factor-1α (Ef1α); thus, it is considered as a species complex [9-13].

Assemblages A and B can infect a wide range of hosts. Assemblages C-H are considered host-adapted [1, 9, 13, 14]. Dogs can be infected by assemblages A, B, C and D, and cats can be infected by A, B and F. Because of the host specificity and the genetic distinction among *G. duodenalis* assemblages, revision of the nomenclature for this species has been proposed (Table 1.2) [3]. Assemblage H was recently discovered [13], therefore, the novel nomenclature for this assemblage has not yet been proposed. However, this novel nomenclature is not generally accepted.

Assemblages A and B are potentially zoonotic genotypes

Because Assemblages A and B can infect a wide range of mammalian hosts they are considered potential zoonotic genotypes. However, subgroups of assemblages A and B have been identified and not all subgroups have a broad range of hosts [11, 15, 16].

Sub-assemblage level of *Giardia duodenalis* Assemblages A and B

Originally, assemblages A and B were sub-classified into A-I and A-II, B-III and B-IV using allozymes analysis, and supported by the DNA analysis based on gdh locus [15]. Using allozymes analyses, members in assemblages A and B have been divided into eight subgroups: A-I to A-IV, and B-I to B-IV. A-I has been shown to have a broad range of hosts and is

considered to be zoonotic. A-II can be found in animals, but has been identified primarily from human isolates. Assemblages A-III and A-IV have been detected exclusively in animals. B-I to B-IV have not shown host specificity [11]. Recently a study based the analyses of gdh, tpi, and β-giardin (bg) loci on human and animal isolates from European countries as well as the nucleotide sequence database from GenBank, confirmed the presence of sub-assemblages A-I to A-III as well as B-III and B-IV [16]. A-I and A-II are found in both animals and humans, with A-I primarily detected in livestock and pets, whereas sub-assemblage A-II is predominantly found in humans. Subgroup A-III is almost exclusively reported in wild hoofed animals and is most likely a host-adapted genotype; however, it is also found in cats and cattle but has not been found in dogs, goats, pigs or humans [16].

Assemblages B-III and B-IV can be found in both humans and animals. Interestingly, the distribution of B-III and B-IV infections in humans varied depending on the geographic region. B-III was predominantly detected in Africa, Asia, the Middle East, and Central and South America, whereas B-IV was more likely to be detected in North America. The frequencies of B-III and B-IV were not much different in Australia and Europe [16]. Both B-III and B-IV can be detected in wildlife as well as marine mammals. Taken altogether, *G. duodenalis* sub-assemblages A-I, A-II, B-III and B-IV are considered potentially zoonotic [9, 16].

1.1.3 Genetic characterization of *Giardia* using molecular techniques

The limitation of using morphological characteristics to describe *Giardia* has been recognized, and has resulted in many species being placed under the *G. duodenalis* umbrella [17]. Therefore several molecular techniques have been developed in an attempt to solve this problem, and have become crucial methods for epidemiological studies of this organism.

Enzyme electrophoresis

Molecular analyses using isozyme or allozyme electrophoreses have revealed extensive genetic variation and host-specific patterns within the *G. duodenalis* morphologic group [3], and the existence of substructure has been confirmed by the use of DNA-based analyses [15, 18]. Recently, using a 21-enzyme system, Monis et al. [11] demonstrated seven distinct assemblages of *G. duodenalis*. However, most of the enzyme electrophoretic studies of *G. duodenalis* have limited usefulness by either using too few characters, a small number of samples, or both. In addition, in some studies the methods are open to criticism and the interpretation of the results for environmental samples is questionable [3].

DNA-based study

The majority of DNA-based studies of genetic polymorphism have focused on *G. duodenalis*. A variety of techniques such as Polymerase Chain Reaction (PCR), Restriction Fragment Length Polymorphism (RFLP), Random Amplified Polymorphic DNA (RAPD), M13 fingerprinting and nucleotide sequencing have been used to characterize *G. duodenalis* isolates. The list of target genes, type of assay and main use of amplification-based techniques for *Giardia* is shown in Table 1.3.

RFLP

RFLP is a technique where the pattern of DNA fragments resulting from the restriction endonuclease is examined and compared among isolates or compared to a reference strain. One of earliest RFLP studies was done by Nash et al. [19], who examined the RFLP patterns from the whole genome of 15 human and animal isolates of *G. duodenalis*. Two major groups were revealed. The RFLP patterns of trophozoite variant-specific surface protein (VSP) gene have also been studied, and two RFLP patterns were identified in ten axenic isolates by Ey et al. [19]. These two patterns corresponded to the genetic groups I and II identified by Andrew et al. [20].

The use of PCR-RFLP on conserved segments from a subset of *G. duodenalis* VSP genes allowed the identification of isolates belonging to genetic groups I and II as well as the Novel livestock group [21, 22]. PCR-RFLP using the gdh gene was used to identify *Giardia* assemblages A and B, and demonstrated that "Polish" and "Belgian" strains were assemblages A and B, respectively [11]. A PCR-RFLP of gdh to characterize *G. duodenalis* isolates from a variety of host species was performed by Read et al. [18] and the representing patterns for Assemblages A-I, A-II, B-III, B-IV, C, D, and E were identified. In that study primers were designed to amplify a 432-bp region of the gdh gene. This PCR-RFLP has proven to be a reproducible, reliable and sensitive method for genotyping *Giardia* from humans, companion animals and livestock and has been widely used [3].

DNA fingerprinting

DNA fingerprinting techniques such as minisatellites and random amplified polymorphic DNA (RAPD) have been useful for differentiating *Giardia* isolates. However, the use of the DNA fingerprinting has been questioned. DNA fingerprinting using the phage M13 or human minisatellite sequences as probes has been able to differentiate isolates of *Giardia* when other methods could not [3]. The first DNA fingerprinting report used the bacteriophage M13 as a hybridization probe to detect minisatellite polymorphisms and found the method to be highly discriminatory [23]. The M13 DNA fingerprinting was also used to demonstrate the mixed infection of two different strains of *G. duodenalis* in a human case of chronic giardiasis [24]. However, the hyper-variability of these minisatellite regions has been shown and the suitability of this marker for epidemiological studies has been questioned. The long-term stability of these markers needs to be verified [3].

RAPDs have also been shown to have a discriminatory power similar to isoenzyme analysis [3, 25-27]. Depending on the primer used, RAPD-PCR has detected polymorphisms between different isolates as well as between clonal lines of the same isolates. This technique may be useful for the examination of strain population structure as well as for the study of localized outbreaks. Genetic analysis of RAPD banding patterns by Morgan et al. [25] demonstrated a correlation with the grouping determined by isoenzyme analysis. Nonetheless, the long-term stability of the banding patterns of isolates also needs to be verified. Recently, Paleyo et al. [27] employed RAPDs to characterize 18 isolates of *G. duodenalis* from humans. They classified the isolates into six clusters and exhibited a good correlation between the cluster and the clinical and epidemiological characters.

Nucleotide sequencing

Nucleotide sequencing was initially used to identify sequences for *Giardia* diagnosis and to study the evolutionary relationship between *Giardia* and other eukaryotes using phylogenetic analysis on sequences to determine the genetic distance among isolates. Many PCR protocols targeting to SSU-rRNA, elongation factor 1 α, gdh, and β-giardin have been developed and applied to epidemiological and clinical samples.

A widely used PCR protocol for *Giardia* SSU-rRNA was developed by Hopkins et al. [28]. This PCR amplified a 292 bp region which was used to compare the same region of *Giardia* isolates from humans and dogs. Of 38 amplified sequences, four genotypes were identified, two for humans and two for dogs. PCR for SSU-rRNA has been able to show the discrimination of *Giardia* isolates from a variety of hosts. However, this protocol cannot differentiate between sub-assemblages A or B [29].

A PCR assay based on gdh was initially developed to characterize human *G. duodenalis* and it correlated with the results from isoenzyme analysis [15]. Several PCR-RFLP protocols were also developed [18, 30]. The nucleotide sequences of this gene are highly polymorphic and distinct assemblages can be successfully characterized. Nucleotide sequence analysis using this gene has been shown to be superior to using the SSU-rRNA sequence since sub-assemblages can be identified using gdh [29].

The genetic characterization of *Giardia* isolates using tpi genes was first described by Sulaiman et al. [31]. However, with this original PCR protocol assemblages A, B, C, E and rat and cat genotypes were identified, but not assemblage D. Later a modified PCR protocol to detect assemblage D was developed by Lebbad et al. [32]. This protocol also showed high discriminatory power in differentiating all eight assemblages as well as other species.

The PCR to amplify β-giardin was first developed in 1992 by Mahbubani et al. [33], and the more sensitive nested PCR protocol was developed by Caccio et al. [34]. In that study the β-giardin PCR protocol was used to analyze 21 reference strains (human, cat, guinea pig, calf and pig) and 30 human clinical samples. Assemblages A, B and E were identified. The results from β-giardin correlated well with SSU-rRNA and gdh genes. The use of PCR targeting β-giardin to determine *Giardia* isolates from various hosts, including humans, calves, dogs, and cats, has been reported and substructuring has also been identified [35].

Multilocus genotyping

The molecular analysis of *Giardia* isolates using PCR and nucleotide sequencing is crucial for epidemiologic study as it provides unbiased information. However, the limitations of PCR have been recognized. Not all PCR works with every sample. Some samples may be amplified with one PCR but not by others. So, a negative result on one PCR does not mean the

sample is free of *Giardia*. In addition, the genotype from one gene may be different from another gene. This problem is significant when a host-adapted genotype is identified based on one gene and a zoonotic genotype from another. Therefore, to achieve more accurate genotype identification, multiple loci genotyping has been suggested for epidemiological studies [32].

1.1.4 Life cycle

Giardia has two stages in its life cycle: the trophozoite and the cyst. The trophozoite is the active motile form that colonizes in the host's intestinal tract. *Giardia* trophozoites are approximately 12-15 μm long and 6-8 μm wide with the exception of *G. agilis* that has a trophozoite measuring 20-30 μm long and 4-5 μm wide. Median bodies can be observed as either claw-, round- or club-shaped, depending on species [2]. The cyst has an ellipsoidal shape with a size of 8 to 12 × 7 to 10 μm [36].

The life cycle of *Giardia* is simple and direct and is complete in one host. After the cyst is ingested by the susceptible host, excystation – the process where the immature trophozoites are released from the cyst – occurs in the duodenum after exposure to the acidic environment of the stomach and pancreatic proteases [8]. Two trophozoites then separate and mature quickly, within 15 to 30 minutes after the onset of excystation, and attach to the brush border of the villous epithelium [8]. Trophozoites multiply by binary fission in the intestinal tract and encyst – the process where *Giardia* trophozoites transform into a cyst stage – after exposure to a high level of bile, a low level of cholesterol and a basic pH. Cysts passed along with the feces are immediately infective.

The trophozoite stage can be found in diarrheic feces because of the short transit time for intestinal contents, but it is not environmentally resistant; therefore, it is not the major stage responsible for transmission. The *Giardia* cyst is environmentally resistant and can live for

weeks to months in wet and cold conditions after excretion and is mainly responsible for transmission; however, this stage is sensitive to desiccation and heat [37].

1.1.5 Transmission

Giardia infection in susceptible hosts can be transmitted via the fecal-oral route by infectious cysts, either directly or indirectly. Direct transmission can occur from infected to healthy individuals by direct contact; for example, in humans direct transmission could occur in a day care or by sexual contact [38]. In animals, direct transmission can occur in nursing barns, kennels, catteries, or in a crowded environment such as a shelter. Indirect transmission can occur through the ingestion of *Giardia* cysts in contaminated food or water.

1.1.5.1 Cycles of Transmission

Based on epidemiological and molecular epidemiological data, *G. duodenalis* can be maintained in at least four cycles of transmission. *Giardia* that maintain in each cycle can be transmitted to other cycles via either direct contact or indirectly through food or water contamination [3].

Humans

Molecular epidemiological studies revealed that the *Giardia* maintained in humans are assemblages A-I, A-II, B-III, and B-IV. In humans, person-to-person transmission of *Giardia* can occur directly in environments where hygiene may be compromised such as in a day care center or a disadvantaged community setting in the developing world, or by sexual activities [3, 38].

Livestock

Assemblages A-I, A-II, and E are the primary types that infect livestock. Assemblage E is considered host-adapted, and A-I and A-II are possibly zoonotic. In livestock the major source of

transmission is likely to be direct contact between animals; for example, grouping calves in pens greatly increases the chance of *Giardia* transmission. Contaminated soil is considered to be a potential reservoir that endangers newborn or newly introduced animals [3].

Dogs and cats

Dogs and cats can be infected with host-adapted assemblages C and D for dogs and F for cats as well as zoonotic genotypes assemblages A-I, A-II, B-III, and B-IV in both dogs and cats. *Giardia* infection in dogs and cats can be transmitted directly via direct contact in a crowded environment such as a shelter, kennel or cattery [39-42]. Communal recreation areas may be another potential source of infection, either directly or indirectly. One study by Wang et al. [43] showed that dogs visiting dog parks are more likely to be infected with *Giardia*.

Wildlife

Wildlife such as beaver, nutria, deer, and rodents can have a high prevalence of *Giardia* infection. Recent studies have confirmed the zoonotic genotypes of *G. duodenalis* in wild fallow deer [44], white-tailed deer [45], red deer, roe deer [46] and beavers [31]. With the current knowledge of molecular epidemiology of *Giardia,* wildlife have been shown to carry assemblages A-I, A-II, A-III, B-III and B-IV. A-III is exclusive to wildlife [16].

1.1.6 Pathogenesis

Although giardiasis has a worldwide distribution and *Giardia* has been discovered for more than three hundred years, the pathogenesis of giardiasis and its virulent factors are not completely understood [47]. Based on *in vitro* and *in vivo* studies, the pathogenesis of *Giardia* is a multifactorial process involving the parasite-host interaction [47].

Studies of *Giardia* infection *in vitro* found that parasite-host interactions lead to the up-regulation of genes involved in the apoptotic cascade and the formation of reactive oxygen

species in the intestinal cells. The enterocyte apoptosis may cause a loss of intestinal epithelial barrier function resulting in increased intestinal permeability. This change allows luminal antigens to activate a host immune-dependent pathological pathway. Recent studies have revealed a novel biological process that enhances glucose uptake by activation of sodium coupled glucose transporter-1 (SGLT-1) and may rescue enterocytes from lipopolysaccharide-induced epithelial cell apoptosis [48]. *Giardia* infection also alters the intestinal epithelial barrier by disruption of cellular F-actin and tight junctional ZO-1, as well as the alpha-actinin, a component of the actomyosin ring that regulates paracellular flow across intestinal epithelia. *Giardia* also alters the claudin proteins which are critical components of the sealing properties of tight junctions [48].

Giardia clinical abnormalities can be observed even in the absence of villus atrophy or mucosal injury. Studies using *in vitro* and *in vivo* models as well as from humans infected with *Giardia duodenalis* reported that this organism stimulates the diffuse shortening and/or loss of brush border microvilli. A loss of epithelial absorptive surface area leads to malabsorption of glucose, sodium and water, and reduced disaccharidase activity. Chloride secretion is also induced by *Giardia* [48].

The epithelial barrier alterations, epithelial brush border injury, and disaccharidase deficiencies appear to be mediated by $CD8^+$ T-lymphocytes. During the course of disease, increased numbers of intra-epithelial lymphocytes can be observed in associated with the sodium/glucose malabsorption [48].

Diarrhea in giardiasis appears to result from the combination of intestinal epithelial malabsorption of nutrients and electrolytes and hypersecretion of chloride and water [48]. In some cases of giardiasis, reduced lipase activity may be also observed and result in occasional

production of steatorrhea [49]. In addition, the loss of epithelial barrier caused by *Giardia* infection leads to the uptake of intestinal antigen to activate a host immune-dependent pathological pathway. Mast cell hyperplasia has been observed to follow infection-induced loss of the intestinal barrier and may be responsible for hypersensitive reactions observed in chronic giardiasis [50]. The chronic disorders include food allergy, inflammatory bowel disease (IBD), and irritable bowel syndrome (IBS) [48].

1.1.7 Canine and Feline giardiasis

1.1.7.1 Prevalence and molecular analysis of giardiasis in dogs and cats

Giardia duodenalis is one of the common causes of gastrointestinal disease in dogs and cats worldwide [37, 51]. It has been estimated that the prevalence of giardiasis in dogs is up to 10% in household dogs, up to 50% in puppies, and up to 100% in shelter dogs [52, 53]. Nevertheless, the prevalence of giardiasis in dogs and cats varies depending on the location, population tested, diagnostic tests and time (Table 1.4 and Table 1.5). For example, in one study in Australia the overall prevalence in dogs was 22.1%. However, when divided into sub-populations, the prevalence in samples from veterinary clinics or exercise areas was as low (7.4% - 7.8%), whereas the prevalence was higher (29% - 37%) in samples from breeding kennels, refuge dogs and pet shops [54]. In another study, four diagnostic tests (zinc sulfate flotation, immunofluorescent antigen test, fecal ELISA, and PCR) applied on the same samples revealed prevalences ranging from 7.9% to 95.9% [55]. A seasonal effect has also been reported on *Giardia* prevalence in dogs with prevalence higher in the summer than in the winter [39]. From Table 1.4 and Table 1.5, it seems that when tests with higher analytical sensitivity are used, i.e. IFA, ELISA or PCR, the higher the probability *Giardia* will be detected in the study population.

Dogs and cats are commonly infected with the host-adapted genotypes: assemblages C and D for dogs and assemblage F for cats (Table 1.6 and Table 1.7). However, it has been shown that in some geographic regions the majority of dog and cat isolates were zoonotic genotypes; for example, this has been described in samples from Europe [16], Germany [56], Portugal [57], Italy [58, 59], Canada [60], Mexico [61], India [62] and Thailand [55].

1.1.7.2 Clinical abnormalities

Giardia infection in dogs and cats has long been recognized: *Giardia* in cats has been reported since 1925 [63, 64] and *Giardia* infection in dogs has been reported since 1946 [65]. However, the clinical relevance of this organism was not recognized until the 1980s [66, 67]. Puppies and kittens are at the most susceptible age for *Giardia* infection, but the organism is not always an effective primary pathogen. Many infected dogs and cats are subclinical carriers. A prepatent period of 5-12 days was reported for giardiasis in experimentally infected dogs [68, 69], and 5-16 days in naturally and experimentally infected cats [66]. Most dogs and cats infected with *Giardia* are subclinical; however, in sick animals diarrhea is the most common clinical sign. Dogs and cats may have acute, short-lived, intermittent or chronic diarrhea. The feces are often pale, malodorous, and steatorrheic. Most infected dogs and cats are afebrile, bright, and alert and have a normal appetite. Severe diarrhea can be observed in young or immune-compromised animals and may result in dehydration, lethargy, and anorexia. Acute vomiting may occasionally be observed [70].

In some animals, chronic malabsorption occurs and therefore weight loss and poor body condition may be detected. On physical examination the small intestine may be slightly thickened and the animal can appear unthrifty. The severity of the disease may be related to interaction of both host and strain factors. Presence of immunosuppressive diseases or co-

infection with other pathogens may also potentiate the development of clinical signs of disease [71-73].

1.1.7.3 Mode of transmission

Giardia duodenalis infection in dogs and cat is transmitted by the fecal-oral route. Direct transmission can be from infected to healthy individuals by direct contact especially in high density or crowded settings such as in kennels, catteries, or in stray animals. Indirect transmission can occur through the ingestion of cysts in contaminated food or water. [55].

1.1.7.4 Diagnosis

The clinical signs and results from standard laboratory tests in *Giardia*-infected animals are not pathognomonic. Therefore, the diagnosis of *Giardia* infection is based solely on the detection of its two stages, the trophozoite and the cyst, in the feces or samples collected from the intestinal tract [37]. The traditional diagnostic tool is a microscopic fecal examination; however, more rapid and more sensitive techniques have been developed such as immunofluorescent assays and enzyme linked immunosorbent assays. In addition, several molecular techniques have been developed for species and genotype determination for molecular epidemiological studies and to determine zoonotic genotypes [74].

Detection of *Giardia* is a diagnostic dilemma. *Giardia* is one of the most commonly misdiagnosed, underdiagnosed and overdiagnosed parasites [36]. False-positive and false-negative fecal examination results are commonly reported from inexperienced workers. False-positive fecal exams can result from mistakenly identifying pseudoparasites, such as yeasts, plant remnants and debris, as *Giardia*. False-negative results, through the failure to identify *Giardia* cysts, can be from deterioration of *Giardia* cysts in the fecal flotation or inability to identify the *Giardia* cyst due to its small size. In addition, *Giardia* cysts are shed intermittently [52] and this

variation may mask the detection of *Giardia* cysts in feces from infected dogs and cats [66, 75, 76]. It has been reported that young dogs shed an average of 200 cysts per gram of feces, and that the average cyst count per gram of feces for all infected dogs was 705.8 [76]. Another study found that the number of cysts shed by dogs ranged between 26 and 114,486 cysts per gram of feces [75]. Shedding of *Giardia* cysts by cats may vary from undetectable to concentrations of >1,000,000 cysts per gram of feces [66]. It has been shown that peaks of fecal shedding occur sporadically rather than cyclically, and the duration between any two given peaks is generally from two to seven days [66]. Therefore, a single negative test result cannot definitively rule out *Giardia* infection, and repeated fecal examination may be needed to confirm an infection. Three fecal samples collection over 7 days has been suggested to confirm *Giardia* infection in dogs and cats [36]

Fecal microscopic examination

Direct smear

The direct smear or fecal wet mount is simple to perform. It can be used to evaluate for the presence of trophozoites of *Giardia* spp. with or without staining agents and using light microscope [77]. This procedure is important in the detection of *Giardia* in diarrheic fecal samples; however, it can be performed using either diarrheic or formed fecal samples. The trophozoites are short-lived outside the host; therefore, examining a sample several hours after collection or refrigerating the sample greatly reduces the likelihood of identifying the organism in that sample. Furthermore, morphological differentiation of *Giardia* and *T. foetus* trophozoites may be difficult, so fecal concentration techniques, an antigen test, or PCR specific for the organism can be used to confirm the infection. The disadvantage of the direct smear technique is

its low sensitivity. Sensitivity of 30% - 40% has been reported even when feces were examined in three different days [78].

Concentration techniques

Concentration techniques to detect *Giardia* cysts are recommended when the trophozoites are not seen on the direct smear [52, 78]. Although sedimentation can be performed, most samples are concentrated with fecal flotation. Common flotation solution recipes can be found elsewhere [79]. These solutions are effective and are easy to make or purchase. However, fecal flotation with zinc sulfate ($ZnSO_4$, specific gravity 1.18) or sugar (specific gravity 1.27) centrifugal flotations are the optimal techniques for the demonstration of *Giardia* cysts [37]. Passive flotation is not recommended as it has been shown to be less sensitive [36, 80]. Sugar solution is hypertonic and pulls the cytoplasm of the cysts to one side, makes it appear as a half or quarter moon. Thus, some parasitologists prefer zinc sulfate solution over sugar since the appearance of the cysts is preserved when examining the slide [37].

The sensitivity and specificity of concentration techniques range from 50% to more than 90% [52, 81]. In cases with diarrhea, combining fecal flotation with a wet mount examination or with a fecal antigen assay will increase the sensitivity. In addition, the sensitivity of fecal flotation increases to more than 90% if at least three fecal specimens are examined within 7 days [36].

Although the sensitivity is not perfect when a single sample is evaluated, fecal flotation remains the primary *Giardia* diagnostic test because it can be used to identify many other potential parasites. If the feces contain much fat, formalin-ethyl acetate sedimentation is the best technique for detecting cysts [78].

Fecal monoclonal immunofluorescent assay (IFA)

Several fluorescein-labeled monoclonal antibody systems have been developed. These include the Merifluor® *Cryptosporidium*/*Giardia* direct immunofluorescence assay (Meridian Bioscience, Inc., Cincinnati, OH, USA), Crypto/*Giardia* Cel (Cellabs Pty, Ltd., Brookvale, NSW, Australia), and Cyst-a-Glo™ (Waterborne, Inc., New Orleans, LA, USA). These systems utilize monoclonal antibodies reacting with *Giardia* spp. cysts and *Cryptosporidium* spp. oocysts. The Merifluor® kit is widely used for *Giardia* detection. Although this test has been developed from human isolates, multiple studies have evaluated the test in dog and cat samples [82-86]. These studies revealed that this assay is superior to $ZnSO_4$ flotation and antigen detection techniques [81, 85, 86]. Although in one study the IFA test was shown to be comparable to zinc sulfate centrifugal flotation and the *Giardia*-antigen assay (Heska Corporation, Fort Collins, CO) [87], with its high sensitivity and specificity, some researchers have considered IFA as a gold standard test [81, 86].

The advantage of IFA is that a false positive is unlikely because with immunofluorescent detection the observer can base the diagnosis not only on the fluorescence but also on the morphology (size and shape) of the organism. In addition, co-infection of *Cryptosporidium* in *Giardia*-infected dogs and cats has been reported [71, 73, 88], so *Giardia* and *Cryptosporidium* infection can be diagnosed simultaneously using this technique. Veterinarians can take the advantage of this dual detection for their patients. Compared with *Giardia* antigen assays, the primary disadvantages of the IFA include the need for a fluorescence microscope and additional technician time. A false negative result is possible due to the lower detection limit of this test at about 10^5 cysts per gram of feces [89] and poor performance of technician/examiner, such as rigorously wash the slide or use low magnification power to observe the cyst/oocyst.

Fecal antigen detection

Many enzyme-linked immunosorbent assays (ELISA) for detection of *Giardia* antigens in feces have been developed and marketed mainly for humans. One point-of-care *Giardia* antigen test is commercially available and licensed for use with dog or cat feces (SNAP *Giardia* Test, IDEXX Laboratories). ELISAs have been evaluated for the detection of *Giardia* infection in humans and animals; however, the results varied among methods and were inconsistent [85, 90-93].

Using the coproscopical method, *Giardia* cysts were identified in 9.5% and 0% of dog and cat fecal specimens, respectively, whereas 29.5% and 22.4% were identified using ELISA (ProSpecT^{TM} *Giardia* Microplate Assay, Remel, Inc.) [94]. One study evaluated fecal flotation and four ELISA based tests (SNAP *Giardia*, ImmunoCardSTAT! *Cryptosporidium*/*Giardia* Rapid assay[1], Xpect *Giardia*/*Cryptsporidium*[2], and ProSpecT^{TM} *Giardia* Microplate Assay) for the detection of *Giardia* infection in feline samples [86]. Using the IFA as a gold standard, ProSpecT *Giardia* Microplate Assay had the highest sensitivity with 91.2%, followed by SNAP *Giardia* (85.3%), $ZnSO_4$ flotation (85.3%), Xpect *Giardia*/*Cryptosporidium* (79.4%), and ImmunoCardSTAT! *Cryptosporidium*/*Giardia* Rapid assay (72.7%). All five tests had a specificity of more than 99% [86]. In another study, the sensitivity and specificity of the *Giardia* SNAP test was reported to be 92% and 99% when compared to ProSpecT *Giardia* Microplate Assay, and 90% and 96% when compared with IFA [95]. In one study in our laboratory, a 94.4% agreement of *Giardia* SNAP test with IFA was shown, and with the ELISA tests (Xpect, ImmunoCardSTAT!, and *Giardia* SNAP test) agreements were 97.2 - 100% [83].

[1] Meridian Diagnostics, Inc., Cincinnati, OH, USA
[2] Remel Inc., Lexana, KS, USA

The advantages of ELISA include batching many samples into a single run and the rapid assay the test can be done within 10 minutes [93]. In addition, for the SNAP *Giardia* test, a spectrophotometer is not necessary. However, these ELISA kits are rapid tests, and a high incidence of false positive and false negative results have been reported for *Giardia* antigen assays. The antigen assays should be supplemental tests and should not replace fecal flotation and wet mount examination [37].

Polymerase chain reaction (PCR)

Giardia infection can be determined using PCR assay to amplify the selected target gene. Several target genes have been evaluated in human and animal fecal samples for *Giardia* DNA detection and have been applied on clinical samples. These target genes include SSU-rRNA [28], gdh, tpi [31, 32] and β-giardin [34]. A PCR assay is more analytical sensitive than conventional microscopic and immunologic techniques; it may be advocated in a case of chronic diarrhea where the conventional tests have failed and the individual did not respond to conventional treatment. The disadvantage of PCR assay is that PCR inhibitor(s) may be present in the fecal DNA samples and cause a failure to amplify *Giardia* DNA.

1.1.7.5 Recommendation

The Companion Animal Parasite Council (www.capcvet.org) recommends testing dogs and cats with diarrhea with the combination of direct smear, fecal centrifugal flotation, and *Giardia* antigen assay. If *Cryptosporidium* spp. co-infection is suspected, it may be prudent to substitute the IFA for the *Giardia* antigen test in the initial diagnostic workup. In addition, repeat testing performed over several days is also recommended to enhance the sensitivity of the detection if the initial results are negative.

1.1.7.6 Treatment and Prevention

Many drugs used to treat *Giardia* in dogs and cats have been extrapolated from human medicine and have been evaluated in both naturally and experimentally infected dogs and cats. However, no drugs have been officially approved for treating canine and feline giardiasis so this use is extra-label. The drugs that have been used to treat giardiasis in dogs and cats are summarized in Table 1.8 [78, 96-106].

Use of metronidazole USP or metronidazole benzoate may be preferentially suggested if clinical findings indicate concurrent *Clostridium perfringens* overgrowth because this drug is also effective against *Clostridium* spp.. In one experimental study, metronidazole benzoate formulated into a tuna suspension was apparently effective and safe in cats [107]. Care should be taken when using metronidazole because central nervous system toxicity can occur [108-110]. If the clinical findings suggest a concurrent infection with nematodes or cestodes, the use of fenbendazole or febantel combination should be indicated. Treatment failures have been attributed to resistance of *Giardia* to drugs, lack of client compliance, failure of the animal to adequately ingest the drug, and re-infection [111]. Re-infection can be from the environment (fecal contaminated water and soil) or from the animal itself (contaminated hair and coprophagia).

The most effective way to prevent *Giardia* infection is to avoid the ingestion of cysts from contaminated environment, especially from water or food. These procedures include filtering or boiling water before drinking, because chlorine disinfection of public drinking water is not completely effective in killing *Giardia* cysts. Feces from infected animals should be removed promptly from the environment. The organism can be inactivated on contaminated surfaces by thorough cleaning followed by steam cleaning or disinfecting with quaternary

ammonium compounds. Mechanical vectors should be controlled. Treatment and bathing of all animals in the same environment should be considered, particularly if repeated bouts of diarrhea are occurring [37].

Giardia vaccines previously licensed by Fort Dodge for dogs and cats were classified as generally not recommended as preventatives by the vaccine guideline committees at the American Animal Hospital Association and the American Association of Feline Practitioners As a result, both products have been discontinued by the manufacturer.

1.1.8 Zoonotic Consideration

Because the species complex *Giardia duodenalis* contains both host-adapted and zoonotic genotypes (Table 1.2) and assemblages A, B, C, D, E and F have been reported in human patients [16, 55, 112], there are public health concerns that dogs and cats can be a potential reservoir for disease transmission.

Healthy pets are not considered significant human health risks by the Centers for Disease Control and Prevention (www.cdc.gov/hiv/pubs/brochure/oi_pets.htm), and there is no current recommendation for testing healthy dogs or cats for *Giardia* infection. However, it is recommended that all healthy dogs and cats should be screened for hookworm and roundworm infection once or twice yearly and by this recommendation some healthy dogs and cats that are harboring *Giardia* cysts will certainly be detected.

Some *Giardia* may be zoonotic, and genotyping of *Giardia* species and/or treatment of healthy infected animals should be considered with each owner. Treatment of subclinical animals is debatable as all of the drugs can potentially cause adverse effects. Moreover, re-infection can occur within days and treatment is unlikely to eliminate the infection. There is also no consensus

on retesting for *Giardia* infection if the animal is subclinical however, if it is necessary, fecal flotation is recommended (www.capcvet.org).

1.2 *Cryptosporidium* spp. and its epidemiology in dogs and cats

1.2.1 Taxonomy and Nomenclature

Cryptosporidium are coccidian protozoan parasites that are classified in Phylum Apicomplexa, Class Coccidea, Order Eucoccidiorida, Family Cryptosporidiidae, [113].

1.2.2 *Cryptosporidium* species

Cryptosporidium was first discovered and described by Ernest Edward Tyzzer who recognized this protozoan parasite from gastric glands of domestic mice in 1907, and at that time the name *Cryptosporidium muris* was proposed [114]. In 1910, greater detail of this parasite was described, including the life cycle by artificial infection through feeding gastric mucosa and gastric contents from infected mice to young uninfected mice [115]. Following that study, Tyzzer added Japanese waltzing mice and English mice as hosts of this parasite.

In 1912, Tyzzer described another new species, *Cryptosporidium parvum*, that infects only the small intestine of tame laboratory mice and has smaller oocysts than *C. muris* [113]. Forty-three years later, in 1955, Slavin described *Cryptosporidium meleagridis* which infects turkeys and is associated with illness and death [113, 116].

Since Tyzzer's discovery in 1907, more than 40 genotypes/species of *Cryptosporidium* have been reported. However, not all are considered valid species names due to the lack of morphologic data, cross-transmission studies, or that a re-examination of the descriptions has resulted in invalidation of its name [113, 117]. Currently, there are at least 24 valid species of *Cryptosporidium* [117-123]. The hosts and the mean oocyst sizes are shown in Table 1.9.

1.2.3 Host specificity

The protozoan parasites in the genus *Cryptosporidium* consist of host-specific and zoonotic species that can infect over 150 mammalian hosts including humans, as well as birds, reptiles, amphibians and fish [113-138]. In general, to determine the host range for a species or genotype of *Cryptosporidium,* oocysts are obtained from the animal of one species and fed to or intubated into animals of another species. If the life-cycle is completed and the excreted oocysts are identical (by either morphology or genetic characterization) to the fed oocysts then the host range is extended [113]. Most *Cryptosporidium* species and genotypes are host-adapted and have a narrow spectrum of natural hosts. Therefore, one species or genotype usually infects only a particular species or group of related animals. However, some species/genotypes demonstrate exceptions to be able to infect a wide range of hosts including humans (Table 1.10) [139].

1.2.4 Genetic characterization of *Cryptosporidium* using molecular techniques

Over the last decade, the development of sensitive molecular techniques for detection, genotyping, and sub-genotyping has allowed the identification of sources of oocyst contamination and routes of transmission in both outbreak and non-outbreak situations [140]. Several techniques have been developed such as SDS-PAGE Western blotting with monoclonal antibodies [141-143], isoenzyme electrophoresis [144], whole DNA restriction fragment patterns [145], random amplified polymorphic analysis [146], PCR-RFLP analysis [147, 148], and PCR generated DNA sequence data of a specific gene [149]. This latter approach includes sequence analysis of SSU-rRNA, hsp70, *Cryptosporidium* oocyst wall protein (COWP), small double strand virus-like RNA, microsatellite and minisatellite analysis and multilocus sequence typing (Table 1.11).

1.2.4 Life cycle

The primary site of infection of *C. hominis* and *C. parvum* is the small intestine, but it can be found at extra-intestinal sites such as the respiratory tract. In some animals, such as mice and calves, *C. parvum* is found in the ileum above the cecal junction. Other species such as *C. muris*, *C. andersoni*, and *C. serpentis* infect the gastric mucosa. In the chicken, *C. baileyi* favors the respiratory tree and cloaca [113].

The life cycle of *Cryptosporidium* is direct and complete within one host. The sporulated oocyst passed in the feces of the infected host is responsible for transmission to the next host. Following excystation, four sporozoites are released in the gastrointestinal tract (or respiratory tract) and infect the mucosal epithelial cells, invading the intestinal cells at the apical end.

After the sporozoite attaches to the host cell, the sporozoite becomes oval or spherical; then the parasitophorous vacuole is formed. The sporozoites in parasitophorous vacuoles are intracellular but extracytoplasmic, as sporozoites are not directly in contact with the cytoplasm of the host cell. At this stage it is called a trophozoite. *Cryptosporidium* has asexual and sexual reproduction. A trophozoite differentiates into a type I meront which contains 4-6 nuclei, each of which develops into a merozoite. Each mature merozoite leaves the meront to infect another host cell and develop into another type I or type II meront. Type II meronts produce 4 merozoites. After a merozoite from a type II meront infects a new host cell, it differentiates into either a microgamont (male) or macrogamont (female) stage. Each microgamont then becomes multinucleate and each nucleus develops into a microgamete which is equivalent to a sperm cell. Macrogamonts are uninucleate and are equivalent to an ovum. After fertilization, the zygotes develop into oocysts. Oocysts sporulate *in situ* and contain four sporozoites. Two types of oocysts are produced, thin-walled and thick-walled. Sporozoites in a thin-walled oocyst can

excyst and autoinfect the host. Thick-walled oocysts pass in the feces and are ready to infect a new host [113].

The prepatent period of cryptosporidiosis varies with the host and species of *Cryptosporidium* as well as the infective dose [113, 118]. Table 1.12 summarizes the prepatent period and days of oocyst excretion in various host species.

Additionally, the observation of free sporozoites in the intestinal tract led to the speculation that autoinfection occurred in the host from either sexual or asexual reproduction.

1.2.5 Transmission

Like *Giardia*, *Cryptosporidium* infection is transmitted via the fecal-oral route by the ingestion of the oocyst stage. In humans, infection can occur directly by close human-to-human contact and while caring for infected livestock, zoo, or companion animals. Drinking water and recreational water can serve as vehicles for transmission and have been reported as sources of infection in many outbreaks. In some foodborne outbreaks oocysts were detected on fresh vegetables and in irrigation water.

Risk factors associated with Cryptosporidiosis

Numerous studies of outbreaks and sporadic disease have been conducted to identify the risk factors associated with *Cryptosporidium* infections. The potential risk factors for cryptosporidiosis in humans are shown in Table 1.13. This information is lacking with regard to dogs and cats. In one study, the potential risk factors associated with *Cryptosporidium* infection in cats were young age and the presence of *Giardia* cysts [150].

1.2.6 Pathogenesis

The pathogenic mechanism of *Cryptosporidium* infection that causes diarrheal disease is not clearly understood. Observational studies of infected humans and experimental infection

studies in animal models have suggested that the pathogenic mechanism of cryptosporidiosis includes both parasite factors and host responses.

Like the pathogenic mechanism of giardiasis, diarrhea in *Cryptosporidium*-infected hosts appears to be due to malabsorption of electrolytes and nutrients, as well as impaired disaccharidase activity and hypersecretion of chloride and water [47]. The overall malabsorption and maldigestion suggests the occurrence of diffuse mucosal injury and shortening and/or loss of brush border microvilli, which have been observed in animal models [151]. However, because the same process is also observed in a variety of other enteric disorders, it is suggested that the insult is a host-mediated event. A recent study revealed increased population and activation of CD8+ TCRgamma-delta T cells in the intra-epithelial compartment during intestinal infection with *Cryptosporidium* [152, 153]. The findings imply that activated T lymphocytes cause microvillus injury during infection. Disaccharidase deficiencies and epithelial malabsorption then result in diarrhea in the infected host.

Observations from *in vitro* and *in vivo* models have suggested that *Cryptosporidium* disrupts intestinal epithelium tight junctions and increases intestinal permeability. This increasing permeability can allow luminal antigens to activate host immune-dependent pathologic pathways. The loss of epithelial barrier function in *Cryptosporidium* is also due to a *Cryptosporidium*-induced apoptosis [47].

1.2.7 Canine and feline cryptosporidiosis

1.2.7.1 Prevalence of cryptosporidiosis in dogs and cats and its distribution

Iseki was the first person to report the occurrence of *Cryptosporidium* oocysts in naturally-infected cats in 1979, giving it the name *C. felis* [128]. Evidence of *Cryptosporidium* infection in dogs was first reported by Tzipori and Campbell [154] when they detected

Cryptosporidium antibody in serum samples. The first clinical case was reported in 1983 when *Cryptosporidium* was identified in an intestinal sample of a 1-week-old puppy with acute diarrhea [155]. Subsequently, *Cryptosporidium* infections have been reported in dogs and cats with or with out clinical signs worldwide (Table 1.14 and Table 1.15). Prevalence has varied among the studies depending on geographic location, population studied, and the diagnostic tests used, with prevalences ranging from 0 to 28% in dogs and 0 to 24.5% in cats.

The *Cryptosporidium* genus is comprised of host-specific and zoonotic species. As indicated based on molecular studies worldwide, dogs are mostly infected with *C. canis* and cats are primarily infected with *C. felis* (Table 1.16 and Table 1.17). There has also been evidence that dogs and cats harbored *C. parvum,* and it raised concerns regarding zoonotic transmission from dogs and cats to humans. However, the link of zoonotic transmission from dogs and cats to humans has not yet been proved.

1.2.7.3 Mode of transmission

Cryptosporidiosis in dogs and cats is transmitted via the fecal-oral route, directly or indirectly. Infection can occur directly by coprophagia or grooming, or indirectly by ingestion of contaminated food or water.

1.2.7.2 Clinical abnormalities

Most dogs and cats infected with *Cryptosporidium* are sub-clinical; however, clinical signs are commonly seen in young animals [156-158]. Common clinical signs are small bowel diarrhea, anorexia, and weight loss. Diarrhea is usually watery without mucous, blood, melena, or straining. Vomiting is uncommon in dogs or cats with *Cryptosporidium* infection unless other abnormalities exist.

1.2.7.4 Diagnosis

Laboratory results for the complete blood count or blood chemistry are usually normal except when severe diarrhea affects these tests, often reflecting the dehydration [159]. *Cryptosporidium* infection in dogs and cats is diagnosed by identifying the oocysts using microscopic techniques, identifying the *Cryptosporidium* antigen using ELISA, or identifying pathogen DNA using PCR in the fecal samples. Infected dogs and cats shed oocysts intermittently, therefore a single negative test result may not completely rule out the infection. Multiple tests in two or three consecutive days may be required [159].

Fecal Microscopic Examination

As small bowel diarrhea in dogs and cats can be caused a variety of pathogens, a combination of wet mount examination and fecal flotation is generally suggested as part of the screening tests [160]. However, due to the transparency and small size of *Cryptosporidium* oocysts, conventional microscopic examination is usually of low sensitivity. A number of staining methods may be used to assist in identification of *Cryptosporidium* oocysts such as modified Ziehl-Neelsen (mZN), auramine-phenol (AP), Wright-Giemsa, safranin-methylene blue, quinacrine, Kinyoun and fluorescein isothiocyanate-conjugated monoclonal antibody (FITC-Mab) [161]. Even with staining the threshold of detection is low. The threshold of detection using an unconcentrated fecal smear and Kinyoun staining was reported at 10^6 oocysts per gram of feces [162]. Using a concentration technique and Kinyoun staining, a threshold of detection of between 1×10^4 and 5×10^5 oocysts per gram of unconcentrated feces was demonstrated [163].

Concentration techniques, including flotation by Sheather's sucrose, zinc sulfate or saturated sodium chloride, or sedimentation by formalin-ethyl-acetate are often the methods of

choice for detecting oocysts. It has been reported that dogs and cats shed a relatively small number of *Cryptosporidium* oocysts as compared to other species. For example, in naturally-infected cats the mean number of oocysts shed with and without diarrhea were 1,817 and 191 oocysts per gram of feces, respectively [164], whereas infected calves shed a mean of 90,867 oocysts per gram of feces [165]. Therefore, fecal concentration methods are usually required to increase the rate of detection in dogs and cats.

Immunofluorescent assay (IFA) has been developed to immunologically detect *Cryptosporidium* oocysts in fecal samples. One study involving humans used direct fluorescent assay and the threshold of detection was 5×10^4 oocysts per gram of feces. Using feline fecal samples spiked with *C. parvum* oocysts, the detection limit with this technique was around 10^4-10^5 oocysts per gram of feces [84]. With the apple green on stained *Cryptosporidium* oocysts, it can reduce the examination time and effort in finding the *Cryptosporidium* oocysts under the microscope compared to conventional techniques. In addition, currently there are several IFA tests that can detect *Giardia* cysts and *Cryptosporidium* oocysts at the same time and are commercially available, such as Merifluor® *Cryptosporidium/Giardia* (Meridian Bioscience, Inc., Cincinnati, OH, USA), Cyst-a-Glo™ Comprehensive Kit (WaterborneTM, Inc., New Orleans, LA, USA) and Crypto/*Giardia* Cel (Cellabs Pty Ltd., Brookvale, NSW, Australia). However, the major disadvantage of this technique is the need for a fluorescent microscope, which may not be available in some diagnostic laboratories.

Fecal antigen detection

There are a number of different ELISA tests available for detection of *C. parvum* antigen in feces. Although the available assays were developed for use with human feces, these tests have also been used to test dogs and cats fecal samples [83, 86, 94, 166]. This technique does not

need an experienced examiner. The results can be available quickly, and the results can be run in a big batch. In dogs, the detection limit of the *Cryptosporidium* ELISA was at a level of 10^5 oocysts per gram of feces. Compared to IFA, the diagnostic sensitivity of ELISA was 71% and the specificity was 94% [166].

Molecular genetic techniques

DNA of *Cryptosporidium* spp. can be amplified from fecal samples by polymerase chain reaction (PCR) and a number of PCR protocols have been developed. Some PCR protocols have been reported to be more sensitive than IFA for the detection of *Cryptosporidium* spp. in cats [167, 168]. In a number of investigations, detection thresholds as low as a single oocyst in fecal samples have been reported [161, 169, 170]. A primary benefit of PCR compared with other assays is that the amplification products can be analyzed by sequencing analysis to determine the species of *Cryptosporidium*.

1.2.7.5 Treatment and prevention

More than 200 substances have been tested for the treatment of cryptosporidiosis in humans, mice, and cattle; however, none of them have showed consistency in eliminating the organism or controlling the clinical signs [171]. Only few reports have been published regarding of the treatment of this disease in dogs and cats (Table 1.18).

Tylosin, at 11 mg/kg BID for 28 days PO, has been reported to resolve the clinical signs in chronically infected cats [172]. The stool became normal within a week after initiating therapy. However, the potential positive results with tylosin may have been due to antibacterial or anti-inflammatory, rather than antiprotozoal, effects for this case. Tylosin can be a gastrointestinal irritant and is not tolerated by most cats because of its unpleasant taste; thus, administration of the drug in capsule form is often required.

Azithromycin has been used with variable results at a dosage of 10 mg/kg daily by the oral route in dogs and cats [159]. The optimal duration of therapy is unknown, but is usually several weeks or until the clinical signs have resolved. This drug appears to be safe in dogs and cats with the most common adverse effect being mild gastrointestinal irritation.

The administration of paromomycin (150 mg/kg, orally, every 12-24 hours for 5 days) to naturally and experimentally infected cats decreased the shedding of oocysts to below the detection limit of the assay used, but it is unknown whether the infection was eliminated [173]. Importantly, this drug should never be given to dogs or cats that have bloody diarrhea because it can be absorbed systemically and renal toxicity and ototoxicity can occur [174]. If the dog or cat appears to respond within the first week of therapy and toxicity has not been noted, treatment should be continued for one week after clinical resolution of diarrhea.

Nitazoxanide, a salicylanide derivative of nitrothiazole, is effective against a broad spectrum of parasites and bacteria and is the only drug approved by the U.S. Food and Drug Administration (FDA) for the treatment of diarrhea caused by Cryptosporidium in humans[175]. Natazoxanide has been administered to *Cryptosporidium* infected dogs, and the diarrhea resolved in 7 of 8 dogs receiving the drug at 25 mg/kg orally every 12 hours for at least 5 days [176]. This drug, however, is a gastrointestinal irritant and frequently causes vomiting.

Prevention of Cryptosporidium infection

Prevention of *Cryptosporidium* infection can be accomplished by avoiding accidental ingestion or inhalation of *Cryptosporidium* oocysts. *Cryptosporidium* oocysts are resistant to extreme temperatures and to most frequently-used disinfectants such as commercial bleach. Concentrated ammonia solutions (50%) effectively inactivate *Cryptosporidium* oocysts after 30 minutes. Moist heat (steam or pasteurization [>55°C]), freezing and thawing, or thorough drying

can rupture the oocysts. Avoiding contact with contaminated food and water or mechanical vectors are the primary ways for prevention of *Cryptosporidium* infection. Feeding cats and dogs with commercial diets instead of raw meat may also decrease the chances of exposure to *Cryptosporidium* spp.. In crowded environments, good sanitation practices and cleaning of the water and food bowls with boiling water will decrease the possibility of contamination. It may be beneficial to separate dogs or cats with diarrhea from other normal animals. However, because infection is possible with a small infective dose, it is difficult to curtail the spread of *Cryptosporidium* within groups of animals.

1.2.8 Zoonotic considerations

A number of case reports have described the transmission of *Cryptosporidium* spp. from infected cattle to farm workers, visitors, and veterinary students [177-182]. In these incidences the infective organism is likely *C. parvum*. Given that there is no effective treatment for cryptosporidiosis, prevention is highly important especially for immunosuppressed individuals. The Center for Disease Control and Prevention recommends hand washing after handling animals, changing diapers, or contacting soil, and before and after food preparation to decrease the risk of cryptosporidiosis in people with HIV [183]. It is also recommended that HIV-infected people avoid contact with animal feces, especially from stray animals or pets aged <6 months, and also to avoid direct exposure to calves and lambs. In addition, HIV-infected people should not drink water directly from lakes or rivers. Boiling untreated water for at least three minutes will eliminate the risk of cryptosporidiosis, and the use of submicron water filters or bottled water might also reduce the risk for infection.

Each *Cryptosporidium* spp. generally only infects a particular species or a group of related animals. Humans are usually infected with *C. hominis* or *C. parvum*. Infrequently the

host-specific *C. felis* and *C. canis* have also been reported in human feces [184-188]. From a total of 22,505 samples from immunocompetent and immunocompromised humans from 40 countries, only 59 isolates were *C. felis* (0.26%) and 4 were *C. canis* (0.02%) [139, 189]. Another study failed to find an association between pet ownership and cryptosporidiosis in HIV-infected individuals [190]. Some studies have detected *C. canis and C. felis* oocysts in pets and humans in the same house [55, 191], but the direction of transmission was difficult to establish. Veterinarians should emphasize the importance of sanitation, particularly in the case of immunocompromised individuals.

1.3 Giardiasis and cryptosporidiosis in Thailand

1.3.1 Human giardiasis and cryptosporidiosis

Giardia and *Cryptosporidium* are leading causes of gastrointestinal disease in both developed and developing worlds. These organisms can produce severe and chronic disease especially in immune-compromised individuals such as HIV-infected patients, the very young or the elderly [192-194]. These infections can also lead to significant morbidity and mortality within human populations in developing countries and have been a worldwide concern, resulting in the inclusion of *Giardia* and *Cryptosporidium* in the WHO 'Neglected Diseases Initiative' since September 2004 [195].

The prevalence of *Giardia* and *Cryptosporidium* infections varies depending on the region, detection method, and population tested. Worldwide, it has been estimated that *G. duodenalis* cause infection in humans at a rate of 2.8×10^8 cases per year [196]. In the US the annual incidence of giardiasis was estimated at 7.3-7.6 cases per 100,000 people, and for *Cryptosporidium* at 2.9 cases per 100,000 people in 2010 [197, 198].

In Thailand, reports regarding *Giardia* and *Cryptosporidium* infections have primarily involved children and HIV patients. The geographic areas that have been studied are shown in Figure 1.1. The reported prevalences of *Giardia* and *Cryptosporidium* infections in humans range from 0%-37.7% (Table 1.19) and 0%-28.7% (Table 1.20), respectively. Based on 131 available genotypes published, 45 (34.3%) were single-infected with assemblage A, 52 (39.7%) were single-infected with assemblage B, 1 was single-infected with assemblage C, 27 (20.6%) were mixed-infected with assemblage A and B, 6 (4.6%) were mixed-infected with dog and human genotypes (Table 1.21). Based on data available for 50 genotypes, 34 (68%) were *C. hominis*, 7 (14%) were *C. meleagridis*, 4 (8%) were *C. parvum*, and 3 (6%) were *C. felis*. One of *C. canis* and one of *C. muris* were also identified in human samples (Table 1.22).

1.3.2 *Giardia* and *Cryptosporidium* infections in livestock

Although *Giardia* and *Cryptosporidium* infections and their molecular characteristics have been reported worldwide, only a few studies regarding these pathogens have been conducted in livestock in Thailand. All the reported studies have been done in cattle [199-202], and the studied provinces are labeled and shown in Figure 1.2.

In the northern region (Chiang Mai), Lwin [203] studied the prevalence of *Cryptosporidium*, *Giardia* and other internal parasites in dairy and beef cattle. In this study she reported a prevalence of *Cryptosporidium* in dairy cows of 0.5% based on simple smear with modified Ziehl-Neelsen staining, and no *Giardia* cysts were detected in the fecal samples. Also, *Giardia* cysts and *Cryptosporidium* oocysts were not detected in the fecal samples from the beef cattle in this study. In another study, Inpankaew et al. [199] reported the seroprevalence of *Cryptosporidium* in dairy cows in Chiang Mai, Chiang Rai and Lumpang provinces. The overall seroprevalence of *Cryptosporidium* infection was 4.4%, and by province the prevalence rates

were 3.3%, 5.1% and 3.0% for dairy cows in Chiang Mai, Chiang Rai and Lumpang provinces, respectively.

In the western region (Ratchaburi and Kanchanaburi), Inpankaew et al. [200] reported a prevalence of *Cryptosporidium* infection in dairy cows of 7% by acid-fast staining and 15.5% by PCR-RFLP. In this study, all positive PCR-RFLPs were determined to be *C. parvum.*

In the northeastern region (Khon Kaen, Udonthani, and Sakon Nakorn provinces) the prevalence of *Giardia* infection was reported to be 2.5% by zinc sulfate flotation and 1% by PCR targeting SSU-rRNA gene. The genotype of the positive isolates was assemblage E [204].

In the southern region (Satun province), the prevalence of *Giardia* infection in goats has been studied. Using microscopic examination after formalin ethyl ether sedimentation, the prevalence was reported to be 2.7% [205].

In the eastern region (Chonburi province), the prevalence of *Cryptosporidium* infection in diary cattle was 13% by acid-fast staining and 9.63% by nested PCR targeting SSU-rRNA. *Cryptosporidium parvum* was identified in all available sequences [206].

1.3.3 *Giardia* and *Cryptosporidium* infections in dogs and cats in Thailand

Information regarding *Giardia* and *Cryptosporidium* infections in dogs and cats in Thailand is limited. To our knowledge there have been only two reports regarding the prevalence and genotype of *Giardia* infections in dogs [55, 207], and no published reports regarding *Cryptosporidium* infections in dogs and cats. In temple dogs in Bangkok, the median estimate of *Giardia* infections in dogs was 56.8% using a Bayesian calculation based on results from microscopic examination, IFA, ELISA, and PCR assays. The genotypic analyses on 42 PCR (SSU-rRNA targeted) positive samples revealed that assemblage A was the most common genotype isolated in these dogs (79%), followed by assemblage D (31%), assemblage B (21%)

and assemblage C (12%). In this paper, the authors concluded that three transmission cycles, anthroponotic, zoonotic and dog-specific cycles, have maintained *Giardia* infections among humans and dogs in the temple community.

1.4 Tables

Table 1.1 Recognized species of *Giardia* based on morphology.

Species	Morphological characteristics of trophozoite	Shape of median body	Trophozoite dimensions length/width (μm)	Susceptible hosts
G. agilis	Long, narrow shaped	Club shaped	20-30/4-5	Amphibians
G. duodenalis	Pear shaped	Claw shaped	12-15/6-8	Humans and other primates, dogs, cats, livestock, rodents and other wild mammals
G. muris	Rounder than *G. duodenalis*	Small round median bodies	9-12/5-7	Rodents
G. ardeae	Rounder with prominent notch in ventral disc and rudimentary caudal flagellum	Round-oval to claw shaped	~10/~6.5	Birds
*G. microti**	Pear shaped	Claw-shaped	12-15/6-8	Rodents
G. psittaci	Pear shaped with no ventrolateral flange	Claw-shaped	~14/~6	Birds

* Mature cysts fully differentiated

Adapted from Monis *et al*. Variation in *Giardia*: towards a taxonomic revision of the genus. Trends Parasitol 2009, 25(2): 93-100

Table 1.2 Genotypic groupings on genetic analyses of *Giardia duodenalis*.

Assemblages	Novel proposed nomenclature	Susceptible host
Assemblage A	*G. duodenalis*	Humans and other primates, dogs, cats, livestock, rodents, and other wild mammals
Assemblage B	*G. enterica*	Humans and other primates, dogs, some species of wild mammals
Assemblages C and D	*G. canis*	Dogs and other canids
Assemblage E	*G. bovis*	Cattle and other hoofed livestock
Assemblage F	*G. cati*	Cats
Assemblage G	*G. simondi*	Rats
Assemblage H	?	Seals

Adapted from Feng and Xiao 2011. Zoonotic potential and molecular epidemiology of *Giardia* species and giardiasis. Clin Microb Rev: 24 (1), 110-140.

Table 1.3 List of the targets, type of assay and main use of amplification-based techniques for *Giardia.*

Amplification target[a]	Assay type	Main application
SSU-rRNA	PCR, nested PCR, microarray	Species and genotype identification
gdh	PCR, nested PCR, sequencing, PCR-RFLP	Species and genotype identification
tpi	PCR, nested PCR, sequencing, real-time PCR, microarray	Species and genotype identification
β-giardin	PCR, nested PCR, sequencing, PCR-RFLP	Genotype identification
ef-1α	PCR, nested PCR, sequencing	Species and genotype identification
GLORF-C4	PCR, sequencing, PCR-RFLP	Genotype identification
IGS	PCR, nested PCR, sequencing	Genotype identification

[a]Abbreviations: rRNA, ribosomal RNA; RFLP, restriction fragment length polymorphism; GDH, glutamate dehydrogenase; TPI, triose phosphate isomerase; EF-1α, Elongation factor - 1α; GLORF-C4, *G. lamblia* open reading frame C4; IGS, intergenic spacer. Adapted from Caccio, S. M., et al. (2005). "Unravelling *Cryptosporidium* and *Giardia* epidemiology." Trends Parasitol 21(9): 430-437. and Lee, J. H., et al. (2006). "Detection and genotyping of *Giardia intestinalis* isolates using intergenic spacers (IGS)-based PCR." Korean Journal of Parasitology 44(4): 343-353.

Table 1.4 Prevalence of *Giardia duodenalis* in dogs worldwide.

Country	Sample size	Prevalence (%)	Method	Reference
Argentina	2193	8.9	Microscopy	[208]
Argentina	106	14.5	Microscopy	[209]
Argentina	1944	1.29	Microscopy	[210]
Australia	1400	9.3	Microscopy	[211]
Australia	421	22.1	Microscopy	[54]
Belgium	1159	22.6	IFA	[212]
Brazil	254	16.9	Microscopy	[213]
Brazil	200	16.5	Microscopy	[214]
Brazil	46	2.2	Microscopy	[215]
Brazil	271	12.8	Microscopy	[216]
Brazil	410	29.0	Microscopy	[40]
Brazil	166	31.33	Microscopy	[217]
Brazil	300	17.3	Microscopy	[218]
Canada	102	7.0	ELISA	[219]
Canada	1,216	7.2	ELISA	[220]
Canada	619	8.1	Microscopy	[221]
Canada	70	7.1	Microscopy	[222]
Canada	241	13.0	SNAP	[91]
China	209	8.6	Microscopy	[223]
China	209	11.0	PCR	[223]
Costa Rica	58	8.6	IFA	[224]
Czech Republic	3,780	0.1	Microscopy	[225]
Czech Republic	458	2.5	Microscopy	[226]
Czech Republic	458	36.5	Serology	[226]
Ecuador	97	5.1	Microscopy	[227]
Finland	150	5.0	IFA	[166]
Germany	8,438	16.6	ELISA	[228]
Germany	24,677	18.6	ELISA	[229]
Germany	341	11.4	SNAP	[230]
Greece	281	4.3	Microscopy	[231]
India	101	3.0	Microscopy	[62]
India	101	20.0	PCR	[62]
Iran	147	0.7	Microscopy	[232]
Italy	183	55.2	ELISA	[53]
Italy	240	26.6	Microscopy	[233]
Italy	406	17.3	Microscopy	[234]
Italy	616	21.3	Microscopy	[235]
Italy	127	11.0	Microscopy	[236]
Italy	127	20.5	PCR	[236]
Japan	361	37.4	ELISA	[237]
Japan	1,035	14.6	Microscopy	[238]
Japan	1,105	12.4	Microscopy	[239]
Japan	1,794	23.4	SNAP	[240]
Japan	2,365	8.3	SNAP	[241]
Korea	472	11.2	SNAP	[242]
Mexico	200	46.5	Microscopy	[39]
Nicaragua	100	8.0	Microscopy	[243]
Poland	148	2.0	Microscopy	[41]
Poland	108	28.0	IFA	[88]
Romania	614	8.5	Microscopy	[244]
Serbia	151	14.6	Microscopy	[245]
Spain	1161	7.0	Microscopy	[101]
Spain	81	4.9	Microscopy	[246]
Spain	1800	1.0	Microscopy	[247]
Spain	251	0.4	Microscopy	[248]
Thailand	229	7.9	Microscopy	[207]
Thailand	229	56.8*	Microscopy, IFA, ELISA, PCR	[55]

Country	Sample size	Prevalence (%)	Method	Reference
UK	80	20.0	Microscopy	[249]
UK	878	21.0	SNAP	[92]
USA	130	5.4	IFA	[250]
USA	1,199,293	4.0	Microscopy	[251]
USA	2294	7.2	Microscopy	[252]
USA	6,555	3.3	Microscopy	[253]
USA	16,064	15.6	SNAP	[90]

* Median prevalence from 4 tests.

Adapted from Scorza, A. V. and M. R. Lappin (2012). Giardiasis - in Enteric Protozoa infection. Infectious diseases of the dog and cat. C. E. Greene. St. Louis, Mo., Elsevier/Saunders**:** 785-792.

Table 1.5 Worldwide prevalence of *Giardia duodenalis* in cats.

Country	Sample size	Prevalence (%)	Method	Reference
Australia	1,063	2.0	Microscopy	[211]
Australia	40	5	Microscopy	[254]
Australia	40	80	PCR	[254]
Australia	120	9.2	SNAP	[255]
Brazil	131	6.1	Microscopy	[256]
Canada	41	2.4	Microscopy	[222]
Canada	16	4.1	SNAP	[91]
Chile	230	19.1	Microscopy	[257]
Colombia	46	13.0	PCR	[258]
Costa Rica	7	57.1	IFA	[224]
Czech Republic	135	0.8	Microscopy	[226]
Czech Republic	135	57.0	Serum	[226]
Egypt	113	2.0	Microscopy	[259]
Finland	402	3.2	ELISA	[260]
Germany	3,167	12.6	ELISA	[228]
Germany	8,560	12.6	ELISA	[229]
Germany	584	6.8	SNAP	[230]
Iran	113	0.9	Microscopy	[261]
Italy	266	15.8	ELISA	[262]
Italy	48	4.2	ELISA	[263]
Japan	600	40.0	ELISA	[264]
Japan	942	1.5	SNAP	[265]
New Zealand	22	32.0	SNAP	[266]
Romania	414	0.7	Microscopy	[267]
Romania	183	27.9	ELISA	[244]
Serbia	81	22.2	Microscopy	[268]
The Netherlands	305	1.0	Microscopy	[269]
The Netherlands	60	13.6	ELISA	[270]
UK	57	7.2	SNAP	[271]
USA	117	31.0	ELISA	[72]
USA	273	7.0	ELISA	[272]
USA	250	13.6	IFA	[71]
USA	153	5.2	IFA	[273]
USA	344	9.9	IFA	[86]
USA	206	2.4	Microscopy	[192]
USA	1,566	2.3	Microscopy	[253]
USA	1,322	8.9	Microscopy	[274]
USA	263	7.2	Microscopy	[275]
USA	757	2.5	Microscopy	[276]
USA	454	3.5	Microscopy	[252]
USA	211,105	0.58	Microscopy	[277]
USA	4,977	10.3	SNAP	[90]

Adapted from Scorza, A. V. and M. R. Lappin (2012). Giardiasis - in Enteric Protozoa infection. Infectious diseases of the dog and cat. C. E. Greene. St. Louis, Mo., Elsevier/Saunders**:** 785-792.

Table 1.6 *Giardia duodenalis* genotypes in dogs[a].

Country	Loci tested	No. of samples genotyped	No. of samples with assemblage					References
			A	B	C	D	Others	
Australia	gdh	11			10	1		[278]
Australia	SSU-rRNA, gdh	9	1	2	4	2		[18]
Australia	SSU-rRNA	88	1		44	41	C+D (2)	[279]
Europe	SSU-rRNA, gdh, bg, tpi	600	137	53	191	215	E (5)	[16]
Belgium	bg	119	40	4	26	49		[212]
Finland	SSU-rRNA	8			3	4	1 (E)	[166]
Germany	SSU-rRNA	55	33		5	2	A+C (15)	[56]
Germany	SSU-rRNA	150	4		54	83	C+D (8), (A+D) 1	[280]
Hungary	SSU-rRNA	15			5	9	C+D (1)	[281]
Italy	bg	9	9					[58]
Italy	SSU-rRNA	17	2		11	1	A+C (2), C+D (1)	[282]
Italy	bg	21	6		1	12	A+D (1)	[283]
Italy	SSU-rRNA	30	8		14	4		[236]
Italy	SSU-rRNA, bg	30	2		3	25		[233]
Poland	bg	2			1	1		[41]
Portugal	bg	31	21		2	2	A+C (2), A+D (4)	[57]
Sweden	SSU-rRNA, gdh, bg, tpi	28	1		8	14	C+D (5)	[32]
The Netherlands	SSU-rRNA, gdh	2				2		[284]
The Netherlands	SSU-rRNA	13	1		7	3	C/D (1), unknown (1)	[270]
UK	SSU-rRNA, bg	41	2		10	29	C+D (1)	[92]
Canada	bg	13	13					[60]
USA	tpi	15			15			[31]
USA	SSU-rRNA	3			1	2		[285]
USA	gdh, bg, tpi	183	4		29	124	AI+D (7), AII+C (1), C+D (18)	[14]
USA	gdh, bg, tpi	2			1	2	C+D (1)	[43]
Mexico	bg	5	4				A+B (1)	[35]
Mexico	bg	19	19					[286]
Costa Rica	gdh, bg	2				2		[224]
Nicaragua	bg	8			2	5	C+D (1)	[243]
Argentina	tpi	1		1				[287]
Brazil	gdh	27			7	20		[288]
Brazil	SSU-rRNA, gdh, bg	36			20	10	C+D (5)	[218]
Brazil	bg	7	7					[289]
Peru	SSU-rRNA	67			9	32	C+D (26)	[290]
China	gdh, bg, ef1α	23	5			18		[223]
Japan	gdh	4				4		[291]
Japan	gdh	24	14		1	6	A+D (3)	[292]
Japan	gdh	29			9	20		[240]
India	SSU-rRNA, elf1α, tpi	7	5	2				[62]
Thailand	SSU-rRNA	13	5		1	3	A+B (3), A+D (1)	[207]
Thailand	SSU-rRNA	60	33	9	5	13		[55]
Vietnam	ND	8				8		[293]

Abbreviations: SSU-rRNA, small subunit ribosomal RNA gene; gdh, glutamate dehydrogenase gene; bg, β-giardin gene.

[a] Modified from Feng, Y. and L. Xiao (2011). "Zoonotic potential and molecular epidemiology of *Giardia* species and giardiasis." Clinical Microbiology Reviews 24(1): 110-140. and Scorza, A. V. and M. R. Lappin (2012). Giardiasis - in Enteric Protozoa infection. Infectious diseases of the dog and cat. C. E. Greene. St. Louis, Mo., Elsevier/Saunders**:** 785-792.

Table 1.7 *Giardia duodenalis* genotypes in cats[a].

Country	Loci tested	No. of samples genotyped	No. of samples with assemblage				References
			A	B	F	Others	
Australia	SSU-rRNA, gdh	18	6	2		C (2), D (7), E (1)	[18]
Australia	SSU-rRNA	8			7	D (1)	[279]
Europe	SSU-rRNA, gdh, bg, tpi	158	68	3	77	C (5), D (3), E (2)	[16]
Italy	SSU-rRNA	1	1				[282]
Italy	bg	1			1		[283]
Italy	SSU-rRNA	10	10				[59]
Italy	SSU-rRNA, gdh, bg	3			3		[294]
Italy	SSU-rRNA	11	3		8		[295]
Italy, Croatia	SSU-rRNA, gdh, bg	3	3				[296]
Sweden	SSU-rRNA, gdh, bg, tpi	18	5		12	E (1)	[32]
The Netherlands	SSU-rRNA	2	1		1		[270]
USA	SSU-rRNA	1			1		[285]
USA	gdh	17	6		11		[297]
USA	gdh, bg, tpi	13	3		7	D (2), C+D (1)	[14]
USA	SSU-rRNA	8			7	D (1)	[298]
Costa Rica	gdh, bg	2	2				[224]
Brazil	gdh	19	8		11		[288]
Brazil	bg	1	1				[289]
Colombia	SSU-rRNA	3			3		[258]
Japan	SSU-rRNA, gdh, tpi	26	6		20		[299]
Japan	gdh	3			3		[292]

Abbreviations: SSU-rRNA, small subunit ribosomal RNA gene; gdh, glutamate dehydrogenase gene; bg, β-giardin gene; elf1α, elongation factor 1- α.

[a]Modified from Feng, Y. and L. Xiao (2011). "Zoonotic potential and molecular epidemiology of *Giardia* species and giardiasis." Clinical Microbiology Reviews 24(1): 110-140. and Scorza, A. V. and M. R. Lappin (2012). Giardiasis - in Enteric Protozoa infection. Infectious diseases of the dog and cat. C. E. Greene. St. Louis, Mo., Elsevier/Saunders**:** 785-792.

Table 1.8 Drugs used for the treatment of *Giardia* infections in dogs and cats.

Drug[a]	Species	Dosage
Febantel	D	15 mg/kg febantel, PO, q24 hour for 3 days
	D	25 mg/kg febantel, PO, q24 hour, for 3 days
	C	56.5 mg/kg febantel, PO, q24 hour for 5 days
Fenbendazole	B	50 mg/kg, PO, q24 hour for 3 days
Furazolidone	C	4 mg/kg, PO, q12 hour, for 7 to 10 days
Ipronidazole	D	126 mg/L drinking water, PO, ad libitum for 7 days
Metronidazole	B	15 to 25 mg/kg, PO, q12 to 24 hour, for 5-7 days
Nitazoxanide	B	100 mg/animal, PO, q12 hour for 3-4 days
Tinidazole	D	44 mg/kg, PO, q24 hour, for 6 days
	C	30 mg/kg, PO, q24 hour, for 7-10 days
Quinacrine	D	9 mg/kg, PO, q24 hour, for 6 days
	C	11 mg/kg, PO, q24 hour, for 12 days

Abbreviations: B, dog and cat; C, cat,: D, dog

[a]Adapted from Scorza, A. V. and M. R. Lappin (2012). Giardiasis - in Enteric Protozoa infection. Infectious diseases of the dog and cat. C. E. Greene. St. Louis, Mo., Elsevier/Saunders**:** 785-792.

Table 1.9 Valid *Cryptosporidium* species, hosts, and oocyst measurements.

Species	Host	Mean oocyst size/Range (μm) per original description	References
C. andersoni	Cattle (*Bos taurus*)	7.4 × 5.5/6.0-8.1 × 5.0-6.5	[124]
C. baileyi	Chicken (*Gallus gallus)*	6.2 × 54.6/5.6-6.3 × 4.5-4.8	[125]
C. bovis	Cattle (*Bos tausus*)	4.9 × 4.6/4.8-5.4 × 4.2-4.8	[126]
C. canis	Dog (*Canis familiaris*)	5.0 ×4.7/3.7-5.9 × 3.7-5.9	[127]
C. cuniculus	Rabbit (*Oryctolagus cuniculus*)	6.0 × 5.4/5.6-6.4 × 5.0-5.9	[118]
C. fayeri	Red Kangaroo (*Macropus rufus*)	4.9 × 4.3/4.5-5.1 × 3.8-5.0	[134]
C. felis	Cat (*Felis catis*)	5.0 × 4.5	[128]
C. fragile	Black Spin toad (*Duttaphrynus melanostictus*)	6.2 × 5.5/ 5.5-7.0 × 5.0-6.5	[135]
C. galli	Chicken (*Gallus gallus)*	8.3 × 6.3/8.0-8.5 × 6.2-6.4	[129]
C. hominis	Human (*Homo sapiens*)	5.2 × 4.9/4.4-5.9 × 4.4-5.4	[130]
C. macropodum	Kangaroo (*Macropus giganteus*)	4.9 × 5.4/4.5-6.0 × 5.0-6.0)	[136]
C. meleagridis	Turkey (*Meleagris gallopavo*)	5.2 × 4.6/4.5-6.0 × 4.2-5.3	[116]
C. molnari	Gilthead sea bream (*Sparus aurata*), European sea bass (*Dicentrarchus labrax*)	4.7 × 4.5/3.2-5.5 × 3.0-5.0	[123]
C. muris	Mouse (*Mus musculus*)	7 × 5	[114]
C. parvum	Mouse (*Mus musculus*)	ovoid or spherical ≥4.5	[115]
C. ryanae	Cattle (*Bos Taurus*)	3.2 × 3.7/2.9-4.4 × 2.9-3.7)	[137]
C. serpentis	Corn snake (*Elaphe guttata*)	2.8 to 3.6	[131]
C. scophthalmi	Turbot (*Scophthalmi maximus*)	4.4 × 3.9/3.7-5.0 × 3.0-4.7	[122]
C. suis	Pig (*Sus scrofa*)	4.6 × 4.2/4.4-4.9 × 4.0-4.3	[132]
C. tyzzeri	Mouse (*Mus musculus*)	4.6 × 4.2	[120]
C. varanii	Emerald monitor (*Varanus prasinus*)	4.8 × 4.7/4.8×-5.1 × 4.4-4.8	[133]
C. ubiquitum	Cattle (*Bos tausus*)	5.0 × 4.7/4.7-5.3 × 4.3-5.0	[119]
C. wrairi	Guinea pig (*Cavia porcellus*)	5.4 × 4.6/4.8-5.6 × 4.0-5.0	[138]
C. xiaoi	Sheep (*Ovis aries)*	3.9 × 3.4/2.9-4.4 × 2.9-4.4	[121]

Adapted from Fayer, R. (2010). "Taxonomy and species delimitation in *Cryptosporidium*." Experimental Parasitology 124(1): 90-97.

Table 1.10 *Cryptosporidium* species that infect humans and selected domesticated animals and wildlife.

Species	Hosts
C. andersoni	Cattle, sheep, Bactrian camel, gerbil, multimammate mouse, wood partridge
C. baileyi	Chicken, duck, Bobwhite quail
C. bovis	Cattle, sheep
C. canis	Dog, fox, coyote, humans
C. cuniculus	Rabbit, humans
C. felis	Cat, cattle
C. hominis	Humans, primates, cattle, sheep, pig, dugong
C. meleagridis	Turkey, chicken, Bobwhite quail, dog, deer, mouse, humans
C. muris	Mouse, hamster, squirrel, Siberian chipmunk, wood mouse, bank vole, rock hyrax, Bactrian camel, mountain goat, cat, coyote, ringed seal, bilby, cynomolgus monkey, tawny frogmouth, humans
C. parvum	Calf, lamb, horse, alpaca, dog, mouse, raccoon dog, eastern squirrel, humans
C. suis	Pig cattle

Adapted from Xiao, L. and R. Fayer (2008). Molecular characterisation of species and genotypes of *Cryptosporidium* and *Giardia* and assessment of zoonotic transmission. International Journal for Parasitology 38(11): 1239-1255.

Table 1.11 List of targets gene, type of assay and main use of amplification for *Cryptosporidium* molecular analysis.

Target gene	Assay type	Main application
SSU-rRNA	PCR, nested PCR, DNA sequencing, PCR-RFLP, real-time PCR, microarray	Species and genotype determination
Hsp70	PCR, nested PCR, DNA sequencing, real-time PCR, microarray	Species and genotype determination
COWP	PCR, nested PCR, DNA sequencing, PCR-RFLP, microarray	Species and genotype determination
Actin	PCR, nested PCR, DNA sequencing,	Species and genotype determination
β-tubulin	PCR, nested PCR, DNA sequencing, PCR-RFLP	Species and genotype determination
GP60	PCR, nested PCR, DNA sequencing,	Sub-genotype determination
Microsatellites	PCR, nested PCR, DNA sequencing,	Sub-genotype determination
Minisatellites	PCR, nested PCR, DNA sequencing,	Sub-genotype determination
Extrachromosomal double-stranded RNA	Reverse transcriptase, PCR, DNA sequencing, heteroduplex mobility assays	Sub-genotype determination

Adapted from Caccio, S. M., et al. (2005). "Unravelling *Cryptosporidium* and *Giardia* epidemiology." Trends Parasitol 21(9): 430-437.

Table 1.12 Prepatent and patent period of cryptosporidiosis for various species.

Cryptosporidium Species	Host species	Prepatent period (days)	Patent period (days)
C. baileyi	Chicken	4-24	Up to 18 days
C. bovis	Cattle	10-12	18
C. cuniculus	Rabbit	4-7	Up to 14 days
C. felis	Cat	5-6	7-10
C. muris	Mice	6-21	-
C. parvum	Calves	2-7	1-12
	Humans	4-22	1-20
C. ryanae	Cattle	11	15-17
C. suis	Pig	2-9	9-15
C. tyzzeri	Mouse	6-7	24-29
C. xiaoi	Sheep	7-8	13-15
C. ubuiquitum	Cattle	6-7	11-12

Adapted from Fayer, R. 2008. General Biology. in *Cryptosporidium* and cryptosporidiosis. R. Fayer and L. Xiao. Boca Raton, CRC Press, IWA Pub

Table 1.13 Risk factors associated with human cryptosporidiosis.

Risk factors	References
Food and drink	
Drinking water	[300-321]
Private water supplies	[322, 323]
Bottled water	[324, 325]
Ice	[326]
Food	[327-331]
Food and drink	[327-339]
Drink	[340, 341]
Unpasteurized milk	[342, 343]
Fruit juice	[341]
Raw salads	[344, 345]
Mollusks	[346-351]
Food handlers	[352]
Recreational water	
Swimming pools	[353-366]
Interactive water features	[367, 368]
Recreational lakes and rivers	[369, 370]
Paddling/wading pools	[368]
Animal to human	
Direct animal contact	[371-377]
Farm visits	[180, 181, 378-381]
Pets	[94, 156, 157, 185, 208, 217, 250, 382-387]
Pet food	[388]
Travel	
Camping	[380]
Visits to the countryside	[389-392]
Travel abroad	[64, 371, 393-398]
Tourist resorts	[399]
Travel away from home	[400]
Cruise and other ships	[401, 402]
Human to human	
Fairs and shows	[403]
Family spread	[404]
Nurseries and schools	[405, 406]
Hospital	[326, 407-411]
Wards with immunocompromised patients	[410]
Changing diapers	[371]
Sexual activity	[412, 413]
Others	
Flies	[414-417]
Weather	[418]

Adapted from Nichols, G. (2008). Epidemiology. In *Cryptosporidium and* cryptosporidiosis. R. Fayer and L. Xiao. Florida, USA, CRC Press.**:** 79-118.

Table 1.14 Worldwide prevalence of *Cryptosporidium* spp. in dogs.

Country	Sample size	Prevalence (%)	Method	Reference
Argentina	2,193	0.2	Microscopy	[208]
Argentina	1,944	0.05	Microscopy	[210]
Australia	1,400	0.6	Microscopy	[211]
Belgium	1,159	0	IFA	[212]
Brazil	254	3.1	Microscopy	[213]
Brazil	410	1.4	Microscopy	[40]
Canada	102	0	ELISA	[219]
Costarica	58	1.7	Microscopy, IFA, PCR	[224]
Czech	3,780	1.4	Microscopy	[225]
Ecuador (Galapagos island)	97	1.1	Microscopy, IFA, PCR	[227]
Greece	281	2.8	Microscopy	[231]
Japan	140	9.3	Microscopy, PCR	[157]
Korea	257	9.7	IFA	[419]
Poland	108	28.0	IFA, PCR	[88]
Spain	81	7.4	Microscopy	[246]
Spain	251	0	Microscopy	[248]

Abbreviation: IFA, immunofluorescent assay; PCR, polymerase chain reaction.
Modified from Scorza, A. V. and M. R. Lappin (2012). Cryptosporidiosis. Infectious diseases of the dog and cat. C. E. Greene. St. Louis, Mo., Elsevier/Saunders**:** 841-849.

Table 1.15 Worldwide prevalence of *Cryptosporidium* spp. in cats.

Country	Sample size	Prevalence (%)	Method	Reference
Australia	1,063	2.2	Microscopy	[211]
Colombia	46	13.0	PCR	[258]
Costa Rica	7	14.0	Microscopy, IFA, PCR	[224]
Italy	200	24.5	Microscopy	[420]
UK	235	8.1	Microscopy	[156]
UK	57	0.0	Microscopy	[271]
USA	600	8.3	ELISA, serum IgG	[421]
USA	153	6.5	IFA	[273]
USA	206	5.4	Microscopy	[192]
USA	344	4.7	Microscopy, IFA, ELISA	[86]
USA	244	2.5	ELISA	[272]
USA	250	12.0	Microscopy, IFA	[150]
USA	263	3.8	Microscopy	[275]
USA	1,322	3.8	Microscopy	[274]

Abbreviation: PCR, polymerase chain reaction; IFA, immunofluorescent assay; ELISA, enzyme-linked immunosorbent assay; IgG, immunoglobulin.

Modified from Scorza, A. V. and M. R. Lappin (2012). Cryptosporidiosis. Infectious diseases of the dog and cat. C. E. Greene. St. Louis, Mo., Elsevier/Saunders**:** 841-849.

Table 1.16 *Cryptosporidium* species reported in dogs[a].

Country	Loci tested	*Cryptosporidium* Species (Number of isolates)	Reference
Australia	SSU-rRNA, hsp70	*C. canis* (8)	[422]
Czech Republic	SSU-rRNA	*C. parvum* (1), *C. meleagridis* (1)	[423]
Italy	COWP	*C. parvum* (7), *C. canis* (1)	[424]
Japan	SSU-rRNA	*C. canis* (13)	[157]
Japan	SSU-rRNA	*C. canis* (1)	[425]
Japan	SSU-rRNA	*C. canis* (1)	[426]
USA	SSU-rRNA	*C. canis* (1)	[427]
USA	SSU-rRNA, hsp70	*C. canis* (1), *C. parvum* (1)	[127]
USA	Actin	*C. canis* (1)	[428]

Abbreviation: SSU-rRNA, small subunit rRNA gene; hsp70, heat shock protein 70 kDa gene; COWP, *Cryptosporidium* oocyst wall protein gene.

[a]Modified from Santin, M. and J. M. Trout (2008). Companion animals. in *Cryptosporidium* and cryptosporidiosis. Boca Raton, FL, CRC Press.

Table 1.17 *Cryptosporidium* species reported in cats[a].

Country	Loci tested	*Cryptosporidium* Species (Number of isolates)	Reference
Australia	SSU-rRNA	*C. felis* (2)	[429]
Australia	SSU-rRNA	*C. felis* (4)	[430]
Colombia	SSU-rRNA	*C. felis* (18), *C. muris* (1)	[258]
Czech Republic	SSU-rRNA, hsp70	*C. felis* (2)	[431]
Czech Republic	SSU-rRNA	*C. felis* (1)	[423]
Czech Republic	SSU-rRNA	*C. muris* (1)	[432]
Portugal	SSU-rRNA	*C. felis* (1)	[433]
USA	SSU-rRNA	*C. felis* (1)	[298]

Abbreviation: SSU-rRNA, small subunit rRNA gene; hsp70, heat shock protein 70 kDa gene.
[a]Modified from Santin, M. and J. M. Trout (2008). Companion animals. in *Cryptosporidium* and cryptosporidiosis. Boca Raton, FL, CRC Press.

Table 1.18 Drug therapy for *Cryptosporidium* infection in dogs and cats.

Drug	Drug protocol
Azithromycin	5-10 mg/kg, PO, every 24 hours until clinical signs resolve.
Nitazoxanide	25 mg/kg, PO, every 12 hours for at least 5 days.
Paromomycin	125 to 165 mg/kg, PO, every 12 to 24 hours for at least 5 days.
Tylosin	10 to 15 mg/kg, PO, every 8 to 12 hours for 21 days.

These doses and duration are based on anecdotal experiences and are not supported by controlled studies. If a dog or cat is improving during the initial treatment period, a longer duration of therapy may be indicated. Paromomycin should not be administered to animals with bloody diarrhea because absorption may occur and cause nephrotoxicity. From Scorza, V. and S. Tangtrongsup (2010). "Update on the diagnosis and management of *Cryptosporidium* spp infections in dogs and cats." Top Companion Anim Med 25(3): 163-169.

Table 1.19 Prevalence of *Giardia* infections in humans in Thailand.

Province	Source	No. of samples	Prevalence (%)	Diagnostic test used	Reference
Bangkok	Stool submitted at Parasitology Unit, of any age	6,231	1.2	Microscopy	[434]
Bangkok	Workers for overseas employment	2,213	1	Microscopy	[435]
Bangkok	Pre-school orphan	266	14.3	Microscopy	[436]
Bangkok	Childcare worker	105	10.5	Microscopy	[436]
Bangkok	Temple community of any age	204	20.3[a]	Microscopy, IFA, PCR, ELISA	[55]
Bangkok	Irritable bowel syndrome patients	59	1.7	Microscopy	[437]
Bangkok	Visitors for health check-up	6,018	0.4	Microscopy, PCR	[438]
Bangkok	HIV patients	64	6.2	Microscopy	[439]
Bangkok	Diarrheic children with HIV+ and HIV-	HIV+: 82 HIV-: 80	2.4 0	Microscopy	[440]
Bangkok	HIV patients	45	4.4	Microscopy	[441]
Bangkok	0-60 months orphanages	205	20	Microscopy	[442]
Central Thailand (Ang Thong, Ayudthaya, and Suphanburi)	Primary school children, 3-12 years old	1037	1.25	Microscopy	[443]
Chacheongsao	Primary school children	531	6.2	Microscopy, PCR	[444]
Chiang Mai	Hill-tribe school children	403	14.9	Microscopy	[445]
Chiang Mai	Karen hill-tribe, 3-19 years old	781	2.21	Microscopy	[446]
Chiang Mai	Hill-tribe children	765	5.2	Microscopy, PCR	[447]
Kanchanaburi	3-5 months old Pre-school children	Diarrhea: 236 Non-diarrhea: 236	13.6 23.3	Microscopy, ELISA	[448]
Khon Kaen	HIV patients	78	1.3	Microscopy	[449]
Nakhon Prathom	7-12 years student	1,920	2.2	Microscopy	[450]
Nakornrachasrima	Military personnel, age up to 60	317	1.3	Microscopy, PCR	[451]
Pathum Tani	10-82 months old pre-school orphan	106	37.7	Microscopy	[452]
Ratchaburi	Community survey, of any age	949	4.1	Microscopy, PCR	[438]
Samut Sakhon	5-7 years old school children	656	3	Microscopy	[453]
Surin	Volunteers of any age	3,358	0.8	Microscopy	[453]

Abbreviations: HIV, human immunodeficiency virus; ELISA, enzyme-linked immunosorbent assay; PCR, polymerase chain reaction.
[a]The median prevalence from 4 tests.

Table 1.20 Prevalence of *Cryptosporidium* infections in humans in Thailand.

Province	Source	No. of samples	Prevalence (%)	Diagnostic test used	Reference
Bangkok	HIV+ patients	64	20.3	Microscopy	[439]
Bangkok	Diarrheic children with HIV+ and HIV-	HIV+: 82 HIV-: 80	6.1 1.2	Microscopy	[440]
Bangkok	0-60 months orphanages	205	8	Microscopy	[442]
Bangkok	Orphanages	Diarrheic: 303 Non-diarrheic: 513	7.3 0	Microscopy	[454]
Bangkok	HIV+	45	20	Microscopy	[441]
Bangkok	HIV+	250	8.8	Microscopy	[455]
Bangkok	HIV+ and HIV- patients/	HIV+: 61 HIV-: 61	10 2	Microscopy	[456]
Kanchanaburi	3-5 months old Pre-school children	Diarrhea - 236 Non-diarrhea - 236	0.8 2.5	Microscopy, ELISA	[448]
Khon Kaen	HIV+ patients	78	11.5	Microscopy	[449]
Lopburi	HIV+ patients	46	28.7	Microscopy, PCR	[206]

Abbreviations: HIV, human immunodeficiency virus; ELISA, enzyme-linked immunosorbent assay; PCR, polymerase chain reaction.

Table 1.21 *Giardia duodenalis* genotypes reported in humans in Thailand.

Province	Source	No. of isolates	Locus tested[a]	Genotype	Reference
Bangkok	Temple community of any age	35	SSU-rRNA	A (25), B (1), C (1), A+B (2), Dog+human genotype (6)	[55]
Bangkok	Visitors for health check-up	22	SSU-rRNA, β-giardin, gdh and tpi	B (12) A+B (10)	[438]
Chacheongsao	Primary school children	12	gdh	A-II (5), B-IV (7)	[444]
Chiang Mai	Hill-tribe children	19	SSU-rRNA, gdh	A-II (10), B-III (1), B-VI (8)	[447]
Nakornrachasrima	Military personnel, age up to 60	4	gdh	B-IV	[451]
Ratchaburi	Community survey, of any age	39	SSU-rRNA, β-giardin, gdh and tpi	A (5), B (19) A+B (15)	[438]

[a]Abbreviations: SSU-rRNA, small subunit ribosomal RNA gene; gdh, glutamate dehydrogenase gene; tpi, triose phosphate isomerase gene.

Table 1.22 *Cryptosporidium* species reported in humans in Thailand.

Province	Source	No. of isolates	Locus	Species	Reference
Bangkok	HIV+ patients	8	SSU-rRNA	*C. parvum* (human) (2) *C. parvum* (bovine) (1) *C. meleagridis* (2) *C. felis* (2) *C. canis* (1)	[187]
Bangkok	HIV+ patients	11	SSU-rRNA	*C. hominis* (8) *C. parvum* (1) *C. meleagridis* (2)	[457]
Bangkok	HIV+ patients	29	SSU-rRNA, ITS1, ITS2, 5.8S rRNA	*C. hominis* (24) *C. meleagridis* (3) *C. felis* (1) *C. muris* (1)	[188]
Lopburi	HIV+ patients	2	SSU-rRNA	*C. parvum*	[206]

Abbreviations: HIV, human immunodeficiency virus; SSU-rRNA, small subunit ribosomal RNA; ITS, internal transcribed spacer.

1.5 Figures

Figure 1.1 Study locations in Thailand for *Giardia* and *Cryptosporidium* infections in humans.

Figure 1.2 Study locations for *Giardia* and *Cryptosporidium* infections in animals in Thailand.

1.6 References

1. Thompson, R.C. and P. Monis, *Taxonomy of Giardia Species*, in *Giardia a model organism*, H.D. Lujan and S. Svard, Editors. 2011, Springer: Wien. p. 3-15.
2. Adam, R.D., *The biology of Giardia spp.* Microbiol Rev, 1991. **55**(4): p. 706-32.
3. Thompson, R.C. and P.T. Monis, *Variation in Giardia: implications for taxonomy and epidemiology.* Adv Parasitol, 2004. **58**: p. 69-137.
4. Erlandsen, S.L. and W.J. Bemrick, *SEM evidence for a new species, Giardia psittaci.* J Parasitol, 1987. **73**(3): p. 623-9.
5. Feely, D.E., *Morphology of the cyst of Giardia microti by light and electron microscopy.* J Protozool, 1988. **35**(1): p. 52-4.
6. Erlandsen, S.L., et al., *Axenic culture and characterization of Giardia ardeae from the great blue heron (Ardea herodias).* J Parasitol, 1990. **76**(5): p. 717-24.
7. Monis, P.T. and R.C. Thompson, *Cryptosporidium and Giardia-zoonoses: fact or fiction?* Infect Genet Evol, 2003. **3**(4): p. 233-44.
8. Adam, R.D., *Biology of Giardia lamblia.* Clin Microbiol Rev, 2001. **14**(3): p. 447-75.
9. Feng, Y. and L. Xiao, *Zoonotic potential and molecular epidemiology of Giardia species and giardiasis.* Clinical Microbiology Reviews, 2011. **24**(1): p. 110-40.
10. Ey, P.L., et al., *Genetic analysis of Giardia from hoofed farm animals reveals artiodactyl-specific and potentially zoonotic genotypes.* J Eukaryot Microbiol, 1997. **44**(6): p. 626-35.
11. Monis, P.T., et al., *Genetic diversity within the morphological species Giardia intestinalis and its relationship to host origin.* Infect Genet Evol, 2003. **3**(1): p. 29-38.
12. Monis, P.T., et al., *Molecular systematics of the parasitic protozoan Giardia intestinalis.* Mol Biol Evol, 1999. **16**(9): p. 1135-44.
13. Lasek-Nesselquist, E., D.M. Welch, and M.L. Sogin, *The identification of a new Giardia duodenalis assemblage in marine vertebrates and a preliminary analysis of G. duodenalis population biology in marine systems.* Int J Parasitol, 2010. **40**(9): p. 1063-74.
14. Scorza, A.V., et al., *Comparisons of mammalian Giardia duodenalis assemblages based on the beta-giardin, glutamate dehydrogenase and triose phosphate isomerase genes.* Veterinary Parasitology, 2012.
15. Monis, P.T., et al., *Molecular genetic analysis of Giardia intestinalis isolates at the glutamate dehydrogenase locus.* Parasitology, 1996. **112 (Pt 1)**: p. 1-12.
16. Sprong, H., S.M. Caccio, and J.W. van der Giessen, *Identification of zoonotic genotypes of Giardia duodenalis.* PLoS Negl Trop Dis, 2009. **3**(12): p. e558.
17. Filice, F.P., *Studies on the cytology and life history of a Giardia from the laboratory rat.* University of California Publications in Zoology, 1952. **57**(2): p. 53-146.
18. Read, C.M., P.T. Monis, and R.C. Thompson, *Discrimination of all genotypes of Giardia duodenalis at the glutamate dehydrogenase locus using PCR-RFLP.* Infect Genet Evol, 2004. **4**(2): p. 125-30.
19. Ey, P.L., et al., *Distinct genetic groups of Giardia intestinalis distinguished by restriction fragment length polymorphisms.* J Gen Microbiol, 1992. **138**(12): p. 2629-37.
20. Andrews, R.H., et al., *Giardia intestinalis: electrophoretic evidence for a species complex.* Int J Parasitol, 1989. **19**(2): p. 183-90.

21. Ey, P.L., R.H. Andrews, and G. Mayrhofer, *Differentiation of major genotypes of Giardia intestinalis by polymerase chain reaction analysis of a gene encoding a trophozoite surface antigen.* Parasitology, 1993. **106 (Pt 4)**: p. 347-56.
22. Ey, P.L., et al., *Comparison of genetic groups determined by molecular and immunological analyses of Giardia isolated from animals and humans in Switzerland and Australia.* Parasitol Res, 1996. **82**(1): p. 52-60.
23. Upcroft, P., R. Mitchell, and P.F. Boreham, *DNA fingerprinting of the intestinal parasite Giardia duodenalis with the M13 phage genome.* Int J Parasitol, 1990. **20**(3): p. 319-23.
24. Butcher, P.D., et al., *Phenotypic and genotypic variation in Giardia lamblia isolates during chronic infection.* Gut, 1994. **35**(1): p. 51-4.
25. Morgan, U.M., et al., *RAPD (random amplified polymorphic DNA) analysis of Giardia DNA and correlation with isoenzyme data.* Trans R Soc Trop Med Hyg, 1993. **87**(6): p. 702-5.
26. van Belkum, A., et al., *Genotyping isolates and clones of Giardia duodenalis by polymerase chain reaction: implications for the detection of genetic variation among protozoan parasite species.* Mol Biochem Parasitol, 1993. **61**(1): p. 69-77.
27. Pelayo, L., et al., *Genetic characterization by random amplified polymorphic DNA analysis (RAPD) of 18 isolates of Giardia lamblia obtained from day care children.* Exp Parasitol, 2003. **104**(3-4): p. 162-6.
28. Hopkins, R.M., et al., *Ribosomal RNA sequencing reveals differences between the genotypes of Giardia isolates recovered from humans and dogs living in the same locality.* J Parasitol, 1997. **83**(1): p. 44-51.
29. Abe, N., I. Kimata, and M. Tokoro, *Genotyping of Giardia isolates from humans in Japan using the small subunit ribosomal RNA and glutamate dehydrogenase gene sequences.* Jpn J Infect Dis, 2005. **58**(1): p. 57-8.
30. Homan, W.L., et al., *Characterization of Giardia duodenalis by polymerase-chain-reaction fingerprinting.* Parasitol Res, 1998. **84**(9): p. 707-14.
31. Sulaiman, I.M., et al., *Triosephosphate isomerase gene characterization and potential zoonotic transmission of Giardia duodenalis.* Emerg Infect Dis, 2003. **9**(11): p. 1444-52.
32. Lebbad, M., et al., *From mouse to moose: multilocus genotyping of Giardia isolates from various animal species.* Vet Parasitol, 2010. **168**(3-4): p. 231-9.
33. Mahbubani, M.H., et al., *Differentiation of Giardia duodenalis from other Giardia spp. by using polymerase chain reaction and gene probes.* J Clin Microbiol, 1992. **30**(1): p. 74-8.
34. Caccio, S.M., M. De Giacomo, and E. Pozio, *Sequence analysis of the beta-giardin gene and development of a polymerase chain reaction-restriction fragment length polymorphism assay to genotype Giardia duodenalis cysts from human faecal samples.* Int J Parasitol, 2002. **32**(8): p. 1023-30.
35. Lalle, M., et al., *Genotyping of Giardia duodenalis from humans and dogs from Mexico using a beta-giardin nested polymerase chain reaction assay.* J Parasitol, 2005. **91**(1): p. 203-5.
36. Dryden, M.W., P.A. Payne, and V. Smith, *Accurate diagnosis of Giardia spp and proper fecal examination procedures.* Vet Ther, 2006. **7**(1): p. 4-14.
37. Tangtrongsup, S. and V. Scorza, *Update on the diagnosis and management of Giardia spp infections in dogs and cats.* Topics in companion animal medicine, 2010. **25**(3): p. 155-62.

38. Shelton, A.A., *Sexually transmitted parasitic diseases.* Clin Colon Rectal Surg, 2004. **17**(4): p. 231-4.
39. Ponce-Macotela, M., G.E. Peralta-Abarca, and M.N. Martinez-Gordillo, *Giardia intestinalis and other zoonotic parasites: prevalence in adult dogs from the southern part of Mexico City.* Vet Parasitol, 2005. **131**(1-2): p. 1-4.
40. Mundim, M.J., et al., *Prevalence of Giardia duodenalis and Cryptosporidium spp. in dogs from different living conditions in Uberlandia, Brazil.* Vet Parasitol, 2007. **144**(3-4): p. 356-9.
41. Solarczyk, P. and A.C. Majewska, *A survey of the prevalence and genotypes of Giardia duodenalis infecting household and sheltered dogs.* Parasitol Res, 2010. **106**(5): p. 1015-9.
42. Kirkpatrick, C.E. and J.P. Laczak, *Giardiasis in a cattery.* J Am Vet Med Assoc, 1985. **187**(2): p. 161-2.
43. Wang, A., et al., *Prevalence of Giardia and Cryptosporidium species in dog park attending dogs compared to non-dog park attending dogs in one region of Colorado.* Veterinary Parasitology, 2012. **184**(2-4): p. 335-40.
44. Lalle, M., et al., *A novel Giardia duodenalis assemblage A subtype in fallow deer.* J Parasitol, 2007. **93**(2): p. 426-8.
45. Trout, J.M., M. Santin, and R. Fayer, *Identification of assemblage A Giardia in white-tailed deer.* J Parasitol, 2003. **89**(6): p. 1254-5.
46. Solarczyk, P., et al., *Multilocus genotyping of Giardia duodenalis isolates from red deer (Cervus elaphus) and roe deer (Capreolus capreolus) from Poland.* Folia Parasitol (Praha), 2012. **59**(3): p. 237-40.
47. Buret, A.G., *Pathogenic mechanism in giardiasis and cryptosporidosis*, in *Giardia and cryptosporidium from molecules to disease*, G. Ortega-Pierres, S. Caccio, and R. Fayer, Editors. 2009, CABI: Wallingford, UK ;. p. 428-441.
48. Buret, A.G., *Pathophysiology of enteric infections with Giardia duodenalius.* Parasite, 2008. **15**(3): p. 261-5.
49. Buret, A.G., K.G. Scott, and A.C. Chin, *Giaridasis: Pathophysiology and Pathogenesis*, in *Giardia The Cosmopolitan Parasite*. 2002, CABI Publishing: New York, NY.
50. Hardin, J.A., et al., *Mast cell hyperplasia and increased macromolecular uptake in an animal model of giardiasis.* J Parasitol, 1997. **83**(5): p. 908-12.
51. Ballweber, L.R., et al., *Giardiasis in dogs and cats: update on epidemiology and public health significance.* Trends Parasitol, 2010. **26**(4): p. 180-9.
52. Barr, S.C. and D.D. Bowman, *Giardiasis in Dogs and Cats.* Compendium on Continuing Education for the Practicing Veterinarian, 1994. **16**(5): p. 603-&.
53. Papini, R., et al., *Survey on giardiosis in shelter dog populations.* Vet Parasitol, 2005. **128**(3-4): p. 333-9.
54. Bugg, R.J., et al., *Gastrointestinal parasites of urban dogs in Perth, Western Australia.* Vet J, 1999. **157**(3): p. 295-301.
55. Traub, R.J., et al., *Transmission cycles of Giardia duodenalis in dogs and humans in Temple communities in Bangkok--a critical evaluation of its prevalence using three diagnostic tests in the field in the absence of a gold standard.* Acta Trop, 2009. **111**(2): p. 125-32.
56. Leonhard, S., et al., *The molecular characterisation of Giardia from dogs in southern Germany.* Vet Parasitol, 2007. **150**(1-2): p. 33-8.

57. Souza, M.C. *Predominance of zoonotic Giardia assemblage A in dogs in Portugal.* in *3rd International Giardia and Cryptosporidium Conference, Oct 11-15*. 2009. Orvieto, Italy.
58. Marangi, M., et al., *Genotyping of Giardia duodenalis among children and dogs in a closed socially deprived community from Italy.* Zoonoses Public Health, 2010. **57**(7-8): p. e54-8.
59. Papini, R., et al., *Detection of Giardia assemblage A in cats in Florence, Italy.* Parasitol Res, 2007. **100**(3): p. 653-6.
60. Himsworth, C.G., et al., *Multiple zoonotic pathogens identified in canine feces collected from a remote Canadian indigenous community.* Am J Trop Med Hyg, 2010. **83**(2): p. 338-41.
61. Eligio-Garcia, L., et al., *Frequency of Giardia intestinalis assemblages isolated from dogs and humans in a community from Culiacan, Sinaloa, Mexico using beta-giardin restriction gene.* Vet Parasitol, 2008. **156**(3-4): p. 205-9.
62. Traub, R.J., et al., *Epidemiological and molecular evidence supports the zoonotic transmission of Giardia among humans and dogs living in the same community.* Parasitology, 2004. **128**(Pt 3): p. 253-62.
63. Hiepe, T. and R. Buchwalder, *[Livestock manure as a vector for parasites--a report of experiences]*. Dtsch Tierarztl Wochenschr, 1991. **98**(7): p. 268-72.
64. Soave, R. and P. Ma, *Cryptosporidiosis. Traveler's diarrhea in two families.* Arch Intern Med, 1985. **145**(1): p. 70-2.
65. Catcott, E.J., *The incidence of intestinal protozoa in the dog.* Journal of the American Veterinary Medical Association, 1946. **108**: p. 34-6.
66. Kirkpatrick, C.E. and J.P. Farrell, *Feline giardiasis: observations on natural and induced infections.* Am J Vet Res, 1984. **45**(10): p. 2182-8.
67. Lopez-Brea, M., *Giardia lamblia: incidence in man and dog.* Trans R Soc Trop Med Hyg, 1982. **76**(4): p. 565.
68. Hewlett, E.L., et al., *Experimental infection of mongrel dogs with Giardia lamblia cysts and cultured trophozoites.* J Infect Dis, 1982. **145**(1): p. 89-93.
69. Olson, M.E., D.W. Morck, and H. Ceri, *Preliminary data on the efficacy of a Giardia vaccine in puppies.* Can Vet J, 1997. **38**(12): p. 777-9.
70. Leib, M.S. and A.M. Zajac, *Giardiasis in dogs and cats.* Veterinary Medicine, 1999. **94**(9): p. 793-+.
71. Vasilopulos, R.J., et al., *Prevalence and factors associated with fecal shedding of Giardia spp. in domestic cats.* J Am Anim Hosp Assoc, 2006. **42**(6): p. 424-9.
72. Gookin, J.L., et al., *Prevalence of and risk factors for feline Tritrichomonas foetus and Giardia infection.* J Clin Microbiol, 2004. **42**(6): p. 2707-10.
73. Scorza, A.V. and M.R. Lappin, *Co-infection of Cryptosporidium and Giardia in naturally infected cats. In Diagnosis and Treatment of Cryptosporidiosis and Giardiasis in Cats and Dogs in the United staes.*, in *Clinical Sciences*2007, Colorado State University: Fort Collins.
74. Caccio, S.M., et al., *Unravelling Cryptosporidium and Giardia epidemiology.* Trends Parasitol, 2005. **21**(9): p. 430-7.
75. Bermudez-Cruz, R.M., et al. *Identification of Giardia genotypes in dogs from kennels and veterinarian clinics in France*. in *3rd International Giardia and Cryptosporidium Conference*. 2009. Orvieto, Italy.

76. Sykes, T.J. and M.T. Fox, *Patterns of infection with Giardia in dogs in London.* Trans R Soc Trop Med Hyg, 1989. **83**(2): p. 239-40.
77. Little, S.E. and D.S. Lindsay, *Laboratory Diagnosis of Protozoal Infections*, in *Infectious Disease of the Dog and Cat*, C.E. Greene, Editor. 2012, Elsevier Saunders: Missouri, USA. p. 711-717.
78. Scorza, A.V. and M.R. Lappin, *Giardiasis - in Enteric Protozoa infection*, in *Infectious diseases of the dog and cat*, C.E. Greene, Editor. 2012, Elsevier/Saunders: St. Louis, Mo. p. 785-792.
79. Dryden, M.W., et al., *Comparison of common fecal flotation techniques for the recovery of parasite eggs and oocysts.* Vet Ther, 2005. **6**(1): p. 15-28.
80. Gates, M.C. and T.J. Nolan, *Comparison of passive fecal flotation run by veterinary students to zinc-sulfate centrifugation flotation run in a diagnostic parasitology laboratory.* J Parasitol, 2009. **95**(5): p. 1213-4.
81. Rishniw, M., et al., *Comparison of 4 Giardia diagnostic tests in diagnosis of naturally acquired canine chronic subclinical giardiasis.* J Vet Intern Med, 2010. **24**(2): p. 293-7.
82. Geurden, T., et al., *A Bayesian evaluation of three diagnostic assays for the detection of Giardia duodenalis in symptomatic and asymptomatic dogs.* Vet Parasitol, 2008. **157**(1-2): p. 14-20.
83. Bachman, D.E., et al. *Evaluation of chromatographic immunoassays for the diagnosis of Giardia spp. and Cryptosporidium spp. of cats and dogs.* in *Proceedings of the American College of Veterinary Internal Medicine Forum, May 31-June 3, 2006*. 2007. Louisville, KY.
84. Scorza, A.V., M.M. Brewer, and M.R. Lappin, *Polymerase chain reaction for the detection of Cryptosporidium spp. in cat feces.* J Parasitol, 2003. **89**(2): p. 423-6.
85. Sokolow, S.H., et al., *Epidemiologic evaluation of diarrhea in dogs in an animal shelter.* Am J Vet Res, 2005. **66**(6): p. 1018-24.
86. Mekaru, S.R., et al., *Comparison of direct immunofluorescence, immunoassays, and fecal flotation for detection of Cryptosporidium spp. and Giardia spp. in naturally exposed cats in 4 Northern California animal shelters.* J Vet Intern Med, 2007. **21**(5): p. 959-65.
87. Lappin, M.R., W.A. Jensen, and G. Taton-Allen. *Comparison of ZnSO4 centrifugation, a fecal antigen assay, and an immuno fluorescent antibody assay for diagnosis of giaridasis in cats.* in *20th Annual ACVIM Forum*. 2002. Dallas, TX.
88. Bajer, A., M. Bednarska, and A. Rodo, *Risk factors and control of intestinal parasite infections in sled dogs in Poland.* Veterinary Parasitology, 2011. **175**(3-4): p. 343-50.
89. Rimhanen-Finne, R., P. Ronkainen, and M.L. Hanninen, *Simultaneous detection of Cryptosporidium parvum and Giardia in sewage sludge by IC-PCR.* J Appl Microbiol, 2001. **91**(6): p. 1030-5.
90. Carlin, E.P., et al., *Prevalence of Giardia in symptomatic dogs and cats throughout the United States as determined by the IDEXX SNAP Giardia test.* Vet Ther, 2006. **7**(3): p. 199-206.
91. Olson, M.E., N.J. Leonard, and J. Strout, *Prevalence and diagnosis of Giardia infection in dogs and cats using a fecal antigen test and fecal smear.* Can Vet J, 2010. **51**(6): p. 640-2.
92. Upjohn, M., et al., *Prevalence, molecular typing and risk factor analysis for Giardia duodenalis infections in dogs in a central London rescue shelter.* Vet Parasitol, 2010. **172**(3-4): p. 341-6.

93. Aldeen, W.E., et al., *Comparison of nine commercially available enzyme-linked immunosorbent assays for detection of Giardia lamblia in fecal specimens.* J Clin Microbiol, 1998. **36**(5): p. 1338-40.
94. Cirak, V.Y. and C. Bauer, *Comparison of conventional coproscopical methods and commercial coproantigen ELISA kits for the detection of Giardia and Cryptosporidium infections in dogs and cats.* Berl Munch Tierarztl Wochenschr, 2004. **117**(9-10): p. 410-3.
95. Groat, R., et al. *Survey of clinic practices and testing for diagnosis of Giardia infections in dogs and cats.* in *21st Annual American College of Veterinary Iinternal Medicine Forum, June 4-7, 2003*. 2003. Charlotte, NC.
96. Rossignol, J.F., *Cryptosporidium and Giardia: treatment options and prospects for new drugs.* Exp Parasitol, 2010. **124**(1): p. 45-53.
97. Barr, S.C., et al., *Efficacy of a drug combination of praziquantel, pyrantel pamoate, and febantel against giardiasis in dogs.* Am J Vet Res, 1998. **59**(9): p. 1134-6.
98. Zimmer, J.F., *Treatment of feline giardiasis with metronidazole.* Cornell Vet, 1987. **77**(4): p. 383-8.
99. Scorza, A.V., S.V. Radecki, and M.R. Lappin, *Efficacy of a combination of febantel, pyrantel, and praziquantel for the treatment of kittens experimentally infected with Giardia species.* J Feline Med Surg, 2006. **8**(1): p. 7-13.
100. Lappin, M.R., M. Clark, and A.V. Scorza. *Treatment of healthy Giardia spp. positive dogs with fenbendazole or nitazoxanide.* in *Proceedings of the American College of Veterinary Internal Medicine Forum*. 2008. San Antonio, TX.
101. Miro, G., et al., *Survey of intestinal parasites in stray dogs in the Madrid area and comparison of the efficacy of three anthelmintics in naturally infected dogs.* Parasitol Res, 2007. **100**(2): p. 317-20.
102. Payne, P.A., et al., *Efficacy of a combination febantel-praziquantel-pyrantel product, with or without vaccination with a commercial Giardia vaccine, for treatment of dogs with naturally occurring giardiasis.* J Am Vet Med Assoc, 2002. **220**(3): p. 330-3.
103. Bowman, D.D., et al., *Treatment of naturally occurring, asymptomatic Giardia sp. in dogs with Drontal Plus flavour tablets.* Parasitol Res, 2009. **105 Suppl 1**: p. S125-34.
104. Giangaspero, A., et al., *Efficacy of pyrantel embonate, febantel and praziquantel against Giardia species in naturally infected adult dogs.* Vet Rec, 2002. **150**(6): p. 184-6.
105. Montoya, A., et al., *Efficacy of Drontal Flavour Plus (50 mg praziquantel, 144 mg pyrantel embonate, 150 mg febantel per tablet) against Giardia sp in naturally infected dogs.* Parasitol Res, 2008. **103**(5): p. 1141-4.
106. Abbitt, B., et al., *Treatment of giardiasis in adult Greyhounds, using ipronidazole-medicated water.* J Am Vet Med Assoc, 1986. **188**(1): p. 67-9.
107. Scorza, A.V. and M.R. Lappin, *Metronidazole for the treatment of feline giardiasis.* J Feline Med Surg, 2004. **6**(3): p. 157-60.
108. Dow, S.W., et al., *Central nervous system toxicosis associated with metronidazole treatment of dogs: five cases (1984-1987).* J Am Vet Med Assoc, 1989. **195**(3): p. 365-8.
109. Caylor, K.B. and M.K. Cassimatis, *Metronidazole neurotoxicosis in two cats.* J Am Anim Hosp Assoc, 2001. **37**(3): p. 258-62.
110. Rosado, T.W., A. Specht, and S.L. Marks, *Neurotoxicosis in 4 cats receiving ronidazole.* J Vet Intern Med, 2007. **21**(2): p. 328-31.

111. Olson, M.E., et al., *The use of a Giardia vaccine as an immunotherapeutic agent in dogs.* Can Vet J, 2001. **42**(11): p. 865-8.
112. Foronda, P., et al., *Identification of genotypes of Giardia intestinalis of human isolates in Egypt.* Parasitol Res, 2008. **103**(5): p. 1177-81.
113. Fayer, R., *General Biology*, in *Cryptosporidium and cryptosporidiosis*, R. Fayer and L. Xiao, Editors. 2008, CRC Press: Boca Raton. p. 1-42.
114. Tyzzer, E.E., *A sporozoan found in the peptic glands of the common mouse.* Proceedings of The Society for Experimental Biology and Medicine, 1907. **5**: p. 12-13.
115. Tyzzer, E.E., *An extracellular Coccidium, Cryptosporidium Muris (Gen. Et Sp. Nov.), of the gastric Glands of the Common Mouse.* J Med Res, 1910. **23**(3): p. 487-510 3.
116. Slavin, D., *Cryptosporidium meleagridis (sp. nov.).* J Comp Pathol, 1955. **65**(3): p. 262-6.
117. Fayer, R., *Taxonomy and species delimitation in Cryptosporidium.* Exp Parasitol, 2010. **124**(1): p. 90-7.
118. Robinson, G., et al., *Re-description of Cryptosporidium cuniculus (Apicomplexa: Cryptosporidiidae): Morphology, biology and phylogeny.* Int J Parasitol, 2010.
119. Fayer, R., M. Santin, and D. Macarisin, *Cryptosporidium ubiquitum n. sp. in animals and humans.* Vet Parasitol, 2010. **172**(1-2): p. 23-32.
120. Ren, X., et al., *Cryptosporidium tyzzeri n. sp. (Apicomplexa: Cryptosporidiidae) in domestic mice (Mus musculus).* Exp Parasitol, 2012. **130**(3): p. 274-81.
121. Fayer, R. and M. Santin, *Cryptosporidium xiaoi n. sp. (Apicomplexa: Cryptosporidiidae) in sheep (Ovis aries).* Vet Parasitol, 2009. **164**(2-4): p. 192-200.
122. Alvarez-Pellitero, P., et al., *Cryptosporidium scophthalmi n. sp. (Apicomplexa: Cryptosporidiidae) from cultured turbot Scophthalmus maximus. Light and electron microscope description and histopathological study.* Dis Aquat Organ, 2004. **62**(1-2): p. 133-45.
123. Alvarez-Pellitero, P. and A. Sitja-Bobadilla, *Cryptosporidium molnari n. sp. (Apicomplexa: Cryptosporidiidae) infecting two marine fish species, Sparus aurata L. and Dicentrarchus labrax L.* Int J Parasitol, 2002. **32**(8): p. 1007-21.
124. Lindsay, D.S., et al., *Cryptosporidium andersoni n. sp. (Apicomplexa: Cryptosporiidae) from cattle, Bos taurus.* J Eukaryot Microbiol, 2000. **47**(1): p. 91-5.
125. Current, W.L., S.J. Upton, and T.B. Haynes, *The life cycle of Cryptosporidium baileyi n. sp. (Apicomplexa, Cryptosporidiidae) infecting chickens.* J Protozool, 1986. **33**(2): p. 289-96.
126. Fayer, R., M. Santin, and L. Xiao, *Cryptosporidium bovis n. sp. (Apicomplexa: Cryptosporidiidae) in cattle (Bos taurus).* J Parasitol, 2005. **91**(3): p. 624-9.
127. Fayer, R., et al., *Cryptosporidium canis n. sp. from domestic dogs.* J Parasitol, 2001. **87**(6): p. 1415-22.
128. Iseki, M., *Cryptosporidium felis sp. n. (Protozoa: Eimeriorina) from the domestic cat.* Jpn. J. Parasitol., 1979. **28**: p. 285-307.
129. Pavlásek, I., *Cryptosporidia: biology, diagnosis, host spectrum specificity and the environment.* Klinicka Mikrobiologie a Infekcni Lekarstvi 1999. **3**: p. 290-301.
130. Morgan-Ryan, U.M., et al., *Cryptosporidium hominis n. sp. (Apicomplexa: Cryptosporidiidae) from Homo sapiens.* J Eukaryot Microbiol, 2002. **49**(6): p. 433-40.
131. Levine, N.D., *Some corrections of coccidian (Apicomplexa: Protozoa) nomenclature.* J Parasitol, 1980. **66**(5): p. 830-4.

132. Ryan, U.M., et al., *Cryptosporidium suis n. sp. (Apicomplexa: Cryptosporidiidae) in pigs (Sus scrofa)*. J Parasitol, 2004. **90**(4): p. 769-73.
133. Pavlasek, I., et al., *Cryptosporidium varanii n. sp. (Apicomplexa, Cryptosporidiidae) in Emerald monitor (Varanus prasinus) Schegel 1893) in captivity at Prague zoo.* Gazella, 1995. **22**: p. 99-108.
134. Ryan, U.M., M. Power, and L. Xiao, *Cryptosporidium fayeri n. sp. (Apicomplexa: Cryptosporidiidae) from the Red Kangaroo (Macropus rufus)*. J Eukaryot Microbiol, 2008. **55**(1): p. 22-6.
135. Jirku, M., et al., *New species of Cryptosporidium Tyzzer, 1907 (Apicomplexa) from amphibian host: morphology, biology and phylogeny*. Folia Parasitol (Praha), 2008. **55**(2): p. 81-94.
136. Power, M.L. and U.M. Ryan, *A new species of Cryptosporidium (Apicomplexa: Cryptosporidiidae) from eastern grey kangaroos (Macropus giganteus)*. J Parasitol, 2008. **94**(5): p. 1114-7.
137. Fayer, R., M. Santin, and J.M. Trout, *Cryptosporidium ryanae n. sp. (Apicomplexa: Cryptosporidiidae) in cattle (Bos taurus)*. Vet Parasitol, 2008. **156**(3-4): p. 191-8.
138. Vetterling, J.M., et al., *Cryptosporidium wrairi sp. n. from the guinea pig Cavia porcellus, with an emendation of the genus.* J Protozool, 1971. **18**(2): p. 243-7.
139. Xiao, L. and R. Fayer, *Molecular characterisation of species and genotypes of Cryptosporidium and Giardia and assessment of zoonotic transmission.* Int J Parasitol, 2008. **38**(11): p. 1239-55.
140. Nichols, G., *Epidemiology*, in *Cryptosporidium and cryptosporidiosis*, R. Fayer and L. Xiao, Editors. 2008, CRC Press.: Florida, USA. p. 79-118.
141. McLauchlin, J., et al., *The epidemiology of cryptosporidiosis: application of experimental sub-typing and antibody detection systems to the investigation of water-borne outbreaks.* Folia Parasitol (Praha), 1998. **45**(2): p. 83-92.
142. Nichols, G.L., J. McLauchlin, and D. Samuel, *A technique for typing Cryptosporidium isolates*. J Protozool, 1991. **38**(6): p. 237S-240S.
143. Nina, J.M., et al., *Comparative study of the antigenic composition of oocyst isolates of Cryptosporidium parvum from different hosts.* Parasite Immunol, 1992. **14**(2): p. 227-32.
144. Awad-el-Kariem, F.M., et al., *Differentiation between human and animal strains of Cryptosporidium parvum using isoenzyme typing.* Parasitology, 1995. **110 (Pt 2)**: p. 129-32.
145. Ortega, Y.R., et al., *Restriction fragment length polymorphism analysis of Cryptosporidium parvum isolates of bovine and human origin.* J Protozool, 1991. **38**(6): p. 40S-41S.
146. Morgan, U.M., et al., *Molecular characterization of Cryptosporidium isolates from humans and other animals using random amplified polymorphic DNA analysis.* Am J Trop Med Hyg, 1995. **52**(6): p. 559-64.
147. Bonnin, A., et al., *Genotyping human and bovine isolates of Cryptosporidium parvum by polymerase chain reaction-restriction fragment length polymorphism analysis of a repetitive DNA sequence.* FEMS Microbiol Lett, 1996. **137**(2-3): p. 207-11.
148. Spano, F., et al., *PCR-RFLP analysis of the Cryptosporidium oocyst wall protein (COWP) gene discriminates between C. wrairi and C. parvum, and between C. parvum isolates of human and animal origin.* FEMS Microbiol Lett, 1997. **150**(2): p. 209-17.

149. Peng, M.M., et al., *Genetic polymorphism among Cryptosporidium parvum isolates: evidence of two distinct human transmission cycles.* Emerg Infect Dis, 1997. **3**(4): p. 567-73.
150. Ballweber, L.R., et al., *Prevalence of and risk factors associated with shedding of Cryptosporidium felis in domestic cats of Mississippi and Alabama.* Vet Parasitol, 2009. **160**(3-4): p. 306-10.
151. Argenzio, R.A., et al., *Villous atrophy, crypt hyperplasia, cellular infiltration, and impaired glucose-Na absorption in enteric cryptosporidiosis of pigs.* Gastroenterology, 1990. **98**(5 Pt 1): p. 1129-40.
152. Chai, J.Y., et al., *Role of intraepithelial lymphocytes in mucosal immune responses of mice experimentally infected with Cryptosporidium parvum.* J Parasitol, 1999. **85**(2): p. 234-9.
153. Guk, S.M., T.S. Yong, and J.Y. Chai, *Role of murine intestinal intraepithelial lymphocytes and lamina propria lymphocytes against primary and challenge infections with Cryptosporidium parvum.* J Parasitol, 2003. **89**(2): p. 270-5.
154. Tzipori, S. and I. Campbell, *Prevalence of Cryptosporidium antibodies in 10 animal species.* J Clin Microbiol, 1981. **14**(4): p. 455-6.
155. Wilson, R.B., M.A. Holscher, and S.J. Lyle, *Cryptosporidiosis in a pup.* J Am Vet Med Assoc, 1983. **183**(9): p. 1005-6, 965.
156. Mtambo, M.M., et al., *Cryptosporidium infection in cats: prevalence of infection in domestic and feral cats in the Glasgow area.* Vet Rec, 1991. **129**(23): p. 502-4.
157. Abe, N., et al., *Cryptosporidium infection in dogs in Osaka, Japan.* Vet Parasitol, 2002. **108**(3): p. 185-93.
158. Asahi, H., et al., *Biological nature of Cryptosporidium sp. isolated from a cat.* Parasitol Res, 1991. **77**(3): p. 237-40.
159. Scorza, V. and S. Tangtrongsup, *Update on the diagnosis and management of Cryptosporidium spp infections in dogs and cats.* Top Companion Anim Med, 2010. **25**(3): p. 163-9.
160. Brown, R.R., et al., *Feline zoonoses guidelines from the American Association of Feline Practitioners.* J Feline Med Surg, 2005. **7**(4): p. 243-74.
161. Webster, K.A., et al., *Detection of Cryptosporidium parvum oocysts in faeces: comparison of conventional coproscopical methods and the polymerase chain reaction.* Vet Parasitol, 1996. **61**(1-2): p. 5-13.
162. Anusz, K.Z., et al., *Detection of Cryptosporidium parvum oocysts in bovine feces by monoclonal antibody capture enzyme-linked immunosorbent assay.* J Clin Microbiol, 1990. **28**(12): p. 2770-4.
163. Weber, R., et al., *Threshold of detection of Cryptosporidium oocysts in human stool specimens: evidence for low sensitivity of current diagnostic methods.* J Clin Microbiol, 1991. **29**(7): p. 1323-7.
164. Uga, S., et al., *Cryptosporidiosis in dogs and cats in Hyogo Prefecture, Japan.* Jpn. J. Parasitol., 1989. **38**: p. 139-143.
165. Nydam, D.V., et al., *Number of Cryptosporidium parvum oocysts or Giardia spp cysts shed by dairy calves after natural infection.* Am J Vet Res, 2001. **62**(10): p. 1612-5.
166. Rimhanen-Finne, R., et al., *Evaluation of immunofluorescence microscopy and enzyme-linked immunosorbent assay in detection of Cryptosporidium and Giardia infections in asymptomatic dogs.* Vet Parasitol, 2007. **145**(3-4): p. 345-8.

167. Castro-Hermida, J.A., et al., *Evaluation of two commercial disinfectants on the viability and infectivity of Cryptosporidium parvum oocysts.* Vet J, 2006. **171**(2): p. 340-5.
168. Chalmers, R.M. and A.P. Davies, *Minireview: clinical cryptosporidiosis.* Exp Parasitol, 2010. **124**(1): p. 138-46.
169. Morgan, U.M., P.A. O'Brien, and R.C. Thompson, *The development of diagnostic PCR primers for Cryptosporidium using RAPD-PCR.* Mol Biochem Parasitol, 1996. **77**(1): p. 103-8.
170. Rochelle, P.A., et al., *Comparison of primers and optimization of PCR conditions for detection of Cryptosporidium parvum and Giardia lamblia in water.* Appl Environ Microbiol, 1997. **63**(1): p. 106-14.
171. Gargala, G., *Drug treatment and novel drug target against Cryptosporidium.* Parasite, 2008. **15**(3): p. 275-81.
172. Lappin, M.R., et al., *Cryptosporidiosis and inflammatory bowel disease in a cat.* Feline Practice, 1997. **25**(3): p. 10-13.
173. Barr, S.C., et al., *Use of paromomycin for treatment of cryptosporidiosis in a cat.* J Am Vet Med Assoc, 1994. **205**(12): p. 1742-3.
174. Gookin, J.L., et al., *Acute renal failure in four cats treated with paromomycin.* J Am Vet Med Assoc, 1999. **215**(12): p. 1821-3, 1806.
175. Hemphill, A., J. Mueller, and M. Esposito, *Nitazoxanide, a broad-spectrum thiazolide anti-infective agent for the treatment of gastrointestinal infections.* Expert Opin Pharmacother, 2006. **7**(7): p. 953-64.
176. Lappin, M.R., M. Spindel, and L. Riggenbach. *Infectious agent prevalence rates in dogs with diarrhea and response to administration of fenbendazole or nitazoxanide*. in *25th American College of Veterinary Internal Medicine Forum*. 2008. Seattle, USA.
177. Anderson, B.C., et al., *Cryptosporidiosis in a veterinary student.* J Am Vet Med Assoc, 1982. **180**(4): p. 408-9.
178. Levine, J.F., et al., *Cryptosporidiosis in veterinary students.* J Am Vet Med Assoc, 1988. **193**(11): p. 1413-4.
179. El-Sherbini, G.T. and K.A. Mohammad, *Zoonotic cryptosporidiosis in man and animal in farms, Giza Governorate, Egypt.* J Egypt Soc Parasitol, 2006. **36**(2 Suppl): p. 49-58.
180. Evans, M.R. and D. Gardner, *Cryptosporidiosis outbreak associated with an educational farm holiday.* Commun Dis Rep CDR Rev, 1996. **6**(3): p. R50-1.
181. Sayers, G.M., et al., *Cryptosporidiosis in children who visited an open farm.* Commun Dis Rep CDR Rev, 1996. **6**(10): p. R140-4.
182. Siwila, J., et al., *Asymptomatic cryptosporidiosis in Zambian dairy farm workers and their household members.* Trans R Soc Trop Med Hyg, 2007. **101**(7): p. 733-4.
183. Kaplan, J.E., et al., *Guidelines for prevention and treatment of opportunistic infections in HIV-infected adults and adolescents: recommendations from CDC, the National Institutes of Health, and the HIV Medicine Association of the Infectious Diseases Society of America.* MMWR Recomm Rep, 2009. **58**(RR-4): p. 1-207; quiz CE1-4.
184. Xiao, L. and Y. Feng, *Zoonotic cryptosporidiosis.* FEMS Immunol Med Microbiol, 2008. **52**(3): p. 309-23.
185. Pedraza-Diaz, S., et al., *Unusual cryptosporidium species recovered from human faeces: first description of Cryptosporidium felis and Cryptosporidium 'dog type' from patients in England.* J Med Microbiol, 2001. **50**(3): p. 293-6.

186. Xiao, L., et al., *Identification of 5 types of Cryptosporidium parasites in children in Lima, Peru.* J Infect Dis, 2001. **183**(3): p. 492-7.
187. Gatei, W., et al., *Zoonotic species of Cryptosporidium are as prevalent as the anthroponotic in HIV-infected patients in Thailand.* Ann Trop Med Parasitol, 2002. **96**(8): p. 797-802.
188. Tiangtip, R. and S. Jongwutiwes, *Molecular analysis of Cryptosporidium species isolated from HIV-infected patients in Thailand.* Trop Med Int Health, 2002. **7**(4): p. 357-64.
189. Lucio-Forster, A., et al., *Minimal zoonotic risk of cryptosporidiosis from pet dogs and cats.* Trends Parasitol, 2010. **26**(4): p. 174-9.
190. Glaser, C.A., et al., *Association between Cryptosporidium infection and animal exposure in HIV-infected individuals.* J Acquir Immune Defic Syndr Hum Retrovirol, 1998. **17**(1): p. 79-82.
191. Traub, R.J., et al., *Humans, dogs and parasitic zoonoses--unravelling the relationships in a remote endemic community in northeast India using molecular tools.* Parasitol Res, 2003. **90 Suppl 3**: p. S156-7.
192. Hill, S.L., et al., *Prevalence of enteric zoonotic organisms in cats.* J Am Vet Med Assoc, 2000. **216**(5): p. 687-92.
193. Robertson, I.D., et al., *The role of companion animals in the emergence of parasitic zoonoses.* Int J Parasitol, 2000. **30**(12-13): p. 1369-77.
194. Irwin, P.J., *Companion animal parasitology: a clinical perspective.* Int J Parasitol, 2002. **32**(5): p. 581-93.
195. Savioli, L., H. Smith, and A. Thompson, *Giardia and Cryptosporidium join the 'Neglected Diseases Initiative'.* Trends Parasitol, 2006. **22**(5): p. 203-8.
196. Lane, S. and D. Lloyd, *Current trends in research into the waterborne parasite Giardia.* Crit Rev Microbiol, 2002. **28**(2): p. 123-47.
197. Yoder, J.S., et al., *Cryptosporidiosis surveillance--United States, 2009-2010.* MMWR Surveill Summ, 2012. **61**(5): p. 1-12.
198. Yoder, J.S., et al., *Giardiasis surveillance--United States, 2009-2010.* MMWR Surveill Summ, 2012. **61**(5): p. 13-23.
199. Inpankaew, T., et al., *Seroprevalence of Cryptosporidium parvum infection of dairy cows in three northern provinces of Thailand determined by enzyme-linked immunosorbent assay using recombinant antigen CpP23.* Onderstepoort J Vet Res, 2009. **76**(2): p. 161-5.
200. Inpankaew, T., et al., *Prevalence and genotyping of Cryptosporidium SPP from dairy cow fecal samples in western Thailand.* Southeast Asian Journal of Tropical Medicine and Public Health, 2010. **41**(4): p. 770-5.
201. Jittapalapong, S., et al., *Prevalence of Cryptosporidium among dairy cows in Thailand.* Ann N Y Acad Sci, 2006. **1081**: p. 328-35.
202. Palagrai, P., et al., *Factors associated with Giardia duodenalis infection in Dairy cows in KhonKaen, Thailand* Chiang Mai Veterinary Journal, 2012. **10**(2): p. 67-74.
203. Lwin, K.S., *Prevalence of Cryptosporidium, Giardia and other internal parasites in dairy and beef cattle of mae on district, Chiang Mai Thailand*, 2011, Chiang Mai University and Freie Universitat Berlin: Chiang Mai, Thailand. p. 126.
204. Inpankaew, T., et al. *Prevalence of giardiasis in dairy cattle in northeastern part of Thailand*. in *48th Kasetsart University Annual Conference*. 2010. Bangkok, Thailand.

205. Jittapalapong, S., et al. *Preliminary study of protozoan and helminth infections of goats of Satun province, Thailand*. in *41th Kasetsart University Annual Conference*. 2003. Bangkok, Thailand.
206. Nuchjangreed, C., et al., *Prevalence and molecular characterization of human and bovine Cryptosporidium isolates in Thailand.* Parasitol Res, 2008. **103**(6): p. 1347-53.
207. Inpankaew, T., et al., *Canine parasitic zoonoses in Bangkok temples.* Southeast Asian J Trop Med Public Health, 2007. **38**(2): p. 247-55.
208. Fontanarrosa, M.F., et al., *An epidemiological study of gastrointestinal parasites of dogs from Southern Greater Buenos Aires (Argentina): age, gender, breed, mixed infections, and seasonal and spatial patterns.* Vet Parasitol, 2006. **136**(3-4): p. 283-95.
209. Taranto, N.J., et al., *[Zoonotic parasitosis transmitted by dogs in the Chaco Salteno, Argentina]*. Medicina (B Aires), 2000. **60**(2): p. 217-20.
210. Soriano, S.V., et al., *A wide diversity of zoonotic intestinal parasites infects urban and rural dogs in Neuquen, Patagonia, Argentina.* Vet Parasitol, 2010. **167**(1): p. 81-5.
211. Palmer, C.S., et al., *National study of the gastrointestinal parasites of dogs and cats in Australia.* Vet Parasitol, 2008. **151**(2-4): p. 181-90.
212. Claerebout, E., et al., *Giardia and other intestinal parasites in different dog populations in Northern Belgium.* Vet Parasitol, 2009. **161**(1-2): p. 41-6.
213. Katagiri, S. and T.C. Oliveira-Sequeira, *Prevalence of dog intestinal parasites and risk perception of zoonotic infection by dog owners in Sao Paulo State, Brazil.* Zoonoses Public Health, 2008. **55**(8-10): p. 406-13.
214. Meireles, P., F. Montiani-Ferreira, and V. Thomaz-Soccol, *Survey of giardiosis in household and shelter dogs from metropolitan areas of Curitiba, Parana state, Southern Brazil.* Vet Parasitol, 2008. **152**(3-4): p. 242-8.
215. Klimpel, S., et al., *Gastrointestinal and ectoparasites from urban stray dogs in Fortaleza (Brazil): high infection risk for humans?* Parasitol Res, 2010. **107**(3): p. 713-9.
216. Oliveira-Sequeira, T.C., et al., *Prevalence of intestinal parasites in dogs from Sao Paulo State, Brazil.* Vet Parasitol, 2002. **103**(1-2): p. 19-27.
217. Huber, F., T.C. Bomfim, and R.S. Gomes, *Comparison between natural infection by Cryptosporidium sp., Giardia sp. in dogs in two living situations in the West Zone of the municipality of Rio de Janeiro.* Vet Parasitol, 2005. **130**(1-2): p. 69-72.
218. Paz e Silva, F.M., et al., *Molecular characterization of Giardia duodenalis in dogs from Brazil.* Parasitology Research, 2012. **110**(1): p. 325-34.
219. Lefebvre, S.L., et al., *Prevalence of zoonotic agents in dogs visiting hospitalized people in Ontario: implications for infection control.* J Hosp Infect, 2006. **62**(4): p. 458-66.
220. Jacobs, S.R., C.P. Forrester, and J. Yang, *A survey of the prevalence of Giardia in dogs presented to Canadian veterinary practices.* Can Vet J, 2001. **42**(1): p. 45-6.
221. Joffe, D., et al., *The prevalence of intestinal parasites in dogs and cats in Calgary, Alberta.* Canadian Veterinary Journal, 2011. **52**(12): p. 1323-8.
222. Shukla, R., et al., *Cryptosporidium spp. and other zoonotic enteric parasites in a sample of domestic dogs and cats in the Niagara region of Ontario.* Canadian Veterinary Journal, 2006. **47**(12): p. 1179-84.
223. Li, J., et al., *Genotype identification and prevalence of Giardia duodenalis in pet dogs of Guangzhou, Southern China.* Veterinary Parasitology, 2012.
224. Scorza, A.V., et al., *Prevalence of selected zoonotic and vector-borne agents in dogs and cats in Costa Rica.* Veterinary Parasitology, 2011.

225. Dubna, S., et al., *The prevalence of intestinal parasites in dogs from Prague, rural areas, and shelters of the Czech Republic.* Vet Parasitol, 2007. **145**(1-2): p. 120-8.
226. Svobodova, V., J. Konvalinova, and M. Svobodova, *Coprological and serological findings in dogs and cats with giardiosis and cryptosporidiosis.* Acta Vet Brno., 1994. **63**: p. 257-262.
227. Gingrich, E.N., et al., *Intestinal parasites of dogs on the Galapagos Islands.* Vet Parasitol, 2010. **169**(3-4): p. 404-7.
228. Barutzki, D. and R. Schaper, *Endoparasites in dogs and cats in Germany 1999-2002.* Parasitology Research, 2003. **90 Suppl 3**: p. S148-50.
229. Barutzki, D. and R. Schaper, *Results of parasitological examinations of faecal samples from cats and dogs in Germany between 2003 and 2010.* Parasitology Research, 2011. **109 Suppl 1**: p. S45-60.
230. Becker, A.C., et al., *Prevalence of endoparasites in stray and fostered dogs and cats in Northern Germany.* Parasitology Research, 2012. **111**(2): p. 849-57.
231. Papazahariadou, M., et al., *Gastrointestinal parasites of shepherd and hunting dogs in the Serres Prefecture, Northern Greece.* Vet Parasitol, 2007. **148**(2): p. 170-3.
232. Jafari Shoorijeh, S., et al., *Giardia spp. and Sarcocystis spp. status in pet dogs of Shiraz, Southern part of Iran.* Trop Biomed, 2008. **25**(2): p. 154-9.
233. Paoletti, B., et al., *Epidemiological scenario of giardiosis in dogs from central Italy.* Ann N Y Acad Sci, 2008. **1149**: p. 371-4.
234. Capelli, G., et al., *Giardia species and other intestinal parasites in dogs in north-east and central Italy.* Vet Rec, 2006. **159**(13): p. 422-4.
235. Capelli, G., et al., *Prevalence of Giardia spp. in dogs and humans in northern and central Italy.* Parasitol Res, 2003. **90 Suppl 3**: p. S154-5.
236. Scaramozzino, P., et al., *A study of the prevalence and genotypes of Giardia duodenalis infecting kennelled dogs.* Vet J, 2009. **182**(2): p. 231-4.
237. Itoh, N., et al., *Prevalence of Giardia intestinalis infection in dogs of breeding kennels in Japan.* J Vet Med Sci, 2005. **67**(7): p. 717-8.
238. Itoh, N., et al., *Prevalence of Giardia lamblia infection in household dogs.* Kansenshogaku Zasshi, 2001. **75**(8): p. 671-7.
239. Itoh, N., et al., *Prevalence of Giardia intestinalis and other zoonotic intestinal parasites in private household dogs of the Hachinohe area in Aomori prefecture, Japan in 1997, 2002 and 2007.* J Vet Sci, 2009. **10**(4): p. 305-8.
240. Itoh, N., et al., *Prevalence of intestinal parasites and genotyping of Giardia intestinalis in pet shop puppies in east Japan.* Veterinary Parasitology, 2011. **176**(1): p. 74-8.
241. Itoh, N., et al., *Giardia and other intestinal parasites in dogs from veterinary clinics in Japan.* Parasitology Research, 2011. **109**(1): p. 253-6.
242. Liu, J., S.E. Lee, and K.H. Song, *Prevalence of canine giardiosis in South Korea.* Res Vet Sci, 2008. **84**(3): p. 416-8.
243. Lebbad, M., et al., *Dominance of Giardia assemblage B in Leon, Nicaragua.* Acta Trop, 2008. **106**(1): p. 44-53.
244. Mircean, V., A. Gyorke, and V. Cozma, *Prevalence and risk factors of Giardia duodenalis in dogs from Romania.* Veterinary Parasitology, 2012. **184**(2-4): p. 325-9.
245. Nikolic, A., et al., *High prevalence of intestinal zoonotic parasites in dogs from Belgrade, Serbia--short communication.* Acta Vet Hung, 2008. **56**(3): p. 335-40.

246. Causape, A.C., et al., *Prevalence of intestinal parasites, including Cryptosporidium parvum, in dogs in Zaragoza city, Spain.* Vet Parasitol, 1996. **67**(3-4): p. 161-7.
247. Martinez-Moreno, F.J., et al., *Estimation of canine intestinal parasites in Cordoba (Spain) and their risk to public health.* Vet Parasitol, 2007. **143**(1): p. 7-13.
248. Martinez-Carrasco, C., et al., *Epidemiological study of non-systemic parasitism in dogs in southeast Mediterranean Spain assessed by coprological and post-mortem examination.* Zoonoses Public Health, 2007. **54**(5): p. 195-203.
249. Winsland, J.K., et al., *Prevalence of Giardia in dogs and cats in the United Kingdom: survey of an Essex veterinary clinic.* Trans R Soc Trop Med Hyg, 1989. **83**(6): p. 791-2.
250. Hackett, T. and M.R. Lappin, *Prevalence of enteric pathogens in dogs of north-central Colorado.* J Am Anim Hosp Assoc, 2003. **39**(1): p. 52-6.
251. Little, S.E., et al., *Prevalence of intestinal parasites in pet dogs in the United States.* Veterinary Parasitology, 2009. **166**(1-2): p. 144-52.
252. Kirkpatrick, C.E., *Epizootiology of endoparasitic infections in pet dogs and cats presented to a veterinary teaching hospital.* Vet Parasitol, 1988. **30**(2): p. 113-24.
253. Gates, M.C. and T.J. Nolan, *Endoparasite prevalence and recurrence across different age groups of dogs and cats.* Vet Parasitol, 2009. **166**(1-2): p. 153-8.
254. McGlade, T.R., et al., *High prevalence of Giardia detected in cats by PCR.* Vet Parasitol, 2003. **110**(3-4): p. 197-205.
255. Bissett, S.A., et al., *Observed occurrence of Tritrichomonas foetus and other enteric parasites in Australian cattery and shelter cats.* J Feline Med Surg, 2009. **11**(10): p. 803-7.
256. Serra, C.M., C.M. Uchoa, and R.A. Coimbra, *[Parasitological study with faecal samples of stray and domiciliated cats (Felis catus domesticus) from the Metropolitan Area of Rio de Janeiro, Brazil]*. Rev Soc Bras Med Trop, 2003. **36**(3): p. 331-4.
257. Lopez, J., et al., *[Intestinal parasites in dogs and cats with gastrointestinal symptoms in Santiago, Chile]*. Rev Med Chil, 2006. **134**(2): p. 193-200.
258. Santin, M., et al., *Cryptosporidium, Giardia and Enterocytozoon bieneusi in cats from Bogota (Colombia) and genotyping of isolates.* Vet Parasitol, 2006. **141**(3-4): p. 334-9.
259. Khalafalla, R.E., *A survey study on gastrointestinal parasites of stray cats in northern region of Nile delta, egypt.* PLoS One, 2011. **6**(7): p. e20283.
260. Nareaho, A., et al., *Feline intestinal parasites in Finland: prevalence, risk factors and anthelmintic treatment practices.* J Feline Med Surg, 2012. **14**(6): p. 378-83.
261. Mohsen, A. and H. Hossein, *Gastrointestinal parasites of stray cats in Kashan, Iran.* Trop Biomed, 2009. **26**(1): p. 16-22.
262. Papini, R., et al., *Survey of feline giardiasis by ELISA test in Italy.* Vet Res Commun, 2007. **31**(3): p. 297-303.
263. Bianciardi, R., et al., *Prevalence of Giardia antigen in stool samples from dogs and cats.* Revue De Medecine Veterinaire, 2004. **155**(8-9): p. 417-421.
264. Itoh, N., et al., *Prevalence of Giardia intestinalis infection in household cats of Tohoku district in Japan.* J Vet Med Sci, 2006. **68**(2): p. 161-3.
265. Itoh, N., et al., *Prevalence of intestinal parasites in private-household cats in Japan.* J Feline Med Surg, 2012. **14**(6): p. 436-9.
266. Kingsbury, D.D., et al., *Identification of Tritrichomonas foetus and Giardia spp. infection in pedigree show cats in New Zealand.* N Z Vet J, 2010. **58**(1): p. 6-10.

267. Mircean, V., A. Titilincu, and C. Vasile, *Prevalence of endoparasites in household cat (Felis catus) populations from Transylvania (Romania) and association with risk factors.* Vet Parasitol, 2010. **171**(1-2): p. 163-6.
268. Nikolic, A., et al., *Giardiasis in dogs and cats in the Belgrade area.* Acta Veterinaria-Beograd, 2002. **52**(1): p. 43-47.
269. Robben, S.R., et al., *[Infections with helminths and/or protozoa in cats in animal shelters in the Netherlands].* Tijdschr Diergeneeskd, 2004. **129**(1): p. 2-6.
270. Overgaauw, P.A., et al., *Zoonotic parasites in fecal samples and fur from dogs and cats in The Netherlands.* Vet Parasitol, 2009. **163**(1-2): p. 115-22.
271. Gow, A.G., et al., *Prevalence of potentially pathogenic enteric organisms in clinically healthy kittens in the UK.* J Feline Med Surg, 2009. **11**(8): p. 655-62.
272. Queen, E.V., S.L. Marks, and T.B. Farver, *Prevalence of selected bacterial and parasitic agents in feces from diarrheic and healthy control cats from Northern California.* Journal of Veterinary Internal Medicine, 2012. **26**(1): p. 54-60.
273. Nutter, F.B., et al., *Seroprevalences of antibodies against Bartonella henselae and Toxoplasma gondii and fecal shedding of Cryptosporidium spp, Giardia spp, and Toxocara cati in feral and pet domestic cats.* J Am Vet Med Assoc, 2004. **225**(9): p. 1394-8.
274. Lucio-Forster, A. and D.D. Bowman, *Prevalence of fecal-borne parasites detected by centrifugal flotation in feline samples from two shelters in upstate New York.* J Feline Med Surg, 2011. **13**(4): p. 300-3.
275. Spain, C.V., et al., *Prevalence of enteric zoonotic agents in cats less than 1 year old in central New York State.* J Vet Intern Med, 2001. **15**(1): p. 33-8.
276. Burrows, R.B. and G.R. Hunt, *Intestinal protozoan infections in cats.* Journal of the American Veterinary Medical Association, 1970. **157**(12): p. 2065-7.
277. De Santis-Kerr, A.C., et al., *Prevalence and risk factors for Giardia and coccidia species of pet cats in 2003-2004.* J Feline Med Surg, 2006. **8**(5): p. 292-301.
278. Monis, P.T., et al., *Novel lineages of Giardia intestinalis identified by genetic analysis of organisms isolated from dogs in Australia.* Parasitology, 1998. **116 (Pt 1)**: p. 7-19.
279. Palmer, C.S., et al., *Determining the zoonotic significance of Giardia and Cryptosporidium in Australian dogs and cats.* Vet Parasitol, 2008. **154**(1-2): p. 142-7.
280. Barutzki, D., et al., *Observations on Giardia infection in dogs from veterinary clinics in Germany.* Parasitology Research, 2007. **101**: p. S153-S156.
281. Szenasi, Z., et al., *Preliminary investigation of the prevalence and genotype distribution of Giardia intestinalis in dogs in Hungary.* Parasitology Research, 2007. **101**: p. S145-S152.
282. Berrilli, F., et al., *Genotype characterisation of Giardia duodenalis isolates from domestic and farm animals by SSU-rRNA gene sequencing.* Vet Parasitol, 2004. **122**(3): p. 193-9.
283. Lalle, M., et al., *Genetic heterogeneity at the beta-giardin locus among human and animal isolates of Giardia duodenalis and identification of potentially zoonotic subgenotypes.* Int J Parasitol, 2005. **35**(2): p. 207-13.
284. van der Giessen, J.W., et al., *Genotyping of Giardia in Dutch patients and animals: a phylogenetic analysis of human and animal isolates.* Int J Parasitol, 2006. **36**(7): p. 849-58.

285. Miska, K.B., et al., *Detection and comparison of Giardia virus (GLV) from different assemblages of Giardia duodenalis.* J Parasitol, 2009. **95**(5): p. 1197-200.
286. Eligio-Garcia, L., A. Cortes-Campos, and E. Jimenez-Cardoso, *Classification of Giardia intestinalis isolates by multiple polymerase chain reaction (multiplex).* Parasitol Res, 2008. **103**(4): p. 797-800.
287. Minvielle, M.C., et al., *First genotyping of Giardia lamblia from human and animal feces in Argentina, South America.* Mem Inst Oswaldo Cruz, 2008. **103**(1): p. 98-103.
288. Souza, S.L., et al., *Molecular identification of Giardia duodenalis isolates from humans, dogs, cats and cattle from the state of Sao Paulo, Brazil, by sequence analysis of fragments of glutamate dehydrogenase (gdh) coding gene.* Vet Parasitol, 2007. **149**(3-4): p. 258-64.
289. Volotao, A.C., et al., *Genotyping of Giardia duodenalis from Southern Brown Howler Monkeys (Alouatta clamitans) from Brazil.* Vet Parasitol, 2008. **158**(1-2): p. 133-7.
290. Cooper, M.A., et al., *Molecular analysis of household transmission of Giardia lamblia in a region of high endemicity in Peru.* J Infect Dis, 2010. **202**(11): p. 1713-21.
291. Abe, N., I. Kimata, and M. Iseki, *Identification of genotypes of Giardia intestinalis isolates from dogs in Japan by direct sequencing of the PCR amplified glutamate dehydrogenase gene.* Journal of Veterinary Medical Science, 2003. **65**(1): p. 29-33.
292. Itagaki, T., et al., *Genotyping of Giardia intestinalis from domestic and wild animals in Japan using glutamete dehydrogenase gene sequencing.* Vet Parasitol, 2005. **133**(4): p. 283-7.
293. Nguyen, L.P., C.D. Phungb, and R.C.A. THompson. *Prevalene and characterization of Giardia and Cryptosporidium in human and domestic animals in a rural area of Vietnam.* in *3rd International Giardia and Cryptosporidium Conference, Oct 11-15*. 2009. Orvieto, Italy.
294. Ravagnan, S., et al. *Comparison of two target genes for detection and genotyping of Giardia duodenalis in mammals*. in *3rd Interantionl Giardia and Cryptosporidium Conference, Oct 11-15*. 2009. Orvieto, Italy.
295. Paoletti, B., et al., *Prevalence and genetic characterization of Giardia and Cryptosporidium in cats from Italy.* Res Vet Sci, 2011. **91**(3): p. 397-9.
296. Caccio, S.M., et al., *Multilocus genotyping of Giardia duodenalis reveals striking differences between assemblages A and B.* Int J Parasitol, 2008. **38**(13): p. 1523-31.
297. Vasilopulos, R.J., et al., *Genotypic analysis of Giardia duodenalis in domestic cats.* J Vet Intern Med, 2007. **21**(2): p. 352-5.
298. Fayer, R., et al., *Detection of Cryptosporidium felis and Giardia duodenalis Assemblage F in a cat colony.* Vet Parasitol, 2006. **140**(1-2): p. 44-53.
299. Suzuki, J., et al., *Risk of human infection with Giardia duodenalis from cats in Japan and genotyping of the isolates to assess the route of infection in cats.* Parasitology, 2011. **138**(4): p. 493-500.
300. Addiss, D.G., et al., *Reduction of risk of watery diarrhea with point-of-use water filters during a massive outbreak of waterborne Cryptosporidium infection in Milwaukee, Wisconsin, 1993.* Am J Trop Med Hyg, 1996. **54**(6): p. 549-53.
301. Atherton, F., C.P. Newman, and D.P. Casemore, *An outbreak of waterborne cryptosporidiosis associated with a public water supply in the UK.* Epidemiol Infect, 1995. **115**(1): p. 123-31.

302. Bridgman, S.A., et al., *Outbreak of cryptosporidiosis associated with a disinfected groundwater supply.* Epidemiol Infect, 1995. **115**(3): p. 555-66.
303. Corso, P.S., et al., *Cost of illness in the 1993 waterborne Cryptosporidium outbreak, Milwaukee, Wisconsin.* Emerg Infect Dis, 2003. **9**(4): p. 426-31.
304. D'Antonio, R.G., et al., *A waterborne outbreak of cryptosporidiosis in normal hosts.* Ann Intern Med, 1985. **103**(6 (Pt 1)): p. 886-8.
305. Dworkin, M.S., et al., *Cryptosporidiosis in Washington State: an outbreak associated with well water.* J Infect Dis, 1996. **174**(6): p. 1372-6.
306. Glaberman, S., et al., *Three drinking-water-associated cryptosporidiosis outbreaks, Northern Ireland.* Emerg Infect Dis, 2002. **8**(6): p. 631-3.
307. Goldstein, S.T., et al., *Cryptosporidiosis: an outbreak associated with drinking water despite state-of-the-art water treatment.* Ann Intern Med, 1996. **124**(5): p. 459-68.
308. Egorov, A., et al., *Contamination of water supplies with Cryptosporidium parvum and Giardia lamblia and diarrheal illness in selected Russian cities.* Int J Hyg Environ Health, 2002. **205**(4): p. 281-9.
309. Hayes, E.B., et al., *Large community outbreak of cryptosporidiosis due to contamination of a filtered public water supply.* N Engl J Med, 1989. **320**(21): p. 1372-6.
310. Howe, A.D., et al., *Cryptosporidium oocysts in a water supply associated with a cryptosporidiosis outbreak.* Emerg Infect Dis, 2002. **8**(6): p. 619-24.
311. Hoxie, N.J., et al., *Cryptosporidiosis-associated mortality following a massive waterborne outbreak in Milwaukee, Wisconsin.* Am J Public Health, 1997. **87**(12): p. 2032-5.
312. Hunter, P., Q. Syed, and E.N. Naumova, *Possible undetected outbreaks of cryptosporidiosis in areas of the north west of England supplied by an unfiltered surface water source.* Commun Dis Public Health, 2001. **4**(2): p. 136-8.
313. Joseph, C., et al., *Cryptosporidiosis in the Isle of Thanet - an Outbreak Associated with Local Drinking-Water.* Epidemiology and Infection, 1991. **107**(3): p. 509-519.
314. Maguire, H.C., et al., *An outbreak of cryptosporidiosis in south London: what value the p value?* Epidemiol Infect, 1995. **115**(2): p. 279-87.
315. McAnulty, J.M., et al., *Contaminated drinking water in one town manifesting as an outbreak of cryptosporidiosis in another.* Epidemiol Infect, 2000. **125**(1): p. 79-86.
316. McDonald, A.C., et al., *Cryptosporidium parvum-specific antibody responses among children residing in Milwaukee during the 1993 waterborne outbreak.* J Infect Dis, 2001. **183**(9): p. 1373-9.
317. Kato, S., et al., *Waterborne Cryptosporidium oocyst identification and genotyping: use of GIS for ecosystem studies in Kenya and Ecuador.* J Eukaryot Microbiol, 2003. **50 Suppl**: p. 548-9.
318. Rush, B.A., P.A. Chapman, and R.W. Ineson, *A probable waterborne outbreak of cryptosporidiosis in the Sheffield area.* J Med Microbiol, 1990. **32**(4): p. 239-42.
319. Smith, H.V., et al., *An outbreak of waterborne cryptosporidiosis caused by post-treatment contamination.* Epidemiol Infect, 1989. **103**(3): p. 703-15.
320. Willocks, L., et al., *A large outbreak of cryptosporidiosis associated with a public water supply from a deep chalk borehole. Outbreak Investigation Team.* Commun Dis Public Health, 1998. **1**(4): p. 239-43.

321. Yamamoto, N., et al., *Outbreak of cryptosporidiosis after contamination of the public water supply in Saitama Prefecture, Japan, in 1996.* Kansenshogaku Zasshi, 2000. **74**(6): p. 518-26.
322. Duke, L.A., et al., *A mixed outbreak of cryptosporidium and campylobacter infection associated with a private water supply.* Epidemiol Infect, 1996. **116**(3): p. 303-8.
323. Said, B., et al., *Outbreaks of infectious disease associated with private drinking water supplies in England and Wales 1970-2000.* Epidemiol Infect, 2003. **130**(3): p. 469-79.
324. Franco, R.M. and R. Cantusio Neto, *Occurrence of cryptosporidial oocysts and giardia cysts in bottled mineral water commercialized in the city of Campinas, State of Sao Paulo, Brazil.* Mem Inst Oswaldo Cruz, 2002. **97**(2): p. 205-7.
325. Leach, C.T., et al., *Prevalence of Cryptosporidium parvum infection in children along the Texas-Mexico border and associated risk factors.* Am J Trop Med Hyg, 2000. **62**(5): p. 656-61.
326. Ravn, P., et al., *Nosocomial outbreak of cryptosporidiosis in AIDS patients.* BMJ, 1991. **302**(6771): p. 277-80.
327. Dawson, D., *Foodborne protozoan parasites.* Int J Food Microbiol, 2005. **103**(2): p. 207-27.
328. Dawson, D.J., et al., *Survival of Cryptosporidium species in environments relevant to foods and beverages.* J Appl Microbiol, 2004. **96**(6): p. 1222-9.
329. Duffy, G. and E.M. Moriarty, *Cryptosporidium and its potential as a food-borne pathogen.* Anim Health Res Rev, 2003. **4**(2): p. 95-107.
330. Nichols, G.L., *Food-borne protozoa.* Br Med Bull, 2000. **56**(1): p. 209-35.
331. Rose, J.B. and T.R. Slifko, *Giardia, Cryptosporidium, and Cyclospora and their impact on foods: a review.* J Food Prot, 1999. **62**(9): p. 1059-70.
332. Bier, J.W., *Isolation of parasites on fruits and vegetables.* Southeast Asian J Trop Med Public Health, 1991. **22 Suppl**: p. 144-5.
333. Caccio, S. and E. Pozio, *Molecular identification of food-borne and water-borne protozoa.* Southeast Asian J Trop Med Public Health, 2001. **32 Suppl 2**: p. 156-8.
334. Cook, N., *The use of NASBA for the detection of microbial pathogens in food and environmental samples.* J Microbiol Methods, 2003. **53**(2): p. 165-74.
335. Deng, M.Q. and D.O. Cliver, *Comparative detection of Cryptosporidium parvum oocysts from apple juice.* Int J Food Microbiol, 2000. **54**(3): p. 155-62.
336. Deng, M.Q., K.M. Lam, and D.O. Cliver, *Immunomagnetic separation of Cryptosporidium parvum oocysts using MACS MicroBeads and high gradient separation columns.* J Microbiol Methods, 2000. **40**(1): p. 11-7.
337. Kniel, K.E. and M.C. Jenkins, *Detection of Cryptosporidium parvum oocysts on fresh vegetables and 1 herbs using antibodies specific for a Cryptosporidium parvum viral antigen.* J Food Prot, 2005. **68**(5): p. 1093-6.
338. Laberge, I., et al., *Detection of Cryptosporidium parvum in raw milk by PCR and oligonucleotide probe hybridization.* Appl Environ Microbiol, 1996. **62**(9): p. 3259-64.
339. Robertson, L.J. and B. Gjerde, *Factors affecting recovery efficiency in isolation of Cryptosporidium oocysts and Giardia cysts from vegetables for standard method development.* J Food Prot, 2001. **64**(11): p. 1799-805.
340. Garcia, L., et al., *Potential sources of microbial contamination in unpasteurized apple cider.* J Food Prot, 2006. **69**(1): p. 137-44.

341. Millard, P.S., et al., *An outbreak of cryptosporidiosis from fresh-pressed apple cider.* JAMA, 1994. **272**(20): p. 1592-6.
342. Djuretic, T., P.G. Wall, and G. Nichols, *General outbreaks of infectious intestinal disease associated with milk and dairy products in England and Wales: 1992 to 1996.* Commun Dis Rep CDR Rev, 1997. **7**(3): p. R41-5.
343. Gelletlie, R., et al., *Cryptosporidiosis associated with school milk.* Lancet, 1997. **350**(9083): p. 1005-6.
344. Robertson, L.J., et al., *The potential for acquiring cryptosporidiosis or giardiosis from consumption of mung bean sprouts in Norway: a preliminary step-wise risk assessment.* Int J Food Microbiol, 2005. **98**(3): p. 291-300.
345. Robertson, L.J. and B. Gjerde, *Occurrence of parasites on fruits and vegetables in Norway.* J Food Prot, 2001. **64**(11): p. 1793-8.
346. Chalmers, R.M., et al., *Cryptosporidium parvum in environmental samples in the Sligo area, Republic of Ireland: a preliminary report.* Lett Appl Microbiol, 1997. **25**(5): p. 380-4.
347. Collins, M.V., et al., *The effects of E-beam irradiation and microwave energy on Eastern Oysters (Crassostrea virginica) experimentally infected with Cryptosporidium parvum.* J Eukaryot Microbiol, 2005. **52**(6): p. 484-8.
348. Collins, M.V., et al., *The effect of high-pressure processing on infectivity of Cryptosporidium parvum oocysts recovered from experimentally exposed Eastern oysters (Crassostrea virginica).* J Eukaryot Microbiol, 2005. **52**(6): p. 500-4.
349. Graczyk, T.K. and K.J. Schwab, *Foodborne infections vectored by molluscan shellfish.* Curr Gastroenterol Rep, 2000. **2**(4): p. 305-9.
350. Li, X., et al., *Cryptosporidium oocysts in mussels (Mytilus edulis) from Normandy (France).* Int J Food Microbiol, 2006. **108**(3): p. 321-5.
351. MacRae, M., et al., *The detection of Cryptosporidium parvum and Escherichia coli O157 in UK bivalve shellfish.* J Microbiol Methods, 2005. **60**(3): p. 395-401.
352. Quiroz, E.S., et al., *An outbreak of cryptosporidiosis linked to a foodhandler.* J Infect Dis, 2000. **181**(2): p. 695-700.
353. Bell, A., et al., *A swimming pool-associated outbreak of Cryptosporidiosis in British Columbia.* Can J Public Health, 1993. **84**(5): p. 334-7.
354. Hunt, D.A., et al., *Cryptosporidiosis associated with a swimming pool complex.* Commun Dis Rep CDR Rev, 1994. **4**(2): p. R20-2.
355. Insulander, M., et al., *An outbreak of cryptosporidiosis associated with exposure to swimming pool water.* Scand J Infect Dis, 2005. **37**(5): p. 354-60.
356. Joce, R.E., et al., *An outbreak of cryptosporidiosis associated with a swimming pool.* Epidemiol Infect, 1991. **107**(3): p. 497-508.
357. Lim, L.S., et al., *Cryptosporidiosis outbreak in a recreational swimming pool in Minnesota.* J Environ Health, 2004. **67**(1): p. 16-20, 28, 27; quiz 31-2.
358. Louie, K., et al., *An outbreak of Cryptosporidium parvum in a Surrey pool with detection in pool water sampling.* Can Commun Dis Rep, 2004. **30**(7): p. 61-6.
359. MacKenzie, W.R., et al., *Massive outbreak of waterborne cryptosporidium infection in Milwaukee, Wisconsin: recurrence of illness and risk of secondary transmission.* Clin Infect Dis, 1995. **21**(1): p. 57-62.

360. Mathieu, E., et al., *Epidemiologic and environmental investigation of a recreational water outbreak caused by two genotypes of Cryptosporidium parvum in Ohio in 2000.* Am J Trop Med Hyg, 2004. **71**(5): p. 582-9.
361. McAnulty, J.M., D.W. Fleming, and A.H. Gonzalez, *A community-wide outbreak of cryptosporidiosis associated with swimming at a wave pool.* JAMA, 1994. **272**(20): p. 1597-600.
362. Puech, M.C., et al., *A statewide outbreak of cryptosporidiosis in New South Wales associated with swimming at public pools.* Epidemiol Infect, 2001. **126**(3): p. 389-96.
363. Sorvillo, F.J., et al., *Swimming-associated cryptosporidiosis.* Am J Public Health, 1992. **82**(5): p. 742-4.
364. Stafford, R., et al., *A community outbreak of Cryptosporidium infection associated with a swimming pool complex.* Commun Dis Intell, 2000. **24**(8): p. 236-9.
365. Sundkvist, T., et al., *Outbreak of cryptosporidiosis associated with a swimming pool in Andover.* Commun Dis Rep CDR Rev, 1997. **7**(12): p. R190-2.
366. Wilberschied, L., *A swimming-pool-associated outbreak of cryptosporidiosis.* Kans Med, 1995. **96**(2): p. 67-8.
367. Jones, M., et al., *Cryptosporidium outbreak linked to interactive water feature, UK: importance of guidelines.* Euro Surveill, 2006. **11**(4): p. 126-8.
368. Nichols, G., *Infection risks from water in natural and man-made environments.* Euro Surveill, 2006. **11**(4): p. 76-8.
369. Causer, L.M., et al., *An outbreak of Cryptosporidium hominis infection at an Illinois recreational waterpark.* Epidemiol Infect, 2006. **134**(1): p. 147-56.
370. Kramer, M.H., et al., *First reported outbreak in the United States of cryptosporidiosis associated with a recreational lake.* Clin Infect Dis, 1998. **26**(1): p. 27-33.
371. Hunter, P.R., et al., *Sporadic cryptosporidiosis case-control study with genotyping.* Emerg Infect Dis, 2004. **10**(7): p. 1241-9.
372. Mahdi, N.K. and N.H. Ali, *Cryptosporidiosis among animal handlers and their livestock in Basrah, Iraq.* East Afr Med J, 2002. **79**(10): p. 550-3.
373. Pohjola, S., et al., *Outbreak of cryptosporidiosis among veterinary students.* Scand J Infect Dis, 1986. **18**(2): p. 173-8.
374. Preiser, G., L. Preiser, and L. Madeo, *An outbreak of cryptosporidiosis among veterinary science students who work with calves.* J Am Coll Health, 2003. **51**(5): p. 213-5.
375. Rahman, A.S., et al., *Cryptosporidium diarrhoea in calves & their handlers in Bangladesh.* Indian J Med Res, 1985. **82**: p. 510-6.
376. Reif, J.S., et al., *Human cryptosporidiosis associated with an epizootic in calves.* Am J Public Health, 1989. **79**(11): p. 1528-30.
377. Stantic-Pavlinic, M., et al., *Cryptosporidiosis associated with animal contacts.* Wien Klin Wochenschr, 2003. **115**(3-4): p. 125-7.
378. Dawson, A., et al., *Farm visits and zoonoses.* Commun Dis Rep CDR Rev, 1995. **5**(6): p. R81-6.
379. Elwin, K., et al., *Modification of a rapid method for the identification of gene-specific polymorphisms in Cryptosporidium parvum and its application to clinical and epidemiological investigations.* Appl Environ Microbiol, 2001. **67**(12): p. 5581-4.
380. Smith, K.E., et al., *Outbreaks of enteric infections caused by multiple pathogens associated with calves at a farm day camp.* Pediatr Infect Dis J, 2004. **23**(12): p. 1098-104.

381. Stefanogiannis, N., M. McLean, and H. Van Mil, *Outbreak of cryptosporidiosis linked with a farm event.* N Z Med J, 2001. **114**(1144): p. 519-21.
382. Chalmers, R.M., et al., *Laboratory ascertainment of Cryptosporidium and local authority policies for investigating sporadic cases of cryptosporidiosis in two regions of the United Kingdom.* Commun Dis Public Health, 2002. **5**(2): p. 114-8.
383. Ederli, B.B., M.F. Rodrigues, and C.B. Carvalho, *[Oocysts of the genus Cryptosporidium in domiciliated dogs from the city of Campos dos Goytacazes, the State of Rio de Janeiro].* Rev Bras Parasitol Vet, 2005. **14**(3): p. 129-31.
384. Lallo, M.A. and E.F. Bondan, *[Prevalence of Cryptosporidium sp. in institutionalized dogs in the city of Sao Paulo, Brazil].* Rev Saude Publica, 2006. **40**(1): p. 120-5.
385. Leoni, F., et al., *Molecular epidemiological analysis of Cryptosporidium isolates from humans and animals by using a heteroduplex mobility assay and nucleic acid sequencing based on a small double-stranded RNA element.* J Clin Microbiol, 2003. **41**(3): p. 981-92.
386. McGlade, T.R., et al., *Gastrointestinal parasites of domestic cats in Perth, Western Australia.* Vet Parasitol, 2003. **117**(4): p. 251-62.
387. Pedraza-Diaz, S., et al., *Cryptosporidium meleagridis from humans: molecular analysis and description of affected patients.* J Infect, 2001. **42**(4): p. 243-50.
388. Strohmeyer, R.A., et al., *Evaluation of bacterial and protozoal contamination of commercially available raw meat diets for dogs.* J Am Vet Med Assoc, 2006. **228**(4): p. 537-42.
389. Goh, S., et al., *Sporadic cryptosporidiosis decline after membrane filtration of public water supplies, England, 1996-2002.* Emerg Infect Dis, 2005. **11**(2): p. 251-9.
390. Hunter, P.R., et al., *Foot and mouth disease and cryptosporidiosis: possible interaction between two emerging infectious diseases.* Emerg Infect Dis, 2003. **9**(1): p. 109-12.
391. Smerdon, W.J., et al., *Foot and mouth disease in livestock and reduced cryptosporidiosis in humans, England and Wales.* Emerg Infect Dis, 2003. **9**(1): p. 22-8.
392. Strachan, N.J., et al., *Foot and mouth epidemic reduces cases of human cryptosporidiosis in Scotland.* J Infect Dis, 2003. **188**(5): p. 783-6.
393. Black, R.E., *Pathogens that cause travelers' diarrhea in Latin America and Africa.* Rev Infect Dis, 1986. **8 Suppl 2**: p. S131-5.
394. Jokipii, L., S. Pohjola, and A.M. Jokipii, *Cryptosporidiosis associated with traveling and giardiasis.* Gastroenterology, 1985. **89**(4): p. 838-42.
395. Khalakdina, A., et al., *Is drinking water a risk factor for endemic cryptosporidiosis? A case-control study in the immunocompetent general population of the San Francisco Bay Area.* BMC Public Health, 2003. **3**: p. 11.
396. McLauchlin, J., et al., *Molecular epidemiological analysis of Cryptosporidium spp. in the United Kingdom: results of genotyping Cryptosporidium spp. in 1,705 fecal samples from humans and 105 fecal samples from livestock animals.* J Clin Microbiol, 2000. **38**(11): p. 3984-90.
397. Roy, S.L., et al., *Risk factors for sporadic cryptosporidiosis among immunocompetent persons in the United States from 1999 to 2001.* J Clin Microbiol, 2004. **42**(7): p. 2944-51.
398. Sterling, C.R., K. Seegar, and N.A. Sinclair, *Cryptosporidium as a causative agent of traveler's diarrhea.* J Infect Dis, 1986. **153**(2): p. 380-1.
399. Cartwright, R.Y., *Food and waterborne infections associated with package holidays.* J Appl Microbiol, 2003. **94 Suppl**: p. 12S-24S.

400. Nichols, R.A., B.M. Campbell, and H.V. Smith, *Identification of Cryptosporidium spp. oocysts in United Kingdom noncarbonated natural mineral waters and drinking waters by using a modified nested PCR-restriction fragment length polymorphism assay.* Appl Environ Microbiol, 2003. **69**(7): p. 4183-9.
401. Moss, D.M., et al., *Enzyme-linked immunoelectrotransfer blot analysis of a cryptosporidiosis outbreak on a United States Coast Guard cutter.* Am J Trop Med Hyg, 1998. **58**(1): p. 110-8.
402. Rooney, R.M., et al., *A review of outbreaks of waterborne disease associated with ships: evidence for risk management.* Public Health Rep, 2004. **119**(4): p. 435-42.
403. Ashbolt, R.H., et al., *An outbreak of cryptosporidiosis associated with an animal nursery at a regional fair.* Commun Dis Intell Q Rep, 2003. **27**(2): p. 244-9.
404. Ribeiro, C.D. and S.R. Palmer, *Family outbreak of cryptosporidiosis.* Br Med J (Clin Res Ed), 1986. **292**(6517): p. 377.
405. Cruickshank, R., L. Ashdown, and J. Croese, *Human cryptosporidiosis in North Queensland.* Aust N Z J Med, 1988. **18**(4): p. 582-6.
406. Hannah, J. and T. Riordan, *Case to case spread of cryptosporidiosis; evidence from a day nursery outbreak.* Public Health, 1988. **102**(6): p. 539-44.
407. Craven, D.E., K.A. Steger, and L.R. Hirschhorn, *Nosocomial colonization and infection in persons infected with human immunodeficiency virus.* Infect Control Hosp Epidemiol, 1996. **17**(5): p. 304-18.
408. el-Sibaei, M.M., et al., *Nosocomial sources of cryptosporidial infection in newly admitted patients in Ain Shams University Pediatric Hospital.* J Egypt Soc Parasitol, 2003. **33**(1): p. 177-88.
409. Gardner, C., *An outbreak of hospital-acquired cryptosporidiosis.* Br J Nurs, 1994. **3**(4): p. 152, 154-8.
410. Martino, P., et al., *Hospital-acquired cryptosporidiosis in a bone marrow transplantation unit.* J Infect Dis, 1988. **158**(3): p. 647-8.
411. Squier, C., V.L. Yu, and J.E. Stout, *Waterborne Nosocomial Infections.* Curr Infect Dis Rep, 2000. **2**(6): p. 490-496.
412. Hellard, M., et al., *Risk factors leading to Cryptosporidium infection in men who have sex with men.* Sex Transm Infect, 2003. **79**(5): p. 412-4.
413. Pedersen, C., et al., *Epidemiology of cryptosporidiosis among European AIDS patients.* Genitourin Med, 1996. **72**(2): p. 128-31.
414. Graczyk, T.K., et al., *House flies (Musca domestica) as transport hosts of Cryptosporidium parvum.* Am J Trop Med Hyg, 1999. **61**(3): p. 500-4.
415. Graczyk, T.K., et al., *Filth flies are transport hosts of Cryptosporidium parvum.* Emerg Infect Dis, 1999. **5**(5): p. 726-7.
416. Szostakowska, B., et al., *Cryptosporidium parvum and Giardia lamblia recovered from flies on a cattle farm and in a landfill.* Appl Environ Microbiol, 2004. **70**(6): p. 3742-4.
417. Graczyk, T.K., et al., *Mechanical transport and transmission of Cryptosporidium parvum oocysts by wild filth flies.* Am J Trop Med Hyg, 2000. **63**(3-4): p. 178-83.
418. Lake, I.R., et al., *Effects of weather and river flow on cryptosporidiosis.* J Water Health, 2005. **3**(4): p. 469-74.
419. Kim, J.T., S.H. Wee, and C.G. Lee, *Detection of Cryptosporidium oocysts in canine fecal samples by immunofluorescence assay.* Korean J Parasitol, 1998. **36**(2): p. 147-9.

420. Rambozzi, L., et al., *Prevalence of cryptosporidian infection in cats in Turin and analysis of risk factors.* J Feline Med Surg, 2007. **9**(5): p. 392-6.
421. McReynolds, C.A., et al., *Regional seroprevalence of Cryptosporidium parvum-specific IgG of cats in the United States.* Vet Parasitol, 1999. **80**(3): p. 187-95.
422. Morgan, U.M., et al., *Cryptosporidium spp. in domestic dogs: the "dog" genotype.* Appl Environ Microbiol, 2000. **66**(5): p. 2220-3.
423. Hajdusek, O., O. Ditrich, and J. Slapeta, *Molecular identification of Cryptosporidium spp. in animal and human hosts from the Czech Republic.* Vet Parasitol, 2004. **122**(3): p. 183-92.
424. Giangaspero, A., et al., *Molecular evidence for Cryptosporidium infection in dogs in Central Italy.* Parasitol Res, 2006. **99**(3): p. 297-9.
425. Abe, N., I. Kimata, and M. Iseki, *Identification of genotypes of Cryptosporidium parvum isolates from a patient and a dog in Japan.* J Vet Med Sci, 2002. **64**(2): p. 165-8.
426. Satoh, M., et al., *Characterization of Cryptosporidium canis isolated in Japan.* Parasitol Res, 2006. **99**(6): p. 746-8.
427. Xiao, L., et al., *Genetic diversity within Cryptosporidium parvum and related Cryptosporidium species.* Appl Environ Microbiol, 1999. **65**(8): p. 3386-91.
428. Miller, D.L., et al., *Gastrointestinal cryptosporidiosis in a puppy.* Vet Parasitol, 2003. **115**(3): p. 199-204.
429. Sargent, K.D., et al., *Morphological and genetic characterisation of Cryptosporidium oocysts from domestic cats.* Vet Parasitol, 1998. **77**(4): p. 221-7.
430. Morgan, U.M., et al., *Cryptosporidium in cats--additional evidence for C. felis.* Vet J, 1998. **156**(2): p. 159-61.
431. Ryan, U., et al., *Identification of novel Cryptosporidium genotypes from the Czech Republic.* Appl Environ Microbiol, 2003. **69**(7): p. 4302-7.
432. Pavlasek, I. and U. Ryan, *The first finding of a natural infection of Cryptosporidium muris in a cat.* Vet Parasitol, 2007. **144**(3-4): p. 349-52.
433. Alves, M., et al., *Multilocus genotyping of Cryptosporidium isolates from human HIV-infected and animal hosts.* J Eukaryot Microbiol, 2001. **Suppl**: p. 17S-18S.
434. Nuchprayoon, S., et al., *Prevalence of parasitic infections among Thai patients at the King Chulalongkorn Memorial Hospital, Bangkok, Thailand.* Journal of the Medical Association of Thailand, 2002. **85 Suppl 1**: p. S415-23.
435. Saksirisampant, W., et al., *Parasitic infections in Thai workers that pursue overseas employment: the need for a screening program.* Southeast Asian Journal of Tropical Medicine and Public Health, 2002. **33 Suppl 3**: p. 110-2.
436. Termmathurapoj, S., et al., *Cross-sectional Study of Intestinal Protozon Infections in Orphans and Childcare Workers at the Phayathai Babies' Home, Bangkok, Thailand.* J Trop Med Parasitol, 2000. **23**(1): p. 21-27.
437. Tungtrongchitr, A., et al., *Blastocystis hominis infection in irritable bowel syndrome patients.* Southeast Asian J Trop Med Public Health, 2004. **35**(3): p. 705-10.
438. Tungtrongchitr, A., et al., *Giardia intestinalis in Thailand: identification of genotypes.* J Health Popul Nutr, 2010. **28**(1): p. 42-52.
439. Viriyavejakul, P., et al., *High prevalence of Microsporidium infection in HIV-infected patients.* Southeast Asian J Trop Med Public Health, 2009. **40**(2): p. 223-8.

440. Chokephaibulkit, K., et al., *Intestinal parasitic infections among human immunodeficiency virus-infected and -uninfected children hospitalized with diarrhea in Bangkok, Thailand.* Southeast Asian J Trop Med Public Health, 2001. **32**(4): p. 770-5.
441. Manatsathit, S., et al., *Causes of chronic diarrhea in patients with AIDS in Thailand: a prospective clinical and microbiological study.* J Gastroenterol, 1996. **31**(4): p. 533-7.
442. Janoff, E.N., et al., *Endemic Cryptosporidium and Giardia lamblia infections in a Thai orphanage.* Am J Trop Med Hyg, 1990. **43**(3): p. 248-56.
443. Saksirisampant, W., et al., *Prevalence of intestinal parasitic infections among school children in the central region of Thailand.* J Med Assoc Thai, 2006. **89**(11): p. 1928-33.
444. Ratanapo, S., et al., *Multiple modes of transmission of giardiasis in primary schoolchildren of a rural community, Thailand.* Am J Trop Med Hyg, 2008. **78**(4): p. 611-5.
445. Piangjai, S., K. Sukontason, and K.L. Sukontason, *Intestinal parasitic infections in hill-tribe schoolchildren in Chiang Mai, northern Thailand.* Southeast Asian J Trop Med Public Health, 2003. **34 Suppl 2**: p. 90-3.
446. Saksirisampant, W., et al., *Prevalence of parasitism among students of the Karen hill-tribe in Mae Chame district, Chiang Mai province, Thailand.* J Med Assoc Thai, 2004. **87 Suppl 2**: p. S278-83.
447. Wilai, S., et al., *Prevalence of giardiasis and genotypic characterization of Giardia duodenalis in hilltribe children, Northern Thailand.* Trop Biomed, 2012. **29**(3): p. 331-8.
448. Wongstitwilairoong, B., et al., *Intestinal parasitic infections among pre-school children in Sangkhlaburi, Thailand.* Am J Trop Med Hyg, 2007. **76**(2): p. 345-50.
449. Pinlaor, S., et al., *Detection of opportunistic and non-opportunistic intestinal parasites and liver flukes in HIV-positive and HIV-negative subjects.* Southeast Asian J Trop Med Public Health, 2005. **36**(4): p. 841-5.
450. Warunee, N., et al., *Intestinal parasitic infections among school children in Thailand.* Trop Biomed, 2007. **24**(2): p. 83-8.
451. Leelayoova, S., et al., *Prevalence of intestinal parasitic infections in military personnel and military dogs, Thailand.* Journal of the Medical Association of Thailand, 2009. **92 Suppl 1**: p. S53-9.
452. Saksirisampant, W., et al., *Intestinal parasitic infestations among children in an orphanage in Pathum Thani province.* J Med Assoc Thai, 2003. **86 Suppl 2**: p. S263-70.
453. Wongjindanon, N., et al., *Current infection rate of Giardia lamblia in two provinces of Thailand.* Southeast Asian J Trop Med Public Health, 2005. **36 Suppl 4**: p. 21-5.
454. Jongwutiwes, S., et al., *Cryptosporidiosis among orphanage children in Thailand: a one year prospective study.* Southeast Asian J Trop Med Public Health, 1990. **21**(3): p. 458-64.
455. Moolasart, P., et al., *Cryptosporidiosis in HIV infected patients in Thailand.* Southeast Asian J Trop Med Public Health, 1995. **26**(2): p. 335-8.
456. Uga, S., et al., *Cryptosporidium infection in HIV-seropositive and seronegative populations in southern Thailand.* Southeast Asian J Trop Med Public Health, 1998. **29**(1): p. 100-4.
457. Srisuphanunt, M., W. Saksirisampant, and P. Karanis, *Prevalence and genotyping of Cryptosporidium isolated from HIV/AIDS patients in urban areas of Thailand.* Annals of Tropical Medicine and Parasitology, 2011. **105**(6): p. 463-8.

CHAPTER 2: PREVALENCE AND MOLECULAR ANALYSIS OF *GIARDIA* AND *CRYPTOSPORIDIUM* SPP. INFECTION IN DOGS AND CATS IN CHIANG MAI, THAILAND: A PRELIMINARY FINDING[*]

Summary

Giardia duodenalis and *Cryptosporidium* spp. are common protozoans that can cause diarrhea in humans, domestic animals, and wildlife worldwide. *Giardia duodenalis* Assemblages A and B and *C. parvum* are considered as potential zoonotic disease agents. The available information related to the distribution, spread, and characteristics of these two organisms in dogs and cats in Thailand, however, is limited. Therefore, the objectives of the study were 1) to determine prevalence of *G. duodenalis* and *Cryptosporidium* spp. infections in dogs and cats in Chiang Mai, Thailand, 2) to characterize the organism isolates using molecular techniques in order to determine the potential for zoonotic transmission, and 3) to determine agreements among tests used to detect *Giardia* and *Cryptosporidium* infections. Fecal samples were collected from 109 dogs and 15 cats between July-August 2008. Age, sex, diarrhea status, and housing types were recorded. *Giardia* infection was diagnosed using a combination of conventional zinc sulfate centrifugal flotation, immunofluorescent assay (IFA), and a PCR assay that amplifies the *Giardia* glutamate dehydrogenase (gdh) gene. *Cryptosporidium* infection was determined using both IFA and a PCR assay that amplifies the *Cryptosporidium* heat shock protein 70 KDa (hsp70) gene. A sample was considered positive if any of the test results was

[*] Sahatchai Tangtrongsup[1], A. Valeria Scorza[2], John S. Reif[3], Lora R. Ballweber[4], Michael R. Lappin[2], Mo D. Salman[1], [1]*Animal Population Health Institute, and* [2]*Center for Companion Animal Studies*, *Department of Clinical Sciences,* [3]*Department of Clinical Sciences,* [3]*Department of Environmental and Radiological Health Sciences, and* [4]*Department of Microbiology, Immunology and Pathology, College of Veterinary Medicine and Biomedical Sciences, Colorado State University, Fort Collins, CO 80523*. This chapter is formatted for requirement of the Veterinary Parasitology journal.

positive. In dogs, the *Giardia* and *Cryptosporidium* prevalences were 45.9% (95%CI:23.5-41.7) and 22.0% (95%CI:14.6-31.1), respectively. In cats, the *Giardia* and *Cryptosporidium* prevalences were 40.0% (95%CI:16.3-67.7) and 26.7% (95%CI:7.8-55.1), respectively. The multivariate analysis indicated that *Giardia* infections were more likely to be detected in dogs less than one year of age (OR 6.53, 95%CI:1.76-24.25), dogs having diarrhea (OR 4.78, 95%CI:1.04-21.98) and dogs residing in a breeding kennel or shelter (OR 3.93, 95%CI:1.33-11.65). Sequence analysis of 21 *Giardia* gdh PCR positive samples and 11 *Cryptosporidium* hsp70 PCR positive samples from dogs revealed the presence of *G. duodenalis* Assemblage C (8/21), and D (13/21), and *C. canis* (5/11) and *C. parvum* (6/11). Sequence analysis from *Giardia* and *Cryptosporidium* positive samples from cats revealed the presence of *G. duodenalis* Assemblage D (2/2) and *C. canis* (2/2). The present study suggested that *Giardia* and *Cryptosporidium* infections were common in dogs in Chiang Mai. The presence of *C. parvum* suggested that dogs could be a potential reservoir for zoonotic transmission to humans.

Keywords: Dogs; Cats; *Giardia*; *Cryptosporidium*; Risk factors; Chiang Mai; Thailand

2.1 Introduction

Giardia duodenalis (syn. *G. intestinalis* and *G. lamblia*) and *Cryptosporidium* spp. are common protozoans that can cause diarrhea in humans, domestic animals, and wildlife throughout the world (Fayer, 2004; Thompson and Smith, 2011; Xiao, 2010). The clinical signs in humans range from subclinical to diarrhea with slight abdominal discomfort to severe abdominal pain and cramping. Clinically, diarrhea is usually of short duration and is self-limiting in immune-competent individuals. It can be, however, a serious problem producing severe and chronic disease in immune-compromised individuals such as HIV-infected patients, very young or elderly individuals (Hill et al., 2000; Irwin, 2002; Robertson et al., 2000). These infections can

lead to significant morbidity and mortality among human populations in developing countries and have been a worldwide concern, resulting in the inclusion of *Giardia* and *Cryptosporidium* in the WHO 'Neglected Diseases Initiative' since September 2004 (Savioli et al., 2006).

The prevalence rates of *Giardia* and *Cryptosporidium* in animal populations vary depending on the population tested, the area studied, the diagnostic test used, and the health status of the animal. Worldwide, the prevalence of *Giardia* and *Cryptosporidium* in dogs or cats are commonly 5%-15% (Ballweber et al., 2009; Bugg et al., 1999; Carlin et al., 2006; Fontanarrosa et al., 2006; Hackett and Lappin, 2003; Hill et al., 2000; Itoh et al., 2012; Itoh et al., 2009; Kim et al., 1998; Little et al., 2009); however, previous studies have reported prevalences of *Giardia* of higher than 25% in dogs and cats (see Table 2.1). In addition, it has been estimated that in kennel dogs the prevalence can be as high as 100% (Papini et al., 2005).

The species complex *Giardia duodenalis* and the genus *Cryptosporidium* contain both host-adapted and zoonotic genotypes/species (Monis and Thompson, 2003). At least eight distinct Assemblages (A-H) of *G. duodenalis* have been reported based on genetic analyses (Lasek-Nesselquist et al., 2010; Monis et al., 2009; Scorza et al., 2012). Assemblages A and B have been detected in a wide range of hosts including humans, other primates, dogs, cats, livestock, and wildlife (Ballweber et al., 2010; Monis et al., 2009; Scorza et al., 2012). Assemblages C to H are considered host-adapted in dogs (Assemblages C and D), cats (Assemblage F), livestock (Assemblage E), rats (Assemblage G) and marine mammals (Assemblage H). There are at least 24 accepted *Cryptosporidium* species (Alvarez-Pellitero et al., 2004; Alvarez-Pellitero and Sitja-Bobadilla, 2002; Fayer, 2010; Fayer and Santin, 2009; Fayer et al., 2010; Ren et al., 2012; Robinson et al., 2010) and dogs and cats are usually infected with the relatively species-specific *C. canis* or *C. felis* or the zoonotic *C. parvum* (Bowman and

Lucio-Forster, 2010; Scorza and Tangtrongsup, 2010). Several studies have reported the detection of zoonotic and human-specific genotypes of *G. duodenalis* and *Cryptosporidium* spp. in dogs and cats (Eligio-Garcia et al., 2008; Eligio-Garcia et al., 2005; Inpankaew et al., 2007; Lalle et al., 2005; Scorza et al., 2012; Scorza et al., 2011; Traub et al., 2004). Infrequently, the dog- and cat-specific agents were also recovered from samples obtained from HIV patients and children (Gatei et al., 2002; Srisuphanunt et al., 2011; Xiao et al., 2007). This evidence increases the public health concern that not only the zoonotic species/genotypes, but also the species-specific genotypes, could infect immune-compromised individuals and that dogs and cats might play an important role in transmitting those diseases.

In Thailand, studies regarding giardiasis and cryptosporidiosis in dogs and cats are limited. In one study in the Bangkok area, the prevalence of *Giardia* infection in temple dogs was 7.9% using zinc sulfate and sodium nitrate flotation followed by microscopy (Inpankaew et al., 2007). Using the same samples, the same group later reported an estimated median prevalence of 56.8% in dogs using Bayesian analysis based on zinc sulfate flotation and microscopy, an immunofluorescence antibody test, and nested polymerase chain reaction (PCR) based on the SSU-rRNA gene (Traub et al., 2009). The majority of *Giardia* isolates recovered by this group were Assemblages A, D, B and C. Furthermore, similar genotypes (Assemblage A) were recovered from dogs and humans in the same monastery. To our knowledge the genotypes of *Cryptosporidium* of dogs and of both *Giardia* and *Cryptosporidium* in cats are still unknown in Thailand. Since these protozoal infections are a potential public health concern, determining the prevalence and genotypes of these organisms in dogs and cats living in close proximity to humans and other animals is a priority. Therefore, the aims of this study were 1) to determine the prevalence of *Giardia* and *Cryptosporidium* infections in dogs and cats in Chiang Mai, Thailand,

2) to characterize the organism isolates using molecular techniques in order to determine the potential for zoonotic transmission, and 3) to determine agreements among tests used to detect *Giardia* and *Cryptosporidium* infections.

2.2 Materials and Methods

2.2.1 Study location

Chiang Mai is the second largest province of Thailand. It is located in the northern part of the country at geographic coordinates 18°47' N and 98°59' E (Figure 2.1). This province covers an area of 20,107.057 sq. km with an average elevation of 310 m above sea level. Approximately 70% of the area is covered with forest, 13% is agricultural land, and 17% is for housing and other uses. There are three seasons: rainy ranges from mid-May to October, winter from November to mid-February, and summer from mid-February to mid-May (Chiang Mai Provincial Office, 2012). The average temperature in rainy, summer, and winter are 27.7 °C (average min 22.4°C-average max 36.0°C), and 28.5°C (average min 20.1-average max 39.1), and 23.9°C (average min 14.4°C - average max 34.35°C), respectively. Average rainfall in rainy, summer and winter were 16.9 cm, 8.7 cm, and 0.8 cm, respectively (Thai Meteorological Department Automate Weather System). Chiang Mai is administratively divided into 25 districts (amphoe), 204 sub-districts (tambon) and 2,066 villages (mooban) (Chiang Mai Provincial Office, 2012). Chiang Mai province was chosen for this study because the province includes agricultural, industrial and tourism areas, and the knowledge of *Giardia* and *Cryptosporidium* infections in this area was unknown. In addition, there was limited laboratory access to IFA and PCR for *Giardia* and *Cryptosporidium* detection in dogs and cats. This study could therefore introduce the use of IFA and PCR for canine and feline *Giardia* and *Cryptosporidium* detection in this province.

2.2.2 Sample collection

During July-August 2008, 109 canine and 15 feline fecal samples were obtained from animals visiting the Small Animal Hospital of the Faculty of Veterinary Medicine, Chiang Mai University, other volunteered private clinics, or from a shelter and breeders in Chiang Mai province, Thailand. The samples were collected on a volunteer basis regardless of the health status of the animals. Demographic information (age, sex, and housing types) was recorded. Fecal consistency was estimated using the Nestle Purina Fecal Scoring System for Dogs and Cats (Nestle-Purina Pet Food Co, St Louis, MO). Fecal scores of 1-4 were considered as normal, with 5-6 classified as diarrheic. All fecal samples were stored at -20ºC until fecal concentration was performed.

2.2.3 Diagnostic methods

Giardia infection was diagnosed using conventional zinc sulfate centrifugal flotation, immunofluorescent assay (IFA) and PCR techniques. *Cryptosporidium* infection was determined using IFA and PCR techniques.

2.2.3.1 Fecal concentration and immunofluorescent assay

Prior to IFA and DNA extraction, all fecal samples were concentrated using sugar concentration techniques as previously described (O'Handley et al., 2000; Vasilopulos et al., 2006). In brief, 3 grams of feces were mixed with 4.5 ml PBS-EDTA and strained through cheesecloth then overlaid on 7 ml sugar solution (Sp.g. 1.13). Samples were centrifuged at a speed of 800g for 10 minutes. The top layer was transfer to new falcon tube and centrifuged at a speed of 1,200g for 10 minutes. The pellet was washed twice with PBS-EDTA and centrifuged at 1,200g for 10 minutes. A pellet then re-suspended in 1 ml PBS-EDTA. A thin fecal smear was made on IFA slides using the loop supplied with the kit (Merifluor® *Cryptosporidium*/*Giardia*

IFA kit, Meridian Diagnostic Corporation, Cincinnati, OH) and slides processed in accordance with manufacturer's instructions. The remaining concentrated fecal material was stored at -20°C until DNA extraction was performed

2.2.3.2 Fecal DNA extraction

All fecal samples were subjected to DNA extraction. DNA of concentrated fecal samples was extracted following an established protocol (Scorza et al., 2003). The extracted DNA was stored at -20°C until assayed by PCR.

2.2.3.3 Polymerase chain reaction

To detect *Giardia* and *Cryptosporidium* DNA present in fecal samples, a semi-nested PCR of *Giardia* glutamate dehydrogenase (gdh) gene and a nested PCR of *Cryptosporidium* heat shock protein 70 kDa (hsp70) gene were performed as previously described (Morgan et al., 2001; Read et al., 2004) using a commercial HotStarTaq Master Mix (Qiagen, Valencia, CA).

2.2.4 DNA sequence and phylogenetic analysis

The PCR products were evaluated by nucleotide sequencing using a commercially available service (Proteomics and Metabolomics Facility, Colorado State University). The obtained sequences were compared with nucleotide sequences from the nucleotide database from the GenBank by BLAST analysis (http://blast.ncbi.nlm.nih.gov/Blast.cgi). Amino acid translation was performed on all sequences to determine whether a DNA substitution could lead to an amino acid change.

Phylogenetic and molecular analyses were performed using MEGA 5 (Tamura et al., 2011). Multiple sequence alignment was performed using MUSCLE (Edgar, 2004) The phylogenetic analyses were performed by Maximum Likelihood method based on the Kimura 2-parameter model. The consensus tree was obtained after bootstrap analysis, with 1000

replications. Additional *Giardia* gdh sequences (Assemblages A (L40509), B (L40508), C (DQ414243, EF507623), D (EU769228, EF507633, HQ538710, EU769230), E (AY178740), F (EU769234), G (AY178747), H (GU176089) and *G. ardeae* (AF069060)) and *Cryptosporidium* hsp70 sequences (*C. parvum* bovine isolate (AF221528), a mouse isolate (AF221530) a marsupial isolate (AF221531), a reptile isolate (FJ429597), *C. canis* (AY120920), *C. felis* (AF221538), *C. meleagridis* (AF221537), *C. muris* (AF221542) and *C. hominis* (EF591787), *C. baileyi* (AF221539), *C. serpentis* (AF221541), and *C. wrairi* (AF221536)) were retrieved from the Genbank for comparative phylogenetic analyses.

2.2.5 Data analysis

The sample was considered positive if any of the test results for *Giardia* (zinc sulfate fecal flotation, IFA and PCR) and for *Cryptosporidium* (IFA and PCR) were positive. Overall prevalence and 95% confidence interval (95% CI) were calculated (Fleiss, 2003). Associations between either *Giardia* or *Cryptosporidium* infections and age (less than one year or one year or more), sex, diarrhea status (yes or no), and housing type (household or breeding kennel/shelter) were assessed using Fisher's exact test (Fleiss, 2003). Agreements among the tests used to diagnose the infection in dogs were calculated based on *kappa* statistics (Fleiss, 2003). Odds ratios and 95% CI were estimated using univariate logistic regression analysis to measure the strength of association of each independent variable including age, sex, diarrhea status, housing type and the presence of co-infection (having both *Giardia* and *Cryptosporidium*) with *Giardia* or *Cryptosporidium* infection. A multivariate logistic regression model, against either *Giardia* or *Cryptosporidium* infection in dogs, was constructed using a backward stepwise elimination procedure (Dohoo et al., 2007). Variables found to be associated with *Giardia* or *Cryptosporidium* infection in the univariate logistic regression ($p<0.25$) were included in the

multivariable logistic regression analysis. Variables were retained in the model based on the likelihood ratio $\chi 2$ statistic, at $p < 0.05$. All statistical analyses were performed using the Stata statistical software release 10.1 (Stata Corp., College Station, Texas, USA).

2.3 Results

2.3.1 Detection of *Giardia* and *Cryptosporidium* spp. infection in dogs and cats in Chiang Mai, Thailand

A single fecal sample was collected from 109 dogs and 15 cats. The characteristics of the population sampled are shown in Table 2.2.

The prevalence of *Giardia* infections in dogs and cats was 45.9% (95%CI:36.3-55.7) and 40.0% (95%CI:16.3-67.7), respectively (Table 2.3). The prevalence of *Cryptosporidium* infections in dogs and cats was 22% (95%CI:14.6-31.0) and 26.7% (95%CI:7.8-55.1), respectively. In addition, in dogs, single infections with *Giardia* or *Cryptosporidium* were 33.0% (36/109) and 9.2% (10/109), respectively. Co-infection of *Giardia* and *Cryptosporidium* was shown in 12.8% (14/109) of the canine samples. In cats, 26.7% (4/15) were infected only with *Giardia*, 13.3% (2/15) only with *Cryptosporidium*, and 13.3% (2/15) had a co-infection.

The kappa analysis in dog samples revealed low or no agreement on detection of *Giardia* among three tests (flotation, IFA and PCR) with a *kappa* index of 0.270 and *Cryptosporidium* among two tests (IFA and PCR) with a kappa index of -0.037 in this study (Table 2.4 and Table 2.5).

Age was significantly associated with the prevalence of both *Giardia* and *Cryptosporidium* in dogs (Table 2.6). Other variables were not associated with infection in dogs or cats in this study.

2.3.2 Univariate and multivariate analysis for risk associated with *Giardia* and *Cryptosporidium* infection in dogs and cats in Chiang Mai, Thailand

Univariate logistic regression analyses for categorical variables showed dogs age less than one year were more likely to be infected with *Giardia* (OR 4.52, 95%CI:1.61-12.65) or *Cryptosporidium* (OR 4.94, 95%CI:1.80-13.55) than dogs age one year or older (Table 2.7). Cats from shelters were more likely to be infected with *Giardia* (OR 16, 95%CI:1.09-234.25) than household cats (Table 2.7).

The variables remaining in the model following multivariate logistic regression for *Giardia* infection were age less than one year (OR 6.53, 95%CI:1.76-24.25), having diarrhea (OR 4.78, 95%CI:1.04-21.98), and residing in breeding kennels or a shelter (OR 3.93, 95%CI:1.33-11.65) (Table 2.8). For the cats, since the housing type was the only significant variable the multivariate logistic regression was not performed.

2.3.3 Genotyping of *Giardia* and *Cryptosporidium* isolated from positive dogs and cats in Chiang Mai, Thailand

Twenty-three sequences from *Giardia* gdh PCR-positive samples were available for genotype analysis, 21 from dogs and 2 from cats. Using BLAST analyses, eight dog isolates were typed as Assemblage C and 13 were typed as Assemblage D. Sequences from the two cat isolates were also Assemblage D. Four of all 23 sequences contained ambiguous nucleotides. Sample TH08Dog36 and TH08Dog45 had double peaks at the same position, C or T at position 69 and 177. These two samples were from the same shelter. TH08Dog24 and TH08dog107 contained three ambiguous nucleotides. TH08Dog24 had double peaks at position 189 (A or C), 366 (C or T) and 378 (A or G). TH08Dog107 contained double peaks at position 189 (A or C), 204 (C or T) and 300 (A or G). Neither of the ambiguous nucleotides resulted in amino acid

changes (a synonymous substitution). When translate all gdh DNA sequences to amino acids, TH08Dog19 and TH09Dog22, both identified as assemblage C, were one amino acid different from the rest of assemblage C identified in this study.

Thirteen sequences from hsp70 PCR positive samples were available for analysis, 11 from dogs and two from cats. Six dog isolates were typed as *C. parvum* and five were typed as *C. canis*. Both cat isolates were typed as *C. canis*.

From the phylogenetic analysis of gdh gene, two subgroups of assemblage C were identified, and all D except TH08Dog107 were placed on the same branch (Figure 2.2). Phylogenetic analysis of a 325-bp region of the *Cryptosporidium* hsp70 gene placed all of the close sequences from dog and cats isolates into either *C. parvum* or *C. canis*; however, one cat isolate identified as *C. canis* from BLAST was diverted from its branch (Figure 2.3).

2.4 Discussion

Although there are several studies reporting *Giardia* and *Cryptosporidium* spp. infections in dogs and cats worldwide, information regarding to these two enteric protozoan parasites in dogs and cats in Thailand is limited. To the authors' knowledge, the current study represents the first report of the *Giardia* and *Cryptosporidium* prevalence rates and genotypes/species in dogs and cats in Chiang Mai, Thailand. Elsewhere in the world, the prevalence of *Giardia* and *Cryptosporidium* in dogs and cats is commonly 5%-15% (Ballweber et al., 2009; Bugg et al., 1999; Carlin et al., 2006; Fontanarrosa et al., 2006; Hackett and Lappin, 2003; Hill et al., 2000; Itoh et al., 2012; Itoh et al., 2009; Kim et al., 1998; Little et al., 2009). The prevalence of *Giardia* and *Cryptosporidium* infections, however, has varied owing to the population tested, diagnostic test used, as well as the geographic and chronologic differences between studies. In the present study, overall *Giardia* and *Cryptosporidium* prevalence were 45.9% and 40% in dogs and 22%

and 26.7% in cats, respectively. These high prevalences were derived by considering detection in parallel from three tests for *Giardia* and two tests for *Cryptosporidium*. The prevalence of canine *Giardia* found in this study is comparable to a previous report of 56.8% in Bangkok (Traub et al., 2009) and similarly high rates in other countries such as Japan (Itoh et al., 2005), Mexico (Ponce-Macotela et al., 2005), Brazil (Mundim et al., 2007), Italy (Papini et al., 2005) and Belgium (Claerebout et al., 2009), where most of the studies were from breeding kennels, shelters or abandoned dogs. For cats, the prevalence of 40% is comparable with a study of household cats in Japan (Itoh et al., 2006) and show cats in New Zealand (Kingsbury et al., 2010). The wide 95% confidence interval for the prevalence estimations of *Giardia* and *Cryptosporidium* infections was noted in cat samples. This finding likely relates to the small sample size. The prevalence of *Cryptosporidium* in dogs is comparable to a previous report of sled dogs from Poland (Bajer et al., 2011) and domestic cats from Italy (Rambozzi et al., 2007). Nevertheless, the prevalences of these two organisms in this study may be overestimated due to selection bias. The samples available for this study were not randomly selected but depended on voluntary participation of the owner visiting the small animal hospital and caregivers of breeders and a shelter. The animals might have already had the disease or were exposed to the risk factors (such as young age and living in the crowded environment). Therefore, the selection of the sample may be biased toward the infected animals, resulting in the overestimation of the apparent prevalence.

Young age, presence of diarrhea, feeding a home-cooked diet, presence of other enteric parasites, being an abandoned or stray dog, dogs in kennels or cats in catteries are the risk factors that have been reported associated with *Giardia* and *Cryptosporidium* in dogs and cats (Katagiri and Oliveira-Sequeira, 2008; Mircean et al., 2012; Rambozzi et al., 2007; Scaramozzino et al.,

2009; Upjohn et al., 2010). Similarly, in this study, *Giardia* infection in dogs is shown to be associated with young age, the presence of diarrhea, and being a breeder/shelter resident.

Concurrent infection of *Giardia* and *Cryptosporidium* in dogs and cats has been reported and it compounds the difficulty in eliminating these two parasites (Keith et al., 2003; Scorza and Lappin, 2007). *Cryptosporidium* has been reported as a significant risk for *Giardia* infection in cats, and vice versa (Ballweber et al., 2009; Vasilopulos et al., 2006); however, this finding was not observed in the current study.

Dogs and cats can harbor not only host-adapted species but also the zoonotic genotypes/species of *G. duodenalis* and *Cryptosporidium* (Claerebout et al., 2009; Scorza et al., 2012; Traub et al., 2009; Volotao et al., 2007), which increases the concern for their potential as reservoirs for human infections. In this study, the results from molecular analysis of *Cryptosporidium* revealed the presence of *C. parvum* in dog samples. This finding suggests that dogs could be a potential reservoir for the zoonotic transmission of *Cryptosporidium* spp.; however, further investigation of these parasites among humans and animals living in the same household or in close proximity are needed to confirm this relationship.

Cross-species transmission of a parasite may be possible when animals share the same habitat and the parasite is biologically capable of infecting multiple host species (Xiao and Fayer, 2008). It has been shown that cats can be infected with *G. duodenalis* Assemblages A, B, C, D and E (Read et al., 2004). In another study from Europe, of 158 sequences analyzed, cats were reported to be infected not only with Assemblage F (49%) but also by A (43%), B (1.3%), C (1.9%), D (1.3%) and E (0.6%) (Sprong et al., 2009). In the current study, *Giardia* Assemblage D was identified in a house cat and a shelter cat. In addition, *C. canis* was identified in two house cats from different households. These findings suggest that cats may be infected

with dog-adapted pathogens from dogs living in the same area. Therefore, dogs could also be a reservoir for giardiasis and cryptosporidiosis for cats, or vice versa. It is noted, however, that the cats that were positive with *G. duodenalis* Assemblage D did not have diarrhea but both of the *C. canis* positive cats had diarrhea.

While *C. parvum* and *C. hominis* are significant causes of human cryptosporidiosis, the detection of *C. canis* and *C. felis* in HIV patients in Thailand (Gatei et al., 2002; Srisuphanunt et al., 2011; Tiangtip and Jongwutiwes, 2002) and elsewhere (Alves et al., 2001; Cama et al., 2003), as well as the detection of *C. canis* in children (Xiao et al., 2001) has raised concerns regarding transmission of protozoal diseases from pets to humans even when they harbor the host-adapted pathogens. Therefore, good sanitary practices are recommended for all pet owners to avoid zoonotic transmission to humans.

Phylogenetic analysis for *Giardia* gdh gene revealed possibly two subgroups of assemblage C, and two subgroups of assemblage D. For *Cryptosporidium* hsp70 analysis, all BLAST identified *C. parvum* and *C. canis* were closely placed on its group with the exception of TH08Cat6 that was placed between the *C. felis* and *C. canis* branch. Since the genotypes of *Giardia* and *Cryptosporidium* in this study were identified based on a single gene for each organism, and discrepancies of genotype/species of *Giardia* and *Cryptosporidium* isolates using multilocus analyses have been reported regarding which locus identifies the zoonotic genotype versus the host-adapted locus (Beck et al., 2012; Scorza et al., 2012), we are not certain these results are absolute. Further studies on other loci, for example beta-giardin and triose phosphate isomerase (tpi) for *Giardia* and small subunit rRNA (SSU-rRNA) and 60 kDa glycoprotein (gp60), may be suggested to confirm the presence and subtype of the zoonotic organisms in these samples.

The limitations of this study include the small sample size and the convenience nature of the sample collection. Due to the lack of a pet registry in Chiang Mai, random sampling for this study was not possible. Selection bias may have led to either underestimation or overestimation of the prevalence rates. A larger sample size is needed for further study, particularly for cats. This study had inadequate power to detect associations between age or diarrhea and infection in cats. We analyzed age, sex, diarrhea status, and housing type; however, other important risk factors, e.g. season, diet, concurrent parasitic infection could be suggested for future study to help in prevention and control.

2.5 Conclusion

Giardia and *Cryptosporidium* infections were common in young dogs in Chiang Mai, Thailand. Animals living in high-density situations and those having diarrhea were also at risk. The presence of *C. parvum* suggested that dogs could be a potential reservoir for the zoonotic transmission of *C. parvum* in humans. The molecular analyses suggested that cats could be infected with the dog-adapted *G. duodenalis* and *C. canis* and dogs could be a potential reservoir for the transmission of these parasites to cats, or vice versa. Further investigation involving a larger sample size and additional risk factors, (e.g., season, presence of other parasites, indoor/outdoor activity), are suggested in order to better define the risk of the infection. Preventive measures then could be implemented to control *Giardia* and *Cryptosporidium* infections in this location.

2.6 Tables

Table 2.1 List of the sources of animal that have been reported to have high prevalence of *Giardia* infection in dogs and cats worldwide.

Source of sample	Prevalence (%)	Reference
Privately owned animals	28-57	(Itoh et al., 2006; Mircean et al., 2012; Scorza et al., 2011)
Pet shops	37	(Bugg et al., 1999)
Stray animals	28-51	(Mundim et al., 2007; Paz e Silva et al., 2012; Ponce-Macotela et al., 2005)
Sled dogs	28	(Bajer et al., 2011)
Breeding kennels	28.6-43.9	(Bugg et al., 1999; Claerebout et al., 2009; Itoh et al., 2005; Mundim et al., 2007; Paoletti et al., 2008; Paz e Silva et al., 2012)
Shelter	55.2	(Papini et al., 2005)
Show cats	31-32	(Gookin et al., 2004; Kingsbury et al., 2010)

Table 2.2 Characteristics of samples included in the current study.

	No. of samples in this study (%)	
	Dog (n=109)	Cat (n=15)
Age		
< 1 year	21.1 (23)	20.0 (3)
> 1 year	76.1 (83)	73.3 (11)
Unknown	3 (2.8)	6.7 (1)
Sex		
Male	31.2 (34)	46.7 (7)
Female	60.6 (66)	46.7 (7)
Unknown	8.3 (9)	6.7 (1)
Diarrhea status		
Yes (17)	15.6 (17)	20.0 (3)
No (89)	81.7 (89)	73.3 (11)
Unknown (3)	2.8 (3)	6.7 (1)
Housing type		
Breeder & Shelter (64)	58.7 (64)	33.3 (5)
Household (45)	41.3 (45)	66.7 (10)

Table 2.3 Prevalence of *Giardia* and *Cryptosporidium* infections by flotation, IFA, PCR and combined results. The results were from 109 canine and 15 feline fecal samples. The prevalence is shown in percent with the 95% confidence interval (CI).

	Prevalence % (95%CI)	
	Dog	Cat
Giardia duodenalis		
Flotation	30.3 (21.8-39.8)	13.3 (1.7-40.5)
IFA	21.1 (13.9-30.0)	26.7 (7.8-55.1)
PCR-gdh	19.3 (12.3-27.9)	13.3 (1.7-40.5)
Combined	45.9 (36.3-55.7)	40.0 (16.3-67.7)
Cryptosporidium		
IFA	12.8 (7.2-20.6)	13.3 (1.7-40.5)
PCR-hsp70	10.1 (5.1-17.3)	13.3 (1.7-40.5)
Combined	22.0 (14.6-31.0)	26.7 (7.8-55.1)

Table 2.4 Tabulation of the *Giardia*-test results based on detection by conventional flotation, IFA, and PCR in dog samples. Numbers represent the count in each cell.

Diagnostic test*	PCR+		PCR -		Total
	IFA+	IFA-	IFA+	IFA-	
Flotation +	7	4	7	15	33
Flotation -	2	8	7	59	76
Total	9	12	14	74	109

**Kappa* statistic for agreement was 0.270 ($p<0.001$).

Table 2.5 Tabulation of the *Cryptosporidium*-test results based on detection by IFA and PCR in dog samples. Numbers represent the count in each cell.

Diagnostic test*	PCR+	PCR-	Total
IFA+	1	13	14
IFA -	10	85	95
Total	11	98	109

**Kappa* statistic for agreement was -0.037 (p=0.653).

Table 2.6 Prevalence of *Giardia* and *Cryptosporidium* infections by age, sex, diarrhea status, and housing type. Number in parentheses represents the number of samples in each category.

	G. duodenalis % (95%CI*)		*P* value	*Cryptosporidium spp.* % (95%CI*)		*P* value
Dog (109)	45.9	(36.3-55.7)		22.0	(14.6-31.0)	
Age			0.003			0.001
< 1 year (23)	73.9	(51.5-89.8)		47.8	(26.8-69.4)	
> 1 year (83)	38.6	(28.1-49.9)		15.7	(8.6-25.3)	
Sex			0.666			0.942
Male (34)	50.0	(32.4-67.6)		20.6	(8.7-37.9)	
Female (66)	45.5	(33.1-58.2)		21.2	(12.1-33.0)	
Diarrhea status			0.065			0.842
Yes (17)	64.7	(38.3-85.5)		23.5	(6.8-49.9)	
No (89)	40.4	(30.2-51.4)		21.3	(13.4-31.3)	
Housing type			0.070			0.370
Breeder & Shelter (64)	53.1	(40.2-65.7)		25.0	(15.0-37.4)	
Household (45)	35.6	(21.9-51.2)		17.8	(8.0-32.1)	
Cat (15)	40.0	(16.3-67.7)		26.7	(7.8-55.1)	
Age			≅1.000			≅1.000
< 1 year (3)	33.3	(0.8-90.6)		33.3	(0.8-90.6)	
≥1 year (11)	45.5	(16.7-76.6)		27.3	(6.0-61.0)	
Sex			0.592			0.192
Male (7)	28.6	(3.7-71.0)		0		
Female (7)	57.1	(18.4-90.1)		42.9	(9.9-81.6)	
Diarrhea status			0.258			0.176
Yes (3)	0			66.7	(9.4-99.2)	
No (11)	45.5	(16.7-76.6)		18.2	(2.3-51.8)	
Housing type			0.089			≅1.000
Breeder & Shelter (5)	80	(28.4-99.5)		20	(0.5-71.6)	
Household (10)	20	(2.5-55.6)		30	(6.7-35.2)	

* 95%CI = 95% confidence interval

Table 2.7 Univariate logistic regression analysis of variables associated with *Giardia* and *Cryptosporidium* spp. infections in dogs and cats in Chiang Mai, Thailand.

	Odds Ratio (OR)	95%CI*	*P* Value
Dog			
Giardia duodenalis			
Age <1 year (n=106)	4.52	1.61-12.65	0.004
Sex (male) (n=100)	1.20	0.52-2.75	0.666
Diarrhea (n=106)	2.70	0.92-7.96	0.072
Breeder & Shelter (n=109)	2.05	0.94-4.50	0.072
Presence of *Cryptosporidium* infection	1.91	0.76-4.77	0.169
Cryptosporidium spp.			
Age <1 year (n=106)	4.94	1.80-13.55	0.002
Sex (male) (n=100)	0.96	0.35-2.67	0.942
Diarrhea (n=106)	1.13	0.33-3.88	0.842
Breeder & Shelter (n=109)	1.54	0.60-3.99	0.372
Presence of *Giardia* infection	1.91	0.76-4.77	0.169
Cat			
Giardia duodenalis			
Age <1 year (n=14)	0.60	0.04-8.73	0.708
Sex (male) (n=14)	0.30	0.03-2.76	0.288
Diarrhea (n=14)	N/A**		
Breeder & Shelter (n=15)	16.0	1.09-234.25	0.043
Presence of *Cryptosporidium* infection	1.75	0.17-17.69	0.635
Cryptosporidium spp.			
Age <1 year (n=14)	1.33	0.09-20.71	0.837
Sex (male) (n=14)	N/A**		
Diarrhea (n=14)	9.0	0.52-155.24	0.130
Breeder & Shelter (n=15)	0.58	0.04-4.66	0.682
Presence of *Giardia* infection	1.75	0.17-17.69	0.635

* 95%CI = 95% confidence interval
**Abbreviation

Table 2.8 Multivariate logistic regression analysis of variables associated with *Giardia duodenalis* infection in dogs in Chiang Mai, Thailand (n=97).

Variables	Odds Ratio	[95%CI*]	*P* Value
Age <1 year	6.53	1.76-24.25	0.005
Diarrhea	4.78	1.04-21.98	0.045
Breeder/shelter	3.93	1.33-11.65	0.013

* 95%CI = 95% confidence interval

2.7 Figures

Figure 2.1 Map of Thailand. Chiang Mai province is in yellow with geographic coordinates at 18°47’ N and 98°59’ E.

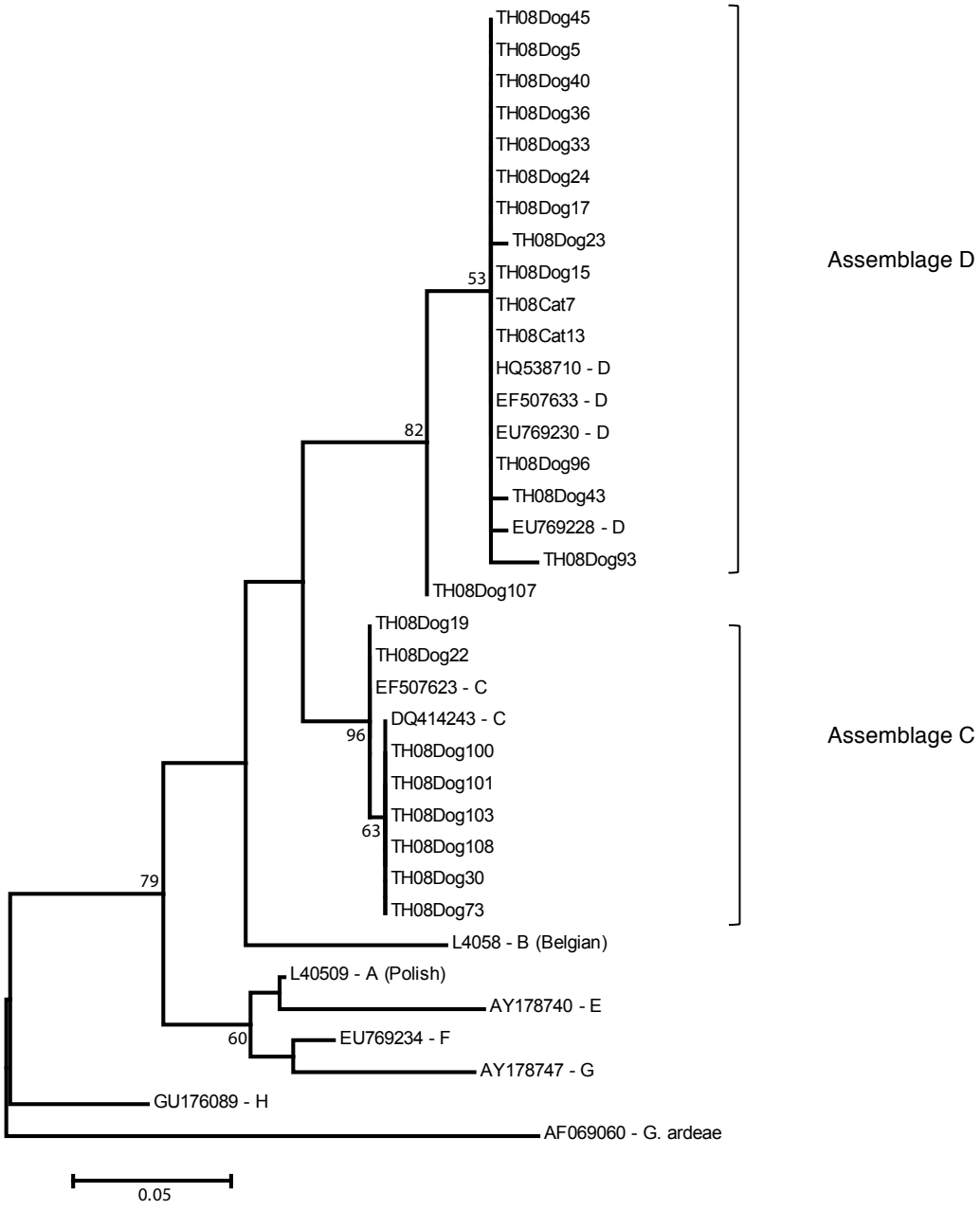


Figure 2.2 A phylogenetic tree of the *Giardia* isolates from dogs and cats based on the glutamate dehydrogenase (gdh) gene. The tree was constructed by a Maximum Likelihood method based on the Kimura 2-parameter model using MEGA5 program. The percentage of trees in which the associated taxa clustered together is shown next to the branches. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. Sequences obtained from GenBank are indicated by their accession numbers.

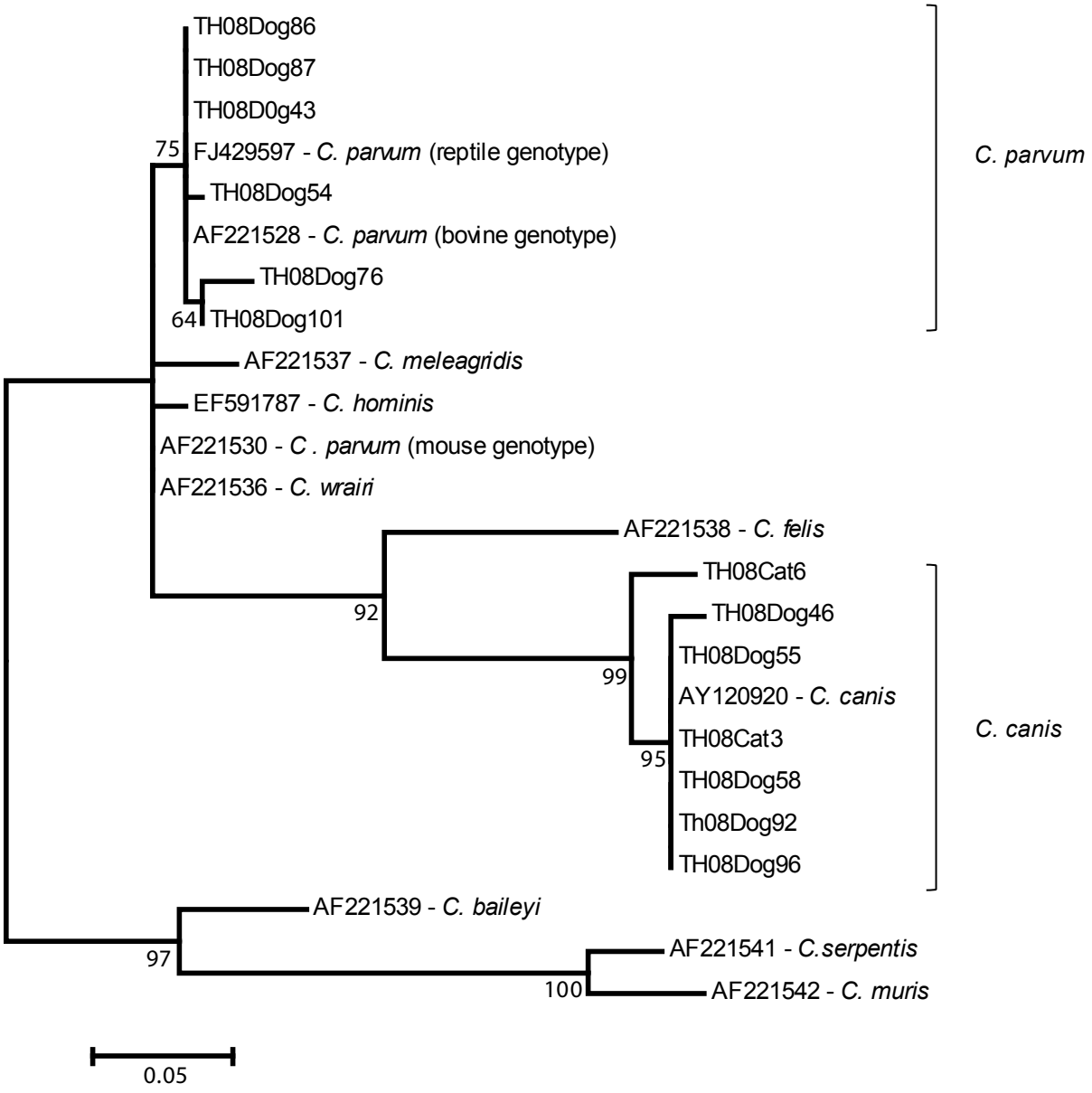


Figure 2.3 A phylogenetic tree of the *Cryptosporidium* isolates from dogs and cats based on the heat shock protein 70 kDa (hsp70) gene. The tree was constructed by a Maximum Likelihood method based on the Kimura 2-parameter model using MEGA5 program. The percentage of trees in which the associated taxa clustered together is shown next to the branches. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. Sequences obtained from GenBank are indicated by their accession numbers.

2.8 References

Alvarez-Pellitero, P., Quiroga, M.I., Sitja-Bobadilla, A., Redondo, M.J., Palenzuela, O., Padros, F., Vazquez, S., Nieto, J.M., 2004. *Cryptosporidium scophthalmi* n. sp. (Apicomplexa: Cryptosporidiidae) from cultured turbot *Scophthalmus maximus*. Light and electron microscope description and histopathological study. Dis. Aquat. Organ. 62, 133-145.

Alvarez-Pellitero, P., Sitja-Bobadilla, A., 2002. *Cryptosporidium molnari* n. sp. (Apicomplexa: Cryptosporidiidae) infecting two marine fish species, *Sparus aurata* L. and *Dicentrarchus labrax* L. Int. J. Parasitol. 32, 1007-1021.

Alves, M., Matos, O., Pereira Da Fonseca, I., Delgado, E., Lourenco, A.M., Antunes, F., 2001. Multilocus genotyping of *Cryptosporidium* isolates from human HIV-infected and animal hosts. J. Eukaryot. Microbiol. Suppl, 17S-18S.

Bajer, A., Bednarska, M., Rodo, A., 2011. Risk factors and control of intestinal parasite infections in sled dogs in Poland. Vet. Parasitol. 175, 343-350.

Ballweber, L.R., Panuska, C., Huston, C.L., Vasilopulos, R., Pharr, G.T., Mackin, A., 2009. Prevalence of and risk factors associated with shedding of *Cryptosporidium felis* in domestic cats of Mississippi and Alabama. Vet. Parasitol. 160, 306-310.

Ballweber, L.R., Xiao, L., Bowman, D.D., Kahn, G., Cama, V.A., 2010. Giardiasis in dogs and cats: update on epidemiology and public health significance. Trends Parasitol 26, 180-189.

Beck, R., Sprong, H., Pozio, E., Caccio, S.M., 2012. Genotyping *Giardia duodenalis* isolates from dogs: lessons from a multilocus sequence typing study. Vector Borne Zoonotic Dis 12, 206-213.

Bowman, D.D., Lucio-Forster, A., 2010. Cryptosporidiosis and giardiasis in dogs and cats: veterinary and public health importance. Exp. Parasitol. 124, 121-127.

Bugg, R.J., Robertson, I.D., Elliot, A.D., Thompson, R.C., 1999. Gastrointestinal parasites of urban dogs in Perth, Western Australia. Vet. J. 157, 295-301.

Cama, V.A., Bern, C., Sulaiman, I.M., Gilman, R.H., Ticona, E., Vivar, A., Kawai, V., Vargas, D., Zhou, L., Xiao, L., 2003. *Cryptosporidium* species and genotypes in HIV-positive patients in Lima, Peru. J. Eukaryot. Microbiol. 50 Suppl, 531-533.

Carlin, E.P., Bowman, D.D., Scarlett, J.M., Garrett, J., Lorentzen, L., 2006. Prevalence of *Giardia* in symptomatic dogs and cats throughout the United States as determined by the IDEXX SNAP *Giardia* test. Vet Ther 7, 199-206.

Chiang Mai Provincial Office 2012. Chiang Mai Database (http://www.chiangmai.go.th/meet_file/sarupCM.pdf).

Claerebout, E., Casaert, S., Dalemans, A.C., De Wilde, N., Levecke, B., Vercruysse, J., Geurden, T., 2009. *Giardia* and other intestinal parasites in different dog populations in Northern Belgium. Vet. Parasitol. 161, 41-46.

Dohoo, I., Martin, W., Stryhn, H., 2007. Veterinary epidemiologic research, 2nd Edition. AVC, Inc., Charlotte, P.E.I., Canada.

Edgar, R.C., 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Res 32, 1792-1797.

Eligio-Garcia, L., Cortes-Campos, A., Cota-Guajardo, S., Gaxiola, S., Jimenez-Cardoso, E., 2008. Frequency of *Giardia intestinalis* assemblages isolated from dogs and humans in a community from Culiacan, Sinaloa, Mexico using beta-giardin restriction gene. Vet. Parasitol. 156, 205-209.

Eligio-Garcia, L., Cortes-Campos, A., Jimenez-Cardoso, E., 2005. Genotype of *Giardia intestinalis* isolates from children and dogs and its relationship to host origin. Parasitol. Res. 97, 1-6.

Fayer, R., 2004. *Cryptosporidium*: a water-borne zoonotic parasite. Vet. Parasitol. 126, 37-56.

Fayer, R., 2010. Taxonomy and species delimitation in *Cryptosporidium*. Exp. Parasitol. 124, 90-97.

Fayer, R., Santin, M., 2009. *Cryptosporidium xiaoi* n. sp. (Apicomplexa: Cryptosporidiidae) in sheep (*Ovis aries*). Vet. Parasitol. 164, 192-200.

Fayer, R., Santin, M., Macarisin, D., 2010. *Cryptosporidium ubiquitum* n. sp. in animals and humans. Vet. Parasitol. 172, 23-32.

Fleiss, J.L., 2003. Statistical methods for rates and proportions. Wiley-Interscience, Hoboken, N.J.

Fontanarrosa, M.F., Vezzani, D., Basabe, J., Eiras, D.F., 2006. An epidemiological study of gastrointestinal parasites of dogs from Southern Greater Buenos Aires (Argentina): age, gender, breed, mixed infections, and seasonal and spatial patterns. Vet. Parasitol. 136, 283-295.

Gatei, W., Suputtamongkol, Y., Waywa, D., Ashford, R.W., Bailey, J.W., Greensill, J., Beeching, N.J., Hart, C.A., 2002. Zoonotic species of *Cryptosporidium* are as prevalent as the anthroponotic in HIV-infected patients in Thailand. Ann. Trop. Med. Parasitol. 96, 797-802.

Hackett, T., Lappin, M.R., 2003. Prevalence of enteric pathogens in dogs of north-central Colorado. J. Am. Anim. Hosp. Assoc. 39, 52-56.

Hill, S.L., Cheney, J.M., Taton-Allen, G.F., Reif, J.S., Bruns, C., Lappin, M.R., 2000. Prevalence of enteric zoonotic organisms in cats. J. Am. Vet. Med. Assoc. 216, 687-692.

Inpankaew, T., Traub, R., Thompson, R.C., Sukthana, Y., 2007. Canine parasitic zoonoses in Bangkok temples. Southeast Asian J. Trop. Med. Public Health 38, 247-255.

Irwin, P.J., 2002. Companion animal parasitology: a clinical perspective. Int. J. Parasitol. 32, 581-593.

Itoh, N., Ikegami, H., Takagi, M., Ito, Y., Kanai, K., Chikazawa, S., Hori, Y., Hoshi, F., Higuchi, S., 2012. Prevalence of intestinal parasites in private-household cats in Japan. J Feline Med Surg 14, 436-439.

Itoh, N., Kanai, K., Hori, Y., Hoshi, F., Higuchi, S., 2009. Prevalence of *Giardia intestinalis* and other zoonotic intestinal parasites in private household dogs of the Hachinohe area in Aomori prefecture, Japan in 1997, 2002 and 2007. J Vet Sci 10, 305-308.

Itoh, N., Muraoka, N., Kawamata, J., Aoki, M., Itagaki, T., 2006. Prevalence of *Giardia intestinalis* infection in household cats of Tohoku district in Japan. J. Vet. Med. Sci. 68, 161-163.

Itoh, N., Muraoka, N., Saeki, H., Aoki, M., Itagaki, T., 2005. Prevalence of *Giardia intestinalis* infection in dogs of breeding kennels in Japan. J. Vet. Med. Sci. 67, 717-718.

Katagiri, S., Oliveira-Sequeira, T.C., 2008. Prevalence of dog intestinal parasites and risk perception of zoonotic infection by dog owners in Sao Paulo State, Brazil. Zoonoses Public Health 55, 406-413.

Keith, C.L., Radecki, S.V., Lappin, M.R., 2003. Evaluation of fenbendazole for treatment of *Giardia* infection in cats concurrently infected with *Cryptosporidium parvum*. Am. J. Vet. Res. 64, 1027-1029.

Kim, J.T., Wee, S.H., Lee, C.G., 1998. Detection of *Cryptosporidium* oocysts in canine fecal samples by immunofluorescence assay. Korean J. Parasitol. 36, 147-149.

Kingsbury, D.D., Marks, S.L., Cave, N.J., Grahn, R.A., 2010. Identification of *Tritrichomonas foetus* and *Giardia* spp. infection in pedigree show cats in New Zealand. N. Z. Vet. J. 58, 6-10.

Lalle, M., Pozio, E., Capelli, G., Bruschi, F., Crotti, D., Caccio, S.M., 2005. Genetic heterogeneity at the beta-giardin locus among human and animal isolates of *Giardia duodenalis* and identification of potentially zoonotic subgenotypes. Int. J. Parasitol. 35, 207-213.

Lasek-Nesselquist, E., Welch, D.M., Sogin, M.L., 2010. The identification of a new *Giardia duodenalis* assemblage in marine vertebrates and a preliminary analysis of *G. duodenalis* population biology in marine systems. Int. J. Parasitol. 40, 1063-1074.

Little, S.E., Johnson, E.M., Lewis, D., Jaklitsch, R.P., Payton, M.E., Blagburn, B.L., Bowman, D.D., Moroff, S., Tams, T., Rich, L., Aucoin, D., 2009. Prevalence of intestinal parasites in pet dogs in the United States. Vet. Parasitol. 166, 144-152.

Mircean, V., Gyorke, A., Cozma, V., 2012. Prevalence and risk factors of *Giardia duodenalis* in dogs from Romania. Vet. Parasitol. 184, 325-329.

Monis, P.T., Caccio, S.M., Thompson, R.C., 2009. Variation in *Giardia*: towards a taxonomic revision of the genus. Trends Parasitol 25, 93-100.

Monis, P.T., Thompson, R.C., 2003. *Cryptosporidium* and *Giardia*-zoonoses: fact or fiction? Infect Genet Evol 3, 233-244.

Morgan, U.M., Monis, P.T., Xiao, L., Limor, J., Sulaiman, I., Raidal, S., O'Donoghue, P., Gasser, R., Murray, A., Fayer, R., Blagburn, B.L., Lal, A.A., Thompson, R.C., 2001. Molecular and phylogenetic characterisation of *Cryptosporidium* from birds. Int. J. Parasitol. 31, 289-296.

Mundim, M.J., Rosa, L.A., Hortencio, S.M., Faria, E.S., Rodrigues, R.M., Cury, M.C., 2007. Prevalence of *Giardia duodenalis* and *Cryptosporidium* spp. in dogs from different living conditions in Uberlandia, Brazil. Vet. Parasitol. 144, 356-359.

Papini, R., Gorini, G., Spaziani, A., Cardini, G., 2005. Survey on giardiosis in shelter dog populations. Vet. Parasitol. 128, 333-339.

Ponce-Macotela, M., Peralta-Abarca, G.E., Martinez-Gordillo, M.N., 2005. *Giardia intestinalis* and other zoonotic parasites: prevalence in adult dogs from the southern part of Mexico City. Vet. Parasitol. 131, 1-4.

Rambozzi, L., Menzano, A., Mannelli, A., Romano, S., Isaia, M.C., 2007. Prevalence of cryptosporidian infection in cats in Turin and analysis of risk factors. J Feline Med Surg 9, 392-396.

Read, C.M., Monis, P.T., Thompson, R.C., 2004. Discrimination of all genotypes of *Giardia duodenalis* at the glutamate dehydrogenase locus using PCR-RFLP. Infect Genet Evol 4, 125-130.

Ren, X., Zhao, J., Zhang, L., Ning, C., Jian, F., Wang, R., Lv, C., Wang, Q., Arrowood, M.J., Xiao, L., 2012. *Cryptosporidium tyzzeri* n. sp. (Apicomplexa: Cryptosporidiidae) in domestic mice (*Mus musculus*). Exp. Parasitol. 130, 274-281.

Robertson, I.D., Irwin, P.J., Lymbery, A.J., Thompson, R.C., 2000. The role of companion animals in the emergence of parasitic zoonoses. Int. J. Parasitol. 30, 1369-1377.

Robinson, G., Wright, S., Elwin, K., Hadfield, S.J., Katzer, F., Bartley, P.M., Hunter, P.R., Nath, M., Innes, E.A., Chalmers, R.M., 2010. Re-description of *Cryptosporidium cuniculus* (Apicomplexa: Cryptosporidiidae): Morphology, biology and phylogeny. Int. J. Parasitol.

Savioli, L., Smith, H., Thompson, A., 2006. *Giardia* and *Cryptosporidium* join the 'Neglected Diseases Initiative'. Trends Parasitol 22, 203-208.

Scaramozzino, P., Di Cave, D., Berrilli, F., D'Orazi, C., Spaziani, A., Mazzanti, S., Scholl, F., De Liberato, C., 2009. A study of the prevalence and genotypes of *Giardia duodenalis* infecting kennelled dogs. Vet. J. 182, 231-234.

Scorza, A.V., Ballweber, L.R., Tangtrongsup, S., Panuska, C., Lappin, M.R., 2012. Comparisons of mammalian *Giardia duodenalis* assemblages based on the beta-giardin, glutamate dehydrogenase and triose phosphate isomerase genes. Vet. Parasitol.

Scorza, A.V., Brewer, M.M., Lappin, M.R., 2003. Polymerase chain reaction for the detection of *Cryptosporidium* spp. in cat feces. J. Parasitol. 89, 423-426.

Scorza, A.V., Duncan, C., Miles, L., Lappin, M.R., 2011. Prevalence of selected zoonotic and vector-borne agents in dogs and cats in Costa Rica. Vet. Parasitol.

Scorza, A.V., Lappin, M.R., 2007. Co-infection of *Cryptosporidium* and *Giardia* in naturally infected cats. In Diagnosis and Treatment of Cryptosporidiosis and Giardiasis in Cats and Dogs in the United staes. Colorado State University, Fort Collins.

Scorza, V., Tangtrongsup, S., 2010. Update on the diagnosis and management of *Cryptosporidium* spp infections in dogs and cats. Top Companion Anim Med 25, 163-169.

Sprong, H., Caccio, S.M., van der Giessen, J.W., 2009. Identification of zoonotic genotypes of *Giardia duodenalis*. PLoS Negl Trop Dis 3, e558.

Srisuphanunt, M., Saksirisampant, W., Karanis, P., 2011. Prevalence and genotyping of *Cryptosporidium* isolated from HIV/AIDS patients in urban areas of Thailand. Ann. Trop. Med. Parasitol. 105, 463-468.

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M., Kumar, S., 2011. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol. Biol. Evol. 28, 2731-2739.

Thai Meteorological Department Automate Weather System. Data from 2008-2012 (http://www.aws-observation.tmd.go.th/web/reports/weather_months.asp).

Thompson, R.C., Smith, A., 2011. Zoonotic enteric protozoa. Vet. Parasitol. 182, 70-78.

Tiangtip, R., Jongwutiwes, S., 2002. Molecular analysis of *Cryptosporidium* species isolated from HIV-infected patients in Thailand. Trop. Med. Int. Health 7, 357-364.

Traub, R.J., Inpankaew, T., Reid, S.A., Sutthikornchai, C., Sukthana, Y., Robertson, I.D., Thompson, R.C., 2009. Transmission cycles of *Giardia duodenalis* in dogs and humans in Temple communities in Bangkok--a critical evaluation of its prevalence using three diagnostic tests in the field in the absence of a gold standard. Acta Trop. 111, 125-132.

Traub, R.J., Monis, P.T., Robertson, I., Irwin, P., Mencke, N., Thompson, R.C., 2004. Epidemiological and molecular evidence supports the zoonotic transmission of *Giardia* among humans and dogs living in the same community. Parasitology 128, 253-262.

Upjohn, M., Cobb, C., Monger, J., Geurden, T., Claerebout, E., Fox, M., 2010. Prevalence, molecular typing and risk factor analysis for *Giardia duodenalis* infections in dogs in a central London rescue shelter. Vet. Parasitol. 172, 341-346.

Vasilopulos, R.J., Mackin, A.J., Rickard, L.G., Pharr, G.T., Huston, C.L., 2006. Prevalence and factors associated with fecal shedding of *Giardia* spp. in domestic cats. J. Am. Anim. Hosp. Assoc. 42, 424-429.

Volotao, A.C., Costa-Macedo, L.M., Haddad, F.S., Brandao, A., Peralta, J.M., Fernandes, O., 2007. Genotyping of *Giardia duodenalis* from human and animal samples from Brazil using beta-giardin gene: a phylogenetic analysis. Acta Trop. 102, 10-19.

Xiao, L., 2010. Molecular epidemiology of cryptosporidiosis: an update. Exp. Parasitol. 124, 80-89.

Xiao, L., Bern, C., Limor, J., Sulaiman, I., Roberts, J., Checkley, W., Cabrera, L., Gilman, R.H., Lal, A.A., 2001. Identification of 5 types of *Cryptosporidium* parasites in children in Lima, Peru. J. Infect. Dis. 183, 492-497.

Xiao, L., Cama, V.A., Cabrera, L., Ortega, Y., Pearson, J., Gilman, R.H., 2007. Possible transmission of *Cryptosporidium canis* among children and a dog in a household. J. Clin. Microbiol. 45, 2014-2016.

Xiao, L., Fayer, R., 2008. Molecular characterisation of species and genotypes of *Cryptosporidium* and *Giardia* and assessment of zoonotic transmission. Int. J. Parasitol. 38, 1239-1255.

CHAPTER 3: PREVALENCE OF AND RISK FACTORS FOR *GIARDIA* AND *CRYPTOSPORIDIUM* INFECTION IN DOGS AND CATS IN CHIANG MAI, THAILAND: 2009-2010 STUDY*

Summary

This cross-sectional study was designed to determine seasonal and other risk factors associated with *Giardia* and *Cryptosporidium* infections in dogs and cats in Chiang Mai, Thailand. Fecal samples from 301 dogs and 66 cats were collected between August 2009 and February 2010. The fecal samples were analyzed for the presence of *Giardia* cysts and *Cryptosporidium* oocysts using zinc sulfate fecal flotation and immunofluorescent assay (IFA). Demographic and geographic data were recorded. For each animal, potential risk data obtained by a questionnaire were analyzed for associations with *Giardia* and *Cryptosporidium* infections. *Giardia* cysts were identified in 76 dogs (25.3%, 95%CI: 20.3-30.2) and 18 cats (27.3%, 95%CI: 16.2-38.3) and *Cryptosporidium* oocysts were identified in 23 dogs (7.6%, 95%CI: 4.6-10.7) and 8 cats (12.1%, 95%CI: 4.0-20.2). The multivariate analysis indicated that *Giardia* infections were more likely to be detected in dogs infected with *Cryptosporidium* (OR 157.39, 95%CI:17.15-1,444.43), dogs in shelters, breeding farms, or Buddhist temples (OR 8.54, 95%CI:1.94-37.63), dogs with reporting chronic diarrhea (OR 5.85, 95%CI:1.52-22.53), dogs sampled during the rainy season (OR 5.16, 95%CI:1.77-15.03), dogs that drink untreated water (OR 3.22, 95%CI:1.16-8.96) and in dogs having diarrhea (OR 2.68, 95%CI:1.15-6.26).

* Sahatchai Tangtrongsup[1], A. Valeria Scorza[2], John S. Reif[3], Lora R. Ballweber[4], Michael R. Lappin[2], Mo D. Salman[1]: *[1]Animal Population Health Institute, and [2]Center for Companion Animal Studies*, *Department of Clinical Sciences, [3]Department of Clinical Sciences, [3]Department of Environmental and Radiological Health Sciences, and [4]Department of Microbiology, Immunology and Pathology, College of Veterinary Medicine and Biomedical Sciences, Colorado State University, Fort Collins, CO 80523*. This chapter is formatted for requirement of the Veterinary Parasitology journal.

Cryptosporidium infection was more likely to be detected in dogs less than one year of age (OR 3.55, 95%CI: 1.76-11.73), and dogs having diarrhea (OR 3.06, 95%CI:1.17-8.0). Sequence analyses of 19 *Giardia* gdh PCR positive samples and 3 *Cryptosporidium* hsp70 PCR positive samples from dogs revealed the presence of *G. duodenalis* assemblage D (100%), *C. canis* (2/3) and *C. parvum* (1/3). Sequence analysis from 6 *Giardia* PCR positive- and 2 *Cryptosporidium* PCR positive samples from cats revealed the presence of *Giardia duodenalis* assemblage C (4/6), D (1/6) and A (1/6) and *C. parvum* (2/2*)*. Additional studies are needed to address the zoonotic potential of dogs and cats in this area. Multilocus genotyping studies characterizing larger number of *Cryptosporidium* and *Giardia* isolates from dogs and cats are needed to assess their zoonotic potential.

Keywords: Dogs; Cats; *Giardia*; *Cryptosporidium*; Risk factors; Seasonality; Chiang Mai; Thailand

3.1 Introduction

Giardia duodenalis and *Cryptosporidium* spp. are common causes of gastrointestinal disease in mammalian hosts including humans, dogs and cats (Xiao and Fayer, 2008). The clinical signs vary from sub-clinical to gastrointestinal discomfort to severe chronic diarrhea. Diarrhea can be of short duration in immune-competent individuals; however, in immune-compromised, severe, chronic diarrhea can occur and is potentially life threatening (Buret et al., 2002; Tzipori and Ward, 2002). *Giardia duodenalis* is comprised of eight genotypes or assemblages (A-H) (Lasek-Nesselquist et al., 2010; Monis et al., 2009). Humans are usually infected with assemblages A and B, which also infect a wide range of hosts including domestic animals and wildlife, and are considered zoonotic genotypes. Dogs and cats can be infected with species-adapted assemblages C and D (dogs) and F (cats) and the zoonotic assemblages A and B.

The genus *Cryptosporidium* contains at least 24 valid species (Alvarez-Pellitero et al., 2004; Alvarez-Pellitero and Sitja-Bobadilla, 2002; Fayer, 2010; Fayer and Santin, 2009; Fayer et al., 2010; Ren et al., 2012; Robinson et al., 2010). Humans are preferentially infected by *C. hominis* and *C. parvum*, with a lesser extent of *C. meleagridis, C. canis* and *C. felis.* Dogs are infected with *C. canis*, *C. parvum*, *C. meleagridis* and *C. muris* (Santin, 2013). Cats are mostly infected with *C. felis* and *C. muris* (Santin, 2013). Often, the diagnoses of *Cryptosporidium* and *Giardia* are determined at the genus level (as *Cryptosporidium* spp. and *Giardia* spp.). Thus, *Giardia* cysts and *Cryptosporidium* oocysts detected in dogs and cats are always assumed zoonotic and can be a potential reservoir for a zoonotic transmission to humans unless molecular genotyping is performed.

Worldwide, it has been estimated that *G. duodenalis* causes human infections at a rate of 2.8×10^8 cases per year (Lane and Lloyd, 2002). In the US, the annual incidence of giardiasis was estimated at 7.3-7.6 cases per 100,000 people and for *Cryptosporidium*, at 2.9 cases per 100,000 people in 2010 (Yoder et al., 2012a; Yoder et al., 2012b). The prevalence of *Giardia* and *Cryptosporidium* infections vary depending on the region, detection method, and population tested. Worldwide, the prevalence of *Giardia* in dogs and cats range from 0 to 57% (Feng and Xiao, 2011) and that of *Cryptosporidium* in dogs and cats ranges from 0 to 28% (Fayer, 2008).

In Thailand, information regarding these organisms is limited. Reports regarding *Giardia* and *Cryptosporidium* infections were mostly done in children and HIV patients. The reported prevalences of *Giardia* and *Cryptosporidium* infection in humans range from 1.3-37.7% and 0.3-32.9%, respectively (Dib et al., 2008; Srisuphanunt et al., 2011; Wongstitwilairoong et al., 2007). The only report in dogs showed a prevalence of *Giardia* of 56.8% (Traub et al., 2009).

Several significant risk factors associated with infections of these parasites have been reported such as season of the year (Batchelor et al., 2008; Fontanarrosa et al., 2006), breed of dog (Gates and Nolan, 2009) and presence of other enteric parasites such as *Giardia* cysts for cryptosporidiosis (Ballweber et al., 2009) and presence of *Cryptosporidium* and coccidial oocysts as a risk of giardiasis in cats (Vasilopulos et al., 2006).

Our research group performed a small pilot study on the epidemiology of giardiasis and cryptosporidiosis in dogs and cats in Chiang Mai, Thailand (see Chapter 2). In that study, prevalence rates of *Giardia* spp. infections in dogs and cats were 45.9% and 40% respectively. Prevalence rates of *Cryptosporidium* spp. infections in dogs and cats were 22% and 26.7%, respectively. The risks associated with *Giardia* infection in dogs were being less than one year of age, having diarrhea and residing in a shelter or breeder setting. In our pilot study, some risks factors like co-infections and association with season were not reported in the dog and cat populations in Chiang Mai, Thailand.

Seasonality of these infections was considered in previous studies. A study of endoparasites in dogs in the UK revealed that the *Cryptosporidium* infection was more prevalent in autumn than any other season (Batchelor et al., 2008). A study in Argentina reported that *Giardia* infection in dogs was more prevalent in winter than at any other time (Fontanarrosa et al., 2006). Thailand has three seasons: summer (March-May), rainy (June-October), and winter (November-February), the latter of which is considered a dry period. Understanding risk factors associated with *Giardia* and *Cryptosporidium* infections in dogs and cats in this region could aid veterinarians in prevention and control of these important diseases, In addition, the potential zoonotic transmission will be reduced.

In this study, we hypothesized that the prevalence of *Giardia* and *Cryptosporidium* infection in dogs and cats is higher in the rainy season than in winter. The aims of the current study were 1) to determine risk factors associated with *Giardia* and *Cryptosporidium* infections in this population, 2) to explore the seasonal differences of *Giardia* and *Cryptosporidium* prevalences, and 3) to genetically determine the genotype/species of these organisms in dogs and cats in Chiang Mai, Thailand.

3.2 Materials and Methods

3.2.1 Study location

Chiang Mai is the second largest province of Thailand. It is located in the northern part of the country at geographic coordinates 18°47' N and 98°59' E. This province covers an area of 20,107.057 sq. km with an average elevation of 310 m above sea level. Approximately 70% of the area is covered with forest, 13% is agricultural land, and 17% is for housing and other uses. There are three seasons: rainy ranges from mid-May to October, winter from November to mid-February, and summer from mid-February to mid-May (Chiang Mai Provincial Office, 2012). The average temperature in rainy, summer, and winter are 27.7 °C (average min 22.4°C-average max 36.0°C), and 28.5°C (average min 20.1-average max 39.1), and 23.9°C (average min 14.4°C - average max 34.35°C), respectively. Average rainfall in rainy, summer and winter were 16.9 cm, 8.7 cm, and 0.8 cm, respectively (Thai Meteorological Department Automate Weather System). Chiang Mai is administratively divided into 25 districts (amphoe), 204 sub-districts (tambon) and 2,066 villages (mooban) (Chiang Mai Provincial Office, 2012).

3.2.2 Sample collection

A cross-sectional study was conducted. Animals visiting the Small Animal Hospital of the Faculty of Veterinary Medicine, Chiang Mai University, as well as shelters and breeders in

Chiang Mai province, Thailand were the sources of samples. Four hundred and sixty fecal samples were targeted for collection during August 2009 to February 2010 (230 samples from rainy months and 230 samples from dry months). This sample size was calculated based on the preliminary prevalence of *Giardia* in dogs in Chiang Mai (21% by immunofluorescent, IFA) and the assumption that the prevalence is reduced by 10% in the dry months (alpha of 0.05 and power of 0.80) (Fleiss, 2003).

The samples were collected on a volunteer basis regardless of the health status of the animals. Owners or caregivers of the animals were asked to complete a written questionnaire (see Appendix) with information regarding geographic and demographic data, number of animals, details of housing and living conditions, health status, owners or caregivers' socioeconomic status. The fecal consistency was estimated using the Nestle Purina Fecal Scoring System for Dogs and Cats (Nestle-Purina Pet Food Co, St Louis, MO, USA). Fecal scores of 1-4 were considered as normal with scores of 5 or 6 classified as diarrheic.

3.2.3 Diagnostic methods

Fecal consistency was determined upon the receipt of the sample and all fecal samples were stored in closed plastic containers at 4ºC. Microscopic examination of feces after performance of a conventional zinc sulfate centrifugal flotation was used to determine intestinal parasitic infection within 5 days of collection and then samples were stored at -20ºC until shipped to Colorado State University for IFA and molecular analysis. Fecal samples were shipped to the USA on dry ice and stored at -70ºC.

Feces were evaluated for *Giardia* cysts and *Cryptosporidium* oocystsusing a commercially available immunofluorescent assay (IFA) (Merifluor® *Cryptosporidium*/*Giardia* IFA kit, Meridian Diagnostic Corporation, Cincinnati, OH). Prior to IFA, all fecal samples were

thawed at room temperature (20ºC) and concentrated using sugar concentration techniques as previously described (O'Handley et al., 2000; Vasilopulos et al., 2006). In brief, 3 grams of feces were mixed with 4.5 ml PBS-EDTA and strained through cheesecloth then overlaid on 7 ml Sheather's sugar solution. Samples were centrifuged at a speed of 800g for 10 minutes. The top layer was transfer to new falcon tub and centrifuged at a speed of 1,200g for 10 minutes. The pellet was washed twice with PBS-EDTA and centrifuged at 1,200g for 10 minutes. The pellet were then re-suspended in 1 ml PBS-EDTA. A thin fecal smear was made on the IFA slides following the manufacturer's instructions.

3.2.4. Molecular analysis

Genomic DNA was extracted from fecal samples containing *Giardia* cysts or *Cryptosporidium* oocysts following an established protocol (Scorza et al., 2003) and stored at -20ºC until assayed in the different polymerase chain reaction (PCR) assays. A fragment of *Giardia* glutamate dehydrogenase gene and a fragment of *Cryptosporidium* heat shock protein 70 kDa (hsp70) gene were amplified by PCR as previously described with the following modifications (Morgan et al., 2001; Read et al., 2004); use of a commercial master mix (HotStarTaq Master Mix, Qiagen, Valencia, CA) with 10 pmol of each primer and 1 µl of genomic DNA per PCR reaction.

The PCR products were analyzed by nucleotide sequencing using a commercially available service (Proteomics and Metabolomics Facility, Colorado State University). The obtained sequences were compared with nucleotide sequences from the nucleotide database, GenBank, by BLAST analysis (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

3.2.5. Statistical analyses

The sample was considered positive if the test results for *Giardia* (zinc sulfate fecal flotation or IFA) and for *Cryptosporidium* (IFA) were positive. Agreement of *Giardia* detection were assessed using the exact McNemar's test and Kappa statistics (Fleiss, 2003). Samples were grouped by age (< 1 yr, 1-7 yrs and > 7 yrs). Fecal samples collected during August to October 2009 were grouped and represented as samples collected during the wet months or rainy season. Fecal samples collected during November 2009 to February 2010 were grouped and represented as dry months or winter samples. Responses to the questions for each individual animal information were entered into a spreadsheet and all statistical analyses were performed using the Stata statistical software release 10.1 (Stata Corp., College Station, Texas, USA). Overall prevalence and 95% confidence interval (95%CI) were calculated. Association of risk factors and *Giardia* or *Cryptosporidium* positive outcomes were analyzed using χ^2 or Fisher's exact test whenever appropriate (Fleiss, 2003). Odd ratios (OR) and 95%CI were estimated to measure the strength of association using univariate logistic regression. A multivariate logistic regression model was constructed using a backward stepwise elimination procedure, against either *Giardia* or *Cryptosporidium* infection (Dohoo et al., 2007). Variables found to be associated with *Giardia* or *Cryptosporidium* infection in ($p \leq 0.1$) were included in the multivariate logistic regression analysis. Variables were retained in the model based on the likelihood ratio χ^2 statistic, at $p<0.05$. Association of risk factors and *Giardia* infection alone and *Giardia* and *Cryptosporidium* co-infections were assessed with the same methods.

3.3 Results

There were 301 canine and 66 feline fecal samples obtained for this study. Ninety-two percent of dog owners and 71.2% of cat owners or caregivers answered or returned the

questionnaires although not all questions were answered in all questionnaires. Samples were collected from 15 of 22 districts (Figure 3.1). Distributions of samples collected in each month are shown in Table 3.1. For dogs, the number of fecal samples collected in the rainy and winter seasons were 167 (55.5%) and 134 (44.5%), respectively. In cats, the number of samples collected in the rainy and winter seasons were 24 (36.4%) and 42 (63.6%), respectively. The majority of fecal samples were obtained from household dogs and cats (Table 3.2). Of the samples, 58.5% (176/301) of dogs and 65% (43/66) of cats were from central Chiang Mai (Muang district) (Table 3.3 and Table 3.4). Of 301 canine fecal samples 67 (22.3%), 161 (53.5%) and 73 (24.3%) were from dogs less than 1 year of age, 1-7 years of age and more than 7 years of age, respectively. Of 66 feline fecal samples, 20 (30.3%), 40 (60.6%) and 6 (9.1%) were from cats age less than 1 year of age, 1-7 years of age and more than 7 years of age, respectively. Of dog and cat fecal samples, 48.5% (146/301) and 57% (38/66) were from male dogs and cats, respectively.

The overall prevalence of *Giardia* infections in dogs and cats was 25.3% (95%CI: 20.3-30.2) and 27.3% (95%CI: 16.2-38.3), respectively. Overall prevalence of *Cryptosporidium* infections in dogs and cats were 7.6% (95%CI: 4.6-10.7) and 12.1% (95%CI: 4.0-20.2), respectively. Of 301 canine and 66 feline fecal samples, 117 (38.9%, 95%CI: 33.3-44.4) of dogs and 30 (45.5%, 95%CI: 33.1-57.8) of cats were infected with at least one parasite. Distributions of intestinal parasitic infections are shown in Table 3.5. Of these parasitic infections, co-infection patterns in dogs and cats are shown in Figure 3.2 and 3.3.

The univariate analyses of risk factors associated with *Giardia* and *Cryptosporidium* infection in dogs and cats are shown in Table 3.6 and Table 3.7, respectively. Age, diarrhea status, and source of drinking water were significantly associated with both *Giardia* and

Cryptosporidium infection in dogs. In cats, the breed category and type of housing were significantly associated with both *Giardia* and *Cryptosporidium* infections. During the rainy season, the odds of dogs shedding *Giardia* cysts were 2.24 times higher than the dry winter season (95%CI:1.29-3.91). *Giardia* infection was more likely in dogs with diarrhea (OR 2.55, 95%CI:1.43-4.55) than dogs having normal stool. Dogs infected with *Cystoisospora* spp. (OR 3.22, 95%CI:1.23-8.45) or *Cryptosporidium* spp. (OR 91.26, 95%CI:12.03-692.03) were more likely to have *Giardia* co-infection. Dogs living in crowded settings like shelters, breeding farms or temples had higher risk for *Giardia* infection than household dogs (OR 4.03, 95%CI:1.59-10.17). Dogs with diarrhea also had a higher risk of infection with *Cryptosporidium* (OR 2.98, 95%CI:1.24-7.15). The odds of having *Cryptosporidium* infection were also higher in dogs infected with *Giardia* cysts (OR 91.26, 95%CI:12.03-692.03). Persian cats were more likely to be infected with *Giardia* (OR 7.08, 95%CI:1.99-25.27) as well as *Cryptosporidium* (OR 24.6, 95%CI:2.65-228.09). Cats in breeding farms had 18.75 (95%CI:4.21-83.48) times higher risk of being infected with *Giardia* and 10.42 (95%CI:2.07-52.33) times higher risk of being infected with *Cryptosporidium* compared to household cats. The odds of having *Giardia* was 11.5 (95%CI:2.06-64.34) in cats infected with *Cryptosporidium*, and vice versa. Due to the high proportion of the co-infection of *Giardia* and *Cryptosporidium* in dogs in this study, the samples were again categorized to *Giardia* infection alone, *Cryptosporidium* infection alone, *Giardia* and *Cryptosporidium* co-infection and no infection groups (Table 3.10). Univariate analyses of risk factors and these categories were shown in Table 3.11. Rainy season (OR 3.77, 95%CI:1.85-7.69), pure bred dogs (OR 2.17, 95%CI:1.12-4.18), having diarrhea (OR 2.11, 95%CI:1.08-4.18), presence of *Toxocara canis* eggs (OR 4.4, 95%CI:1.06-18.19), presence of *Cystoisospora* oocysts (OR 4.15, 95%CI:1.52-11.34), residing in a shelter, breeding facility, or temple (OR

2.99, 95%CI:1.01-8.79), and having chronic diarrhea (OR 6.0, 95%CI:2.12-17.0) were significantly associated with infection by *Giardia* alone. For co-infection of *Giardia* and *Cryptosporidium* co-infection, the significant associated risk factors were age <1 year (OR 8.71, 95%CI:1.85-40.96), having diarrhea (OR 3.83, 95%CI:1.55-9.49), and residing in in a shelter, breeding facility, or temple (OR 7.03, 95%CI:2.12-23.3). Although non-significant, the dogs with co-infection of *Giardia* and *Cryptosporidium* are more likely to have diarrhea than dogs with *Giardia* infection alone (OR 1.81, 95%CI: 0.66-5.01, p=0.251). In contrast, dogs with *Giardia* infection alone are more likely having chronic diarrhea than dogs with *Giardia* and *Cryptosporidium* co-infection (OR 4.07, 95%CI:0.48-34.46, p=0.198)..

From the univariate analyses (Table 3.6), the candidate variables ($p \leq 0.1$) to be included in the multivariable analysis for *Giardia* infection in dogs were season, age, diarrhea status, presence of *Cystoisospora* oocysts, presence of *Cryptosporidium* oocysts, type of housing, number of animals in the household, defecating area, source for drinking water and reporting chronic diarrhea. For *Cryptosporidium* multivariable analysis in dogs, age, diarrhea status, type of housing, number of animals in the household, free roaming and source for drinking were included. The variables remaining in the multivariate logistic regression model for *Giardia* infection were presence of *Cryptosporidium* oocysts, residing in shelter/breeding setting or temple, reporting chronic diarrhea, rainy season, drinking untreated water, and having diarrhea (Table 3.8). The variables remained in the multivariable model for *Cryptosporidium* infection were age less than 1 year, and having diarrhea (Table 3.9). The results of multivariate analyses when determine an infections as *Giardia* alone, *Cryptosporidium* alone, and co-infection of *Giardia* and *Cryptosporidium* are shown in Table 3.12 and Table 3.13. The significant risk factors for *Giardia* infection alone were residing in shelter/breeding setting or temple, reporting

chronic diarrhea, rainy season and having diarrhea. The significant risk factors for *Giardia* and *Cryptosporidium* co-infection were residing in shelter/breeding setting/temple, age less than 1 year and having diarrhea. For cats, from multivariate analysis, *Giardia* infection was significant high in Persian cats with odds ratio of 7.08 (95%CI:1.99-25.27), whereas, *Cryptosporidium* infection was higher in cats shedding *Giardia* cysts with OR of 11.5 (95%CI:2.06-64.34).

Seventy-six dog and eighteen cat samples containing *Giardia* cysts were genotyped using the *Giardia* gdh PCR assay. Bands of ~ 432 bp fragment of the gdh gene were successfully amplified in 19 dog- and 6 cat samples. All dog gdh sequences were identified as *G. duodenalis* assemblage D. For cats, 1 (16.7%) was identified as assemblage D, 4 (66.7%) as assemblage C, and 1 (16.7%) as assemblage A. For *Cryptosporidium* genotyping, 23 dog and 8 cat samples were analyzed. Bands of ~ 325-bp fragment of hsp70 were successfully amplified in 3 dog and 2 cat samples. Two of three PCR positive dog samples were identified as *C. canis* and a remaining sequence was identified as *C. parvum*. Both PCR positive cat samples were identified as *C. parvum*.

Kappa analysis (K, 0.03, $p = 0.270$) and McNemar's test ($p<0.001$) on the *Giardia* detection by zinc sulfate flotation and IFA revealed that the two tests were inagreement.

3.4 Discussion

The limitations of this study include the small sample size for cats and sample collection based on convenience. Most of the samples in this study were from healthy animals; selection bias may have led to underestimation of the prevalence rates. The prevalence of *Giardia* and *Cryptosporidium* infection in this study, therefore, may not reflect the general population of dogs and cats in this province.

In our previous pilot study in 2008, the prevalence rates of *Giardia* spp. infection in dogs and cats were 45.9% and 40%, and the prevalence rates for *Cryptosporidium* spp. infections in dogs and cats were 22% and 26.7%, respectively. In our current study, performed on samples collected approximately one year later, over a longer period of time, the prevalence of *Giardia* in dogs and cats were 25.2% and 27.3%, and the prevalence of *Cryptosporidium* in dogs and cats were 7.6% and 12.1%. The difference of prevalences of infection may be due to the fact that in the previous study, in 2008, fecal samples the majority of samples were from shelters/breeders (55%) whereas in the current study, 93.3% of dog samples and 80% of cat samples were from household pets. Therefore, the previous study and the current study may represent the prevalence of *Giardia* and *Cryptosporidium* infection in shelter/breeding dogs and the household dogs, respectively.

Most of Thailand has a tropical wet and dry or savanna climate, in which some months of the year have heavier rain fall than other months. As *Giardia* and *Cryptosporidium* are common causes of waterborne gastrointestinal disease, it was hypothesized in the current study that the prevalence of *Giardia* and *Cryptosporidium* in wet months (rainy season) is higher than in the dry months (winter). From the analysis, regardless to co-infection with *Cryptosporidium*, *Giardia* infection was significantly higher in the rainy season than in winter. Dogs in the rainy season were 5.16 times more at risk of having *Giardia* infection when compared to dogs in the winter. This finding was also true when the analysis was done in dogs with *Giardia* infection alone (OR 3.82, 95%CI:1.37-10.70). However, the seasonality was not significantly associated with *Cryptosporidium* infection or *Giardia and Cryptosporidium* co-infection in dogs. A retrospective study of endoparasites in dogs based on fecal samples submitted to a commercial diagnostic laboratory in the United Kingdom revealed that *Cryptosporidium* was more prevalent

in autumn (October-December) than any other period (Batchelor et al., 2008). It has also reported that *Giardia* infection was more prevalent in cool weather than warm weather (Fontanarrosa et al., 2006). Therefore, in Chiang Mai, Thailand, veterinarians should be aware of an increased risk for *Giardia* infection in dogs with diarrhea during the rainy seasons

To authors' knowledge, this is the first report to assess the association of risk factors and co-infection of *Giardia* and *Cryptosporidium.* In this study, it is shown that rate of co-presence of *Giardia* and *Cryptosporidium* infection is high, with 22 of 76 (31.6%) *Giardia* infected dogs had *Cryptosporidium* infection and 22 of 23 (95.7%) *Cryptosporidium* infected dogs had *Giardia* infection. Previous studies have shown the co-presence of *Giardia* and *Cryptosporidium* in cats (Ballweber et al., 2009; Mundim et al., 2007; Scorza and Lappin, 2007; Vasilopulos et al., 2006), but neither of these studies have analyzed the association of risk factors and co-infection of these parasites.

Risk factors associated with the *Giardia* and *Cryptosporidium* infection in dogs and cats have been studied and vary depending on the population studied. Mostly, the infections were associated with young age, having diarrhea or chronic diarrhea, the presence of other intestinal parasites, eating homemade food, living in a crowded setting or being abandoned (i.e., stray animals) (Ballweber et al., 2009; Fontanarrosa et al., 2006; Itoh et al., 2011; Katagiri and Oliveira-Sequeira, 2008; Rambozzi et al., 2007; Vasilopulos et al., 2006). The risk factors evaluated in the current study support these previous studies. When adjusting for factors other than season of the year, significant risks for *Giardia* infection in dogs were presence of *Cryptosporidium* infection, residing in a crowded setting (shelter/breeder/temple), reporting chronic diarrhea and currently having diarrhea. Therefore, the transmission of *Giardia* and *Cryptosporidium* in dogs and cats may be reduced by limiting the exposure to diarrheic dogs.

The infected dogs should be separated from other animals and should be treated and bathed before moving to the new clean cage. A soiled cage or contaminated floor should be thorough cleaned followed by steam cleaning or disinfecting with quaternary ammonium compounds with a minimum contact time of 1 minute (Tangtrongsup and Scorza, 2010). In the diarrheic dogs with co-infection of *Giardia* and *Cryptosporidium*, nitazoxanide at the dose of 25 mg/kg, orally every 12 hours for at least 5 days may be suggested as this drug has been shown to be able to treat both *Giardia* and *Cryptosporidium* infection in dogs and cats (Scorza and Tangtrongsup, 2010; Tangtrongsup and Scorza, 2010).

In the current study, 17 of 54 dogs (31.5%) infected with *Giardia* alone and 10 of 22 dogs (45.45%) with *Giardia* and *Cryptosporidium* co-infection had diarrheic stool. In contrast, 9 of 51 dogs (17.6%) infected with *Giardia* alone and 1 of 21 dogs (5%) with *Giardia* and *Cryptosporidium* co-infections had chronic diarrhea (reported by owners or caregivers). Although, these diarrhea status and reporting chronic diarrhea information were not significant, with power of 0.15 and 0.11, respectively, the larger collection of samples may be suggested to analyze the association between diarrhea (as well as chronic diarrhea) and single or co-infection of *Giardia* and *Cryptosporidium*.

Giardia cysts and *Cryptosporidium* oocysts can be resistant to extreme environmental conditions and survive in soil or water for long periods of time (Fayer, 2008; Pozio, 2003). Therefore, untreated water is always a risk not only for *Giardia* and *Cryptosporidium* but also with other waterborne pathogens (Baldursson and Karanis, 2011; O'Reilly et al., 2007; Smith et al., 2006). The multivariate analysis in this study revealed that sources of water significantly associated with *Giardia* infection. Therefore, choosing safe, reliable water sources or boiling or

filtering untreated water (i.e, river water, underground and well water) with an appropriate filter before giving to animals is recommended.

Infection with intestinal parasites has a negative health effect on the hosts and co-infection of *Giardia* with *Cryptosporidium* or with other enteric parasites has been documented (Ballweber et al., 2009; Itoh et al., 2011; Scorza and Lappin, 2007; Vasilopulos et al., 2006). The co-infection of other parasites can worsen the clinical symptoms of giardiasis and cryptosporidiosis. In this study, the high prevalence of intestinal parasites was detected in both dogs and cats (38.9% and 45.5%); therefore, setting a regular schedule for deworming and testing for enteric parasites is also suggested.

The genera *Giardia* and *Cryptosporidium* comprise host-adapted and zoonotic genotypes or species. Dogs and cats can be infected by *G. duodenalis* assemblage A and B, and *C. parvum,* which means they may be a potential reservoir for zoonotic transmission. The transmission cycle among dogs and humans living in the same community has been documented (Traub et al., 2009; Traub et al., 2004). In the current study all *Giardia* isolates from dogs were the dog-adapted genotype assemblage D, whereas in cats, 3 of 4 of *Giardia* isolates was assemblage C or D. Although only one assemblage A was identified in a cat, there is not enough evidence to support or refute the proposal that dogs and cats have a potential role in zoonotic transmission since only 9-25% of positive samples could be amplified. More studies are needed to clarify the role of dogs and cats in this area in zoonotic transmission, including the using of multilocus genotyping, expanding the study time or focusing on target populations that have a high prevalence, such as breeding farms, shelters, kennels or catteries. In addition, the study of *Giardia* and *Cryptosporidium* isolates from animals and humans in the same household may elucidate zoonotic transmission potential of pets to their owners.

The detection of parasites on unfrozen fecal samples has been suggested to diagnosed for accurately detection (Scorza and Tangtrongsup, 2010; Tangtrongsup and Scorza, 2010); however, in this study, freezing were required because the fecal material needed to be stored for up to 8 months before international shipment. This freezing step might have affected *Giardia* and *Cryptosporidium* detection in later steps. Although the IFA technique used can detect *Giardia* cysts in frozen samples, the number of freeze-thaw cycles likely had a negative effect on the quantity of recovered cysts from the fecal samples (Erlandsen et al., 1990). After three freeze-thaw cycles, a loss of approximately 22-27% of *Giardia muris* cysts in high-concentration fecal samples (>4.6 × 10^5/ml) and a 70-80% loss at the lower cyst concentration (<9 ×10^4/ml) have been reported. In this study, the sugar concentration technique was applied to the frozen samples, which relies on the ability of the cysts or oocysts of these organisms to float. If freezing disrupts the cyst or oocyst wall, *Giardia* and *Cryptosporidium* infection could be underestimated, since the cysts/oocysts would fail to float. Therefore, evaluating concentration techniques for use on frozen samples would be beneficial.

3.5 Tables

Table 3.1 Proportion of fecal samples from dogs and cats in Chiang Mai, Thailand included in the current study distributed by month of collection.

		Dog % (n)	Cat % (n)
Rainy	2009		
	August	12.6 (38)	
	September	22.3 (67)	25.8 (17)
	October	20.6 (62)	10.6 (7)
Winter	November	13.3 (40)	15.2 (10)
	December	7.3 (22)	16.7 (11)
	2010		
	January	10.6 (32)	6.1 (4)
	February	13.3 (40)	25.8 (17)
		100 (301)	100 (66)

Table 3.2 Proportion of fecal samples from dogs and cats in Chiang Mai, Thailand distributed by housing type of animals.

Housing type	Dog % (n)	Cat % (n)
Household	93.4 (281)	80.3 (53)
Shelter	5.3 (16)	-
Breeding farm	1 (3)	19.7 (13)
Temple	0.3 (1)	-
	100 (301)	100 (66)

Table 3.3 Prevalence of *Giardia* and *Cryptosporidium* infection in dogs in Chiang Mai, Thailand distributed by district of sample collected.

District	% of all samples (n)	Prevalence of *G. duodenalis* % (n)	Prevalence of *Cryptosporidium* spp. % (n)
Doi Lor	0.3 (1)	0 (0)	0 (0)
Doi Sa Ket	10.0 (30)	10 (3)	6.7 (2)
Fang	0.3 (1)	0 (0)	0 (0)
Hang Dong	7.6 (23)	47.8 (11)	13.0 (3)
Jom Thong	0.3 (1)	100 (1)	0 (0)
Mae On	0.3 (1)	0 (0)	0 (0)
Mae Rim	10.3 (31)	19.3 (6)	3.2 (1)
Mae Wang	0.7 (2)	0 (0)	0 (0)
Muang	58.5 (176)	26.1 (46)	8.5 (15)
Prao	0.3 (1)	0 (0)	0 (0)
San Kum Paeng	0.7 (2)	0 (0)	0 (0)
San Pa Tong	2.0 (6)	50 (3)	0 (0)
San Sai	6.3 (19)	26.3 (5)	10.5 (2)
Sarapee	2.3 (7)	14.3 (1)	0 (0)
Total	100 (301)	25.2 (76)	7.6 (23)

Table 3.4 Prevalence of *Giardia* and *Cryptosporidium* infection in cats distributed by district of sample collected.

District	% of all samples (n)	Prevalence of *G. duodenalis* % (n)	Prevalence of *Cryptosporidium* spp. % (n)
Doi Sa Ket	19.7 (13)	76.9 (10)	38.5 (5)
Hang Dong	7.6 (5)	0 (0)	0 (0)
Mae Rim	1.5 (1)	0 (0)	0 (0)
Muang	65.2 (43)	16.8 (7)	7.0 (3)
San Sai	4.6 (3)	33.3 (1)	0 (0)
Sarapee	1.5 (1)	0 (0)	0 (0)
Total	100 (66)	27.3 (18)	12.1 (8)

Table 3.5 Prevalence of intestinal parasites detected in dogs and cats in this Chiang Mai, Thailand distributed by species of parasites.

Parasite	Dog (n=301) % positive (n)	Cat (n=66) % positive (n)
At least one parasite	38.9 (117)	45.5 (30)
Giardia duodenalis	25.3 (76)	27.3 (18)
Ancylostoma spp.	12.0 (36)	3.0 (2)
Cryptosporidium spp.	7.6 (23)	12.1 (8)
Cystoisospora spp.	6.0 (18)	10.6 (7)
Toxocara canis	2.7 (8)	-
Coccidian-like	1.7 (5)	3.0 (2)
Trichuris vulpis	2.0 (6)	-
Strongyloides spp.	0.7 (2)	-
Toxascaris leonine	0.7 (2)	1.5 (1)
Toxocara cati	-	3.0 (2)
Spirometra spp.	-	4.6 (3)

Table 3.6 Factors examined for association with *Giardia* and *Cryptosporidium* infections in dogs in Chiang Mai, Thailand. (n=301).

Factor	n[ʒ]	*G. duodenalis* % positive (n)	*P* value[c]	*Cryptosporidium spp.* % positive (n)	*P* value[c]
Season			0.004		0.228
Rainy	167	31.7 (53)		6.0 (10)	
Winter	134	17.2 (23)		9.7 (13)	
Age			0.01		0.005
< 1 year	67	37.3 (25)		17.9 (12)	
1 – 7 years	161	24.8 (40)		4.9 (8)	
> 7 years	73	15.0 (11)		4.1 (3)	
Sex			0.197		0.714
Male	146	21.9 (32)		8.2 (12)	
Female	155	28.4 (44)		7.1 (11)	
Breed			0.154		0.184
Purebred	62	32.3 (20)		3.2 (2)	
Mixed	239	23.4 (56)		8.8 (21)	
Diarrhea status			0.001		0.011
Yes	67	40.3 (27)		14.93 (10)	
No	234	20.9 (49)		5.56 (13)	
Ancylostoma eggs present			0.656		0.868
Yes	36	22.2 (8)		8.3 (3)	
No	265	25.7 (68)		7.6 (20)	
Trichuris vulpis eggs present			N/A		N/A
Yes	6	0 (0)		0 (0)	
No	295	25.8 (76)		7.8 (23)	
Toxocara canis eggs present			0.114		N/A
Yes	8	50.0 (4)		0 (0)	
No	293	24.6 (72)		7.8 (23)	
Toxascarid leonina eggs present			0.422		0.745
Yes	2	50.0 (1)		8.3 (3)	
No	299	25.1 (75)		7.6 (20)	
Cystoisospora oocysts present			0.022		≅1.000
Yes	18	50.0 (9)		5.6 (1)	
No	283	23.7 (67)		7.7 (22)	
Coocidian-like oocysts present			0.603		N/A
Yes	5	40.0 (2)		0 (0)	
No	296	25.0 (74))		7.8 (23)	
Strongyloides eggs present			N/A		N/A
Yes	2	0 (0)		0 (0)	
No	299	25.4 (76)		7..8 (23)	
Cryptosporidium oocysts present			<0.001		
Yes	23	95.7 (22)			
No	278	19.4 (54)			
Giardia cysts present					<0.001
Yes	76			29.0 (22)	
No	225			0.4 (1)	
Type of Housing			0.006		0.012
Household	281	23.1 (65)		6.4 (18)	
Shelter/breeder/Temple	20	55.0 (11)		25.0 (5)	
No of animals			0.062		0.050
1	60	30.0 (18)		6.7 (4)	
2-4	106	24.5 (26)		9.4 (10)	
5-10	70	15.7 (11)		1.4 (1)	
>10	43	37.2 (16)		14.0 (6)	
Cat in the same house			0.454		≅1.000
Yes	44	20.5 (9)		6.8 (3)	
No	231	26.8 (62)		7.8 (18)	

Factor	n[3]	*G. duodenalis* % positive (n)	*P* value[c]	*Cryptosporidium spp.* % positive (n)	*P* value[c]
Free roaming			0.719		N/A
Yes	48	22.9 (11)		0 (0)	
No	234	26.1 (61)		9.0 (21)	
Defecating area			0.019		0.330
In household area	199	25.6 (51)		9.6 (19)	
Out side household area	37	8.1 (3)		2.7 (1)	
Fecal picked immediately			0.745		≅1.000
Yes	86	20.9 (18)		7 .0 (7)	
No	143	23.1 (33)		8.1 (11)	
Drinking water source			0.026		0.013
Bottled water	42	35.7 (15)		16.7 (7)	
Filtered water	13	15.4 (2)		0 (0)	
Tap water	180	20.6 (37)		4.4 (8)	
Untreated water[b]	44	38.6 (17)		13.6 (6)	
Food type			0.329		0.360
Commercial	65	32.3 (21)		9.2 (6)	
Homemade	35	25.7 (9)		11.4 (4)	
Mix	179	22.9 (41)		6.15 (11)	
Ever eaten raw meat			0.192		0.206
Yes	21	38.1 (8)		14.3 (3)	
No	255	24.3 (62)		7.1 (18)	
Regular deworming			0.674		0.607
Yes	142	24.0 (34)		7.8 (11)	
No	130	26.2 (34)		6.2 (8)	
Household ever been flooded			0.477		0.773
Yes	54	22.2 (12)		5.6 (3)	
No	215	27.0 (58)		7.9 (17)	
Chronic diarrhea reported by owner			0.001		≅1.000
Yes	17	58.8 (10)		5.9 (1)	
No	258	23.6 (61)		7.8 (20)	
Owners/Care givers Educational level			0.253		0.236
Higher school and lower	45	31.1 (14)		11.1 (5)	
Bachelor	139	28.8 (40)		8.6 (12)	
Diploma	33	18.2 (6)		0 (0)	
Master&PhD	51	31.1 (14)		5.9 (3)	
Household income (Baht)			0.935		0.522
<10,000	44	25.0 (11)		9.1 (4)	
10,000 – 25,000	64	25.0 (16)		3.1 (2)	
25,001 – 50,000	79	21.5 (17)		7.6 (6)	
> 50,000	71	25.4 (18)		8.5 (6)	

[3]In some factors the total number are less due to lack of response on the questionaire.

* 95%CI = 95% confidence interval

[a] For *Cryptosporidium* calculation n for Yes =116, n for No= 185

[b] Untreated water includes river, underground and well water.

[c]*P* value was calculated using Fisher's exact or χ^2 test whichever appropriated; N/A= not applicable.

Table 3.7 Factors examined for association with *Giardia* and *Cryptosporidium* infections in cats in Chiang Mai, Thailand. (n=66).

Factor	n[ʒ]	*G. duodenalis* no. positive (%)	*P* value[c]	*Cryptosporidium spp.* no. positive (%)	*P* value[c]
Season			0.165		N/A
Rainy	24	16.7 (4)		0 (0)	
Winter	42	33.3 (14)		19.1 (8)	
Age			0.234		≅1.000
< 1 year	20	15.0 (3)		15.0 (3)	
1 – 7 years	40	35.0 (14)		12.5 (5)	
> 7 years	6	16.7 (1)		0 (0)	
Sex			0.446		0.063
Male	38	23.7 (9)		5.3 (2)	
Female	28	32.1 (9)		21.4 (6)	
Breed			0.001		0.001
DSH	42	19.1 (8)		2.4 (1)	
Persian	16	62.5 (10)		37.5 (6)	
Siamese	8	0 (0)		12.5 (1)	
Diarrhea status			0.474		≅1.000
Yes	11	36.4 (4)		9.1 (1)	
No	55	25.5 (14)		12.7 (7)	
Ancylostoma eggs present			N/A		N/A
Yes	2	0 (0)		0 (0)	
No	64	28.1 (18)		12.5 (8)	
Spirometra eggs present			≅1.000		N/A
Yes	3	33.3 (1)		0 (0)	
No	63	27.0 (17)		12.7 (8)	
Toxocara cati eggs present			N/A		N/A
Yes	2	0 (0)		0 (0)	
No	64	28.1 (18)		12.5 (8)	
Toxascarid leonina eggs present			N/A		N/A
Yes	1	0 (0)		0 (0)	
No	65	27.7 (18)		12.3 (8)	
Cystoisospora oocysts present			≅1.000		N/A
Yes	7	28.6 (2)		0 (0)	
No	59	27.1 (16)		13.6 (8)	
Coccidian-like oocysts presence			0.474		N/A
Yes	2	50.0 (1)		(0)	
No	64	26.6 (17)		12.5 (8)	
Cryptosporidium oocysts present			0.004		
Yes	8	75.0 (6)			
No	58	20.7 (12)			
Giardia cysts present					0.004
Yes	18			33.3 (6)	
No	48			4.2 (2)	
Type of Housing			0.001		0.006
Household	53	15.1 (8)		5.7 (3)	
Breeder	13	76.9 (10)		38.5 (5)	
No of animals			<0.001		0.250
1	8	12.5 (1)		25 (2)	
2-4	11	0 (0)		0 (0)	
5-10	8	75.0 (6)		12.5 (1)	
>10	18	55. 0 (10)		27.8 (5)	
Dog in the same house			0.396		N/A
Yes	6	16.7 (1)		0 (0)	
No	41	39.0 (16)		19.5 (8)	
Free roaming			0.375		N/A
Yes	14	7.1 (1)		0 (0)	
No	20	30.0 (6)		15.0 (3)	

Factor	n[3]	*G. duodenalis* no. positive (%)	*P* value[c]	*Cryptosporidium spp.* no. positive (%)	*P* value[c]
Defecating area			0.627		0.627
In household area	12	41.7 (5)		8.3 (1)	
Litter box	10	0 (0)		20.0 (2)	
Out side household area	1	0 (0)		0 (0)	
Fecal picked immediately			0.127		N/A
Yes	6	40.0 (4)		0 (0)	
No	13	7.7 (1)		23.1 (3)	
Drinking water source			≅1.000		0.566
Bottled water	6	16.7 (1)		16.7 (1)	
Filtered water	1	0 (0)		0 (0)	
Tap water	26	23.1 (6)		7.7 (2)	
Untreated water	1	0 (0)		0 (0)	
Food type			0.738		≅1.000
Commercial	19	26.3 (5)		10.5 (2)	
Homemade	1	0 (0)		0 (0)	
Mix	14	14.3 (2)		7.14 (1)	
Ever eaten raw meat			0.384		N/A
Yes	2	50.0 (1)		0 (0)	
No	31	19.4 (6)		9.7 (3)	
Regular deworming			0.426		N/A
Yes	15	13.3 (2)		20.0 (3)	
No	19	26.3 (5)		0 (0)	
Household ever been flooded			N/A		N/A
Yes	2	0 (0)		0 (0)	
No	27	25.9 (7)		11.1 (3)	
Chronic diarrhea reported by owner			0.580		N/A
Yes	6	33.3 (2)		0 (0)	
No	28	17.9 (5)		10.7 (3)	
Owners/Care givers Educational level			0.145		≅1.000
Higher school and lower	9	11.1 (1)		11.1 (1)	
Bachelor	17	35.3 (6)		11.8 (2)	
Master&PhD	7	0 (0)		0 (0)	
Household income (Baht)			0.008		0.141
<10,000	4	0 (0)		25.0 (1)	
10,000 – 25,000	5	0 (0)		0 (0)	
25,001 – 50,000	10	20.0 (2)		20.0 (2)	
> 50,000	14	18.5 (5)		0 (0)	

[3]In some factors the total number are less due to the non-responding.
*95%CI = 95% confidence intervalT
[a] For *Cryptosporidium* calculation n for Yes =28, n for No= 38
[b] Untreated water includes river, underground and well water

Table 3.8 Multivariate logistic regression analysis of significant variables associated with *Giardia duodenalis* infection in dogs in Chiang Mai, Thailand (n=231).

Variables	Odds Ratio	95%CI*	P value
Presence of *Cryptosporidium* oocysts	157.39	17.15-1,444.43	<0.001
Shelter/Breeder/Temple	8.54	1.94-37.63	0.005
Reporting chronic diarrhea	5.85	1.52-22.53	0.010
In rainy season	5.16	1.77-15.03	0.003
Drinking untreated water[a]	3.22	1.16-8.96	0.025
Having diarrhea	2.68	1.15-6.26	0.023

***** 95% Confidence interval

[a] Untreated water includes river, underground and well water

Table 3.9 Multivariate logistic regression analysis of variables associated with *Cryptosporidium* spp. infection in dogs in Chiang Mai, Thailand (n=217).

Variables	Odds Ratio	95%CI*	P value
Age<1 year	3.55	1.76-11.73	0.002
Having diarrhea	3.06	1.17-8.00	0.022

* 95% Confidence interval

Table 3.10 Factors examined for association with *Giardia* and *Cryptosporidium* single and co-infections in dogs in Chiang Mai, Thailand. (n=301)

Factor	n[a]	No. of *Giardia* single infection	No. of *Cryptosporidium* single infection	No. of co-infection	No. of neither infection	*P* value[b]
Season						<0.001
Rainy	167	43	0	10	114	
Winter	134	11	1	12	110	
Age						0.003
< 1 year	67	23	0	12	42	
1 – 7 years	161	32	0	8	121	
> 7 years	73	9	1	2	61	
Sex						0.309
Male	146	21	1	11	113	
Female	155	33	0	11	111	
Breed						0.046
Purebred	62	18	0	2	42	
Mixed	239	36	1	20	182	
Diarrhea status						0.005
Yes	67	17	0	10	40	
No	234	37	1	12	184	
Ancylostoma eggs present						0.806
Yes	36	5	0	3	28	
No	265	49	1	19	196	
Trichuris vulpis eggs present						0.753
Yes	6	0	0	0	6	
No	295	54	1	22	218	
Toxocara canis eggs present						0.085
Yes	8	4	0	0	4	
No	293	50	1	22	220	
Toxascarid leonina eggs present						0.447
Yes	2	1	0	0	1	
No	299	53	1	22	223	
Cystoisospora oocysts present						0.032
Yes	18	8	0	1	9	
No	283	46	1	21	215	
Coocidian-like oocysts present						0.497
Yes	5	2	0	0	3	
No	296	52	1	22	221	
Strongyloides eggs present						N/A
Yes	2	0	0	0	2	
No	299	54	1	22	222	
Type of Housing						0.004
Household	281	48	1	17	215	
Shelter/breeder/Temple	20	6	0	5	9	
No of animals						0.114
1	60	14	0	4	42	
2-4	106	17	1	9	79	
5-10	70	10	0	1	59	
>10	43	10	0	6	27	

Factor	n[a]	No. of *Giardia* single infection	No. of *Cryptosporidium* single infection	No. of co-infection	No. of neither infection	*P* value[b]
Cat in the same house						0.709
Yes	44	6	0	3	35	
No	231	45	1	17	168	
Free roaming						0.102
Yes	48	11	0	0	37	
No	234	41	1	20	172	
Defecating area						0.125
In household area	199	33	1	18	147	
Out side household area	37	2	0	1	34	
Fecal picked immediately						0.707
Yes	86	12	1	6	67	
No	143	22	0	11	110	
Drinking water source						0.041
Bottled water	42	8	0	7	27	
Filtered water	13	2	0	0	11	
Tap water	180	30	1	7	142	
Untreated water[c]	44	11	0	6	27	
Food type						0.287
Commercial	65	15	0	6	44	
Homemade	35	6	1	3	25	
Mix	179	30	0	11	138	
Ever eaten raw meat						0.275
Yes	21	5	0	3	13	
No	255	45	1	17	192	
Regular deworming						0.854
Yes	142	24	1	10	107	
No	130	26	0	8	96	
Household ever been flooded						0.866
Yes	54	9	0	3	42	
No	215	42	1	16	156	
Chronic diarrhea reported by owner						0.004
Yes	17	9	0	1	7	
No	258	42	1	19	196	
Owners/Care givers						0.296
Educational level						
Higher school and lower	45	10	1	4	30	
Bachelor	139	28	0	12	99	
Diploma	33	6	0	0	27	
Master&PhD	51	6	0	3	42	
Household income (Baht)						0.672
<10,000	44	8	1	3	32	
10,000 – 25,000	64	14	0	2	48	
25,001 – 50,000	79	11	0	6	62	
> 50,000	71	12	0	6	53	

[a] In some factors the total number are less due to the non-responding.
[b] *P* value were calculated based on Fisher's exact test.
[c] Untreated water includes river, underground and well water.

Table 3.11 Odds ratios of risk factors examined for association with *Giardia* and *Cryptosporidium* single and co-infections in dogs in Chiang Mai, Thailand.

Factor	*Giardia* single infection OR[a] (95%CI[b])	*Cryptosporidium* single infection OR[a,c] (95%CI[b])	Co-infection OR[a] (95%CI[b])
Season			
Rainy	3.77 (1.85-7.69)	N/A	0.80 (0.33-1.94)
Winter	Ref		Ref
Age			
< 1 year	2.10 (0.82-5.35)	N/A	8.71 (1.85-40.96)
1 – 7 years	1.79 (0.80-3.99)		2.02 (0.42-9.79)
> 7 years	Ref		Ref
Sex			
Male	0.63 (0.34-1.15)	N/A	0.98 (0.41-2.36)
Female	Ref		Ref
Breed			
Purebred	2.17 (1.12-4.18)	N/A	0.433 (0.10-1.92)
Mixed	Ref		Ref
Diarrhea status			
Yes	2.11 (1.08-4.12)	N/A	3.83 (1.55-9.49)
No	Ref		Ref
Ancylostoma eggs present			
Yes	0.71 (0.26-1.95)	N/A	1.11 (0.31-3.98)
No	Ref		Ref
Trichuris vulpis eggs present			
Yes	N/A	N/A	N/A
No			
Toxocara canis eggs present			
Yes	4.4 (1.06-18.19)	N/A	N/A
No	Ref		
Toxascarid leonina eggs present			
Yes	4.21 (0.26-68.36)	N/A	N/A
No	Ref		
Cystoisospora oocysts present			
Yes	4.15 (1.52-11.34)	N/A	1.14 (0.14-9.4)
No	Ref		Ref
Coocidian-like oocysts present			
Yes	2.83 (0.46-17.39)	N/A	N/A
No	Ref		
Strongyloides eggs present			
Yes	N/A	N/A	N/A
No			
Type of Housing			
Household	Ref		Ref
Shelter/breeder/Temple	2.99 (1.01-8.79)	N/A	7.03 (2.12-23.3)
No of animals			
1	Ref	N/A	Ref
2-4	0.65 (0.29-1.44)		1.20 (0.35-4.12)
5-10	0.51 (0.21-1.25)		0.18 (0.02-1.65)
>10	1.11 (0.43-2.86)		2.33 (0.60-9.04)
Cat in the same house			
Yes	0.64 (0.25 – 1.62)	N/A	0.85 (0.24-3.05)
No	Ref		Ref
Free roaming			
Yes	1.25 (0.59-2.65)	N/A	N/A
No	Ref		

Factor	*Giardia* single infection OR[a] (95%CI[b])	*Cryptosporidium* single infection OR[a,c] (95%CI[b])	Co-infection OR[a] (95%CI[b])
Defecating area			
In household area	3.82 (0.87-16.68)	N/A	4.16 (0.54-32.27)
Out side household area	Ref		Ref
Fecal picked immediately			
Yes	0.90 (0.42-1.93)	N/A	0.90 (0.32-2.53)
No	Ref		Ref
Drinking water source			
Bottled water	Ref	N/A	Ref
Filtered water	0.61 (0.11-3.36)		N/A
Tap water	0.71 (0.30-1.72)		0.19 (0.06-0.59)
Untreated water[e]	1.38 (0.48-3.95)		0.86 (0.25-2.88)
Food type			
Commercial	Ref	N/A	Ref
Homemade	0.70 (0.24-2.05)		0.88 (0.20-3.83)
Mix	0.64 (0.31-1.29)		0.58 (0.20-1.67)
Ever eaten raw meat			
Yes	1.64 (0.56-4.84)	N/A	2.61 (0.68-10.05)
No	Ref		Ref
Regular deworming			
Yes	0.83 (0.45-1.54)	N/A	1.12 (0.43-2.96)
No	Ref		Ref
Household ever been flooded			
Yes	0.80 (0.36-1.77)	N/A	0.70 (0.19-2.50)
No	Ref		Ref
Chronic diarrhea reported by owner			
Yes	6.0 (2.12-17.0)	N/A	1.47 (0.17-12.62)
No	Ref		Ref
Owners/Care givers Educational level			
Higher school and lower	1.98 (0.76-5.13)	N/A	1.70 (0.46-6.32)
Diploma	1.56 (0.45-5.32)		N/A
Bachelor	2.33 (0.76-7.12)		1.87 (0.39-8.96)
Master&PhD	Ref		Ref
Household income (Baht)			
<10,000	1.10 (0.41-2.99)	N/A	0.83 (0.19-3.54)
10,000 – 25,000	1.29 (0.54-3.06)		0.37 (0.07-1.91)
25,001 – 50,000	0.78 (0.32-1.92)		0.85 (0.26-2.81)
> 50,000	Ref		Ref

[a] OR=odds ratio, calculate using neither *Giardia* or *Cryptosporidium* infections as a reference group
[b]95%CI=95% confidence interval
[c]OR for *Cryptosporidium* single infection was not applicable due to the single isolate in this category.
[d]N/A= not applicable
[e]Untreated water includes river, underground and well water.

Table 3.12 Multivariate logistic regression analysis of significant variables associated with *Giardia* infection alone in dogs in Chiang Mai, Thailand (n=212).

Variables	Odds Ratio	95%CI*	P value
Shelter/Breeder/Temple	9.86	2.30-42.34	0.002
Reporting chronic diarrhea	5.37	1.40-20.60	0.014
In rainy season	3.82	1.37-10.70	0.011
Having diarrhea	2.50	1.07-5.85	0.034

* 95% Confidence interval

Table 3.13 Multivariate logistic regression analysis of significant variables associated with *Giardia* and *Cryptosporidium* co-infection alone in dogs in Chiang Mai, Thailand (n=214).

Variables	Odds Ratio	95%CI*	P value
Shelter/Breeder/Temple	6.00	1.53-23.57	0.010
Age < 1 year	5.67	2.02-15.9	0.001
Having diarrhea	3.22	1.15-9.03	0.026

* 95% Confidence interval

3.6 Figures

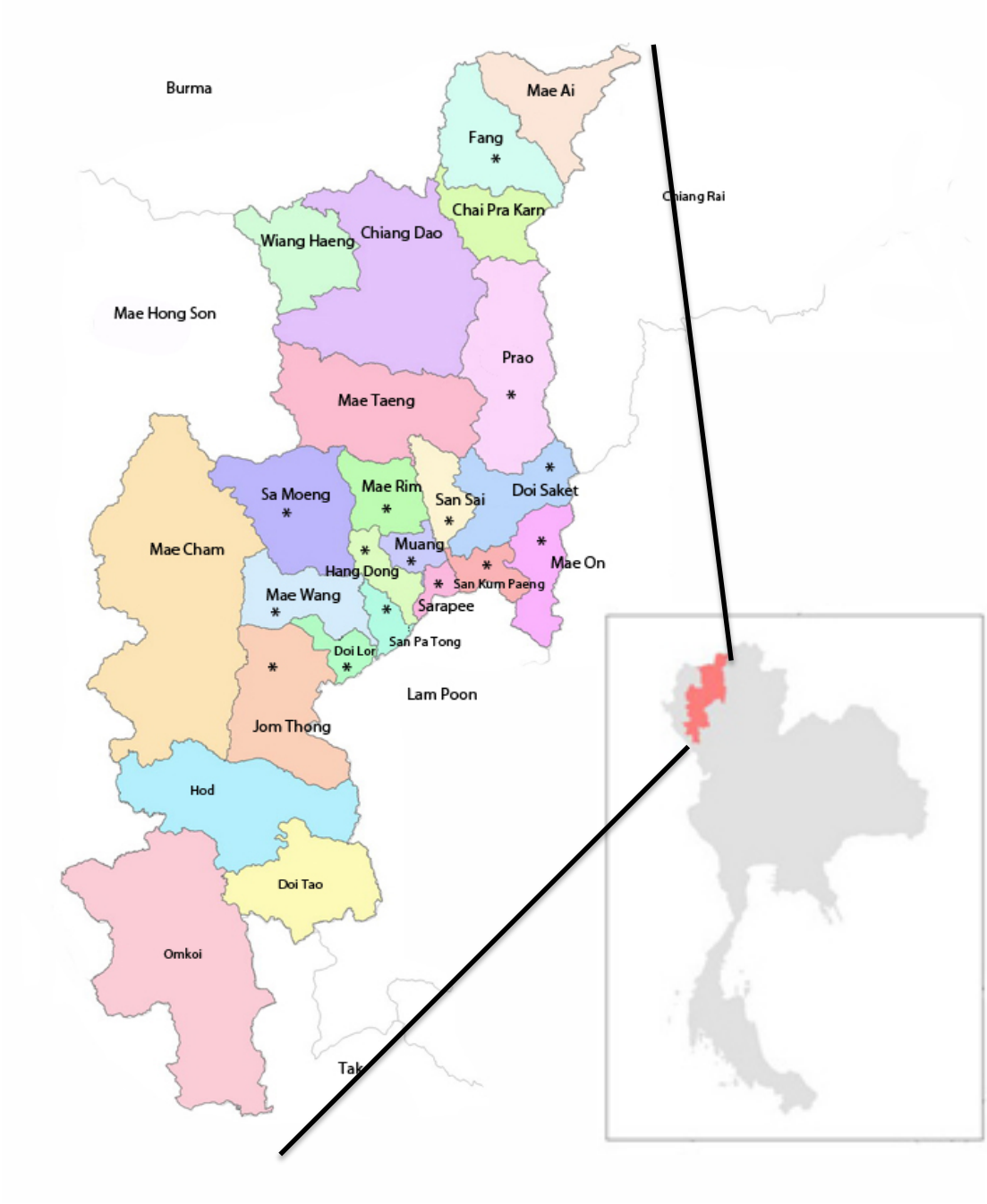


Figure 3.1 Map of Chiang Mai, Thailand, Asterisk indicated the sources of samples include in this study.

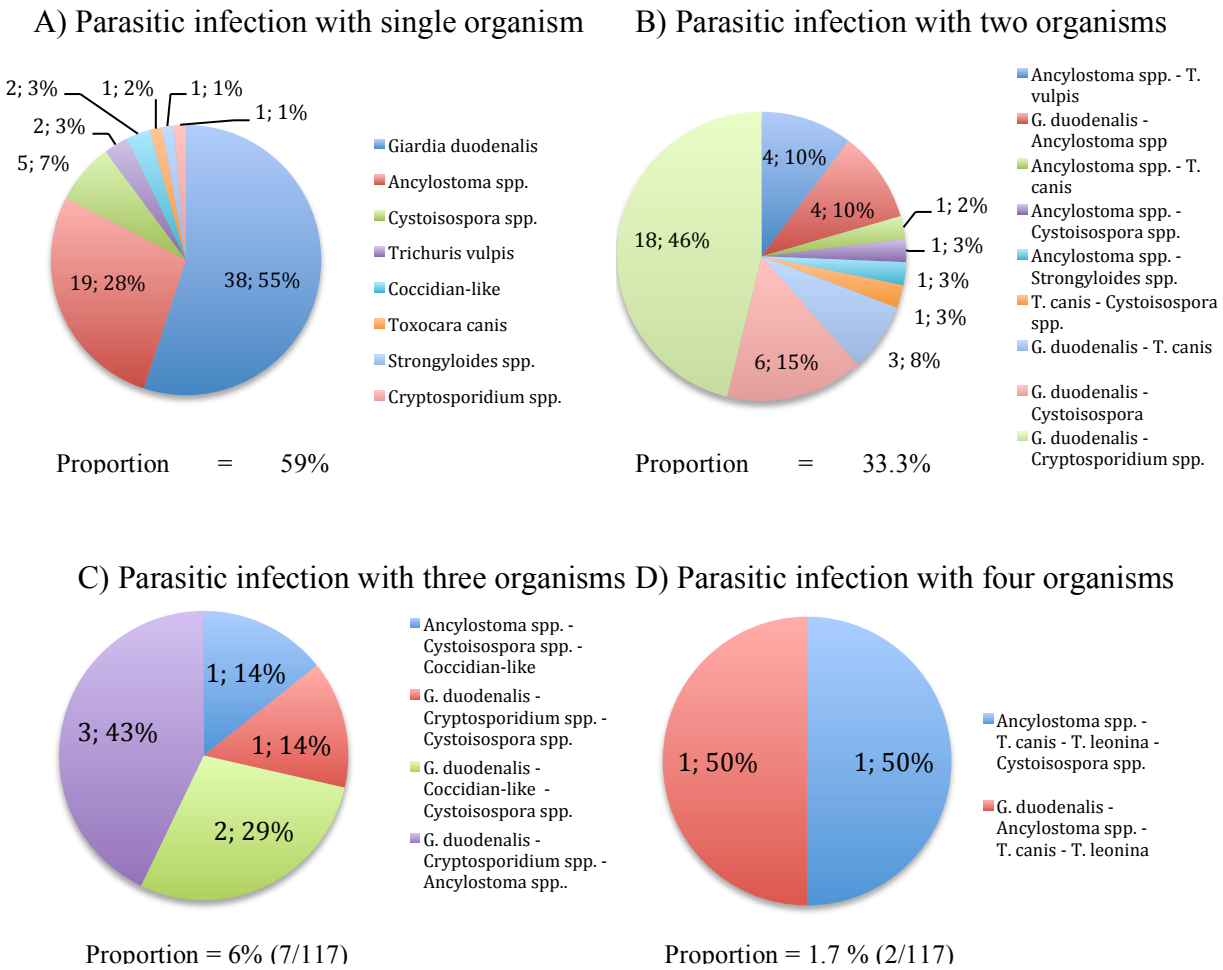
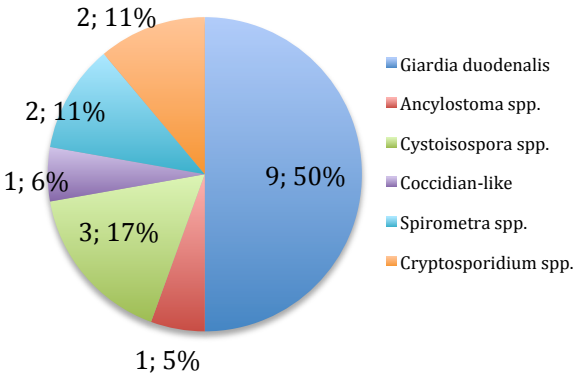


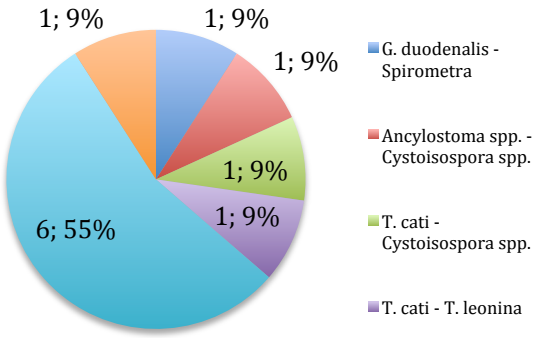
Figure 3.2 Parasitic co-infection among 117 infected dogs in Chiang Mai, Thailand

A) Parasitic infection with one organism



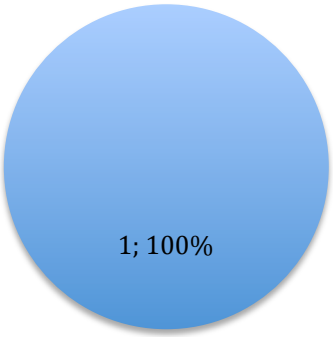
Proportion = 60% (18/30)

B) Parasitic infection with two organisms



Proportion = 36.7% (11/30)

C) Parasitic infection with three organisms



G. duodenalis - Coccidian-like - Cystoisospora spp.

Proportion = 3.3% (1/30)

Figure 3.3 Parasitic co-infection among 30 infected cats in Chiang Mai, Thailand.

3.7 References

Alvarez-Pellitero, P., Quiroga, M.I., Sitja-Bobadilla, A., Redondo, M.J., Palenzuela, O., Padros, F., Vazquez, S., Nieto, J.M., 2004. *Cryptosporidium scophthalmi* n. sp. (Apicomplexa: Cryptosporidiidae) from cultured turbot *Scophthalmus maximus*. Light and electron microscope description and histopathological study. Dis. Aquat. Organ. 62, 133-145.

Alvarez-Pellitero, P., Sitja-Bobadilla, A., 2002. *Cryptosporidium molnari* n. sp. (Apicomplexa: Cryptosporidiidae) infecting two marine fish species, *Sparus aurata* L. and *Dicentrarchus labrax* L. Int. J. Parasitol. 32, 1007-1021.

Baldursson, S., Karanis, P., 2011. Waterborne transmission of protozoan parasites: review of worldwide outbreaks - an update 2004-2010. Water Res 45, 6603-6614.

Ballweber, L.R., Panuska, C., Huston, C.L., Vasilopulos, R., Pharr, G.T., Mackin, A., 2009. Prevalence of and risk factors associated with shedding of *Cryptosporidium felis* in domestic cats of Mississippi and Alabama. Vet. Parasitol. 160, 306-310.

Batchelor, D.J., Tzannes, S., Graham, P.A., Wastling, J.M., Pinchbeck, G.L., German, A.J., 2008. Detection of endoparasites with zoonotic potential in dogs with gastrointestinal disease in the UK. Transbound Emerg Dis 55, 99-104.

Buret, A.G., Scott, K.G., Chin, A.C., 2002. Giaridasis: Pathophysiology and Pathogenesis, In: *Giardia* The Cosmopolitan Parasite. CABI Publishing, New York, NY.

Chiang Mai Provincial Office 2012. Chiang Mai Database (http://www.chiangmai.go.th/meet_file/sarupCM.pdf).

Dib, H.H., Lu, S.Q., Wen, S.F., 2008. Prevalence of *Giardia lamblia* with or without diarrhea in South East, South East Asia and the Far East. Parasitol. Res. 103, 239-251.

Dohoo, I., Martin, W., Stryhn, H., 2007. Veterinary epidemiologic research, 2nd Edition. AVC, Inc., Charlotte, P.E.I., Canada.

Erlandsen, S.L., Sherlock, L.A., Bemrick, W.J., 1990. The detection of *Giardia muris* and *Giardia lamblia* cysts by immunofluorescence in animal tissues and fecal samples subjected to cycles of freezing and thawing. J. Parasitol. 76, 267-271.

Fayer, R., 2008. General Biology, In: Fayer, R., Xiao, L. (Eds.) *Cryptosporidium* and cryptosporidiosis. CRC Press, Boca Raton, pp. 1-42.

Fayer, R., 2010. Taxonomy and species delimitation in *Cryptosporidium*. Exp. Parasitol. 124, 90-97.

Fayer, R., Santin, M., 2009. *Cryptosporidium xiaoi* n. sp. (Apicomplexa: Cryptosporidiidae) in sheep (*Ovis aries*). Vet. Parasitol. 164, 192-200.

Fayer, R., Santin, M., Macarisin, D., 2010. *Cryptosporidium ubiquitum* n. sp. in animals and humans. Vet. Parasitol. 172, 23-32.

Feng, Y., Xiao, L., 2011. Zoonotic potential and molecular epidemiology of *Giardia* species and giardiasis. Clin. Microbiol. Rev. 24, 110-140.

Fleiss, J.L., 2003. Statistical methods for rates and proportions. Wiley-Interscience, Hoboken, N.J.

Fontanarrosa, M.F., Vezzani, D., Basabe, J., Eiras, D.F., 2006. An epidemiological study of gastrointestinal parasites of dogs from Southern Greater Buenos Aires (Argentina): age, gender, breed, mixed infections, and seasonal and spatial patterns. Vet. Parasitol. 136, 283-295.

Gates, M.C., Nolan, T.J., 2009. Risk factors for endoparasitism in dogs: retrospective case-control study of 6578 veterinary teaching hospital cases. J. Small Anim. Pract. 50, 636-640.

Itoh, N., Kanai, K., Tominaga, H., Kawamata, J., Kaneshima, T., Chikazawa, S., Hori, Y., Hoshi, F., Higuchi, S., 2011. *Giardia* and other intestinal parasites in dogs from veterinary clinics in Japan. Parasitol. Res. 109, 253-256.

Katagiri, S., Oliveira-Sequeira, T.C., 2008. Prevalence of dog intestinal parasites and risk perception of zoonotic infection by dog owners in Sao Paulo State, Brazil. Zoonoses Public Health 55, 406-413.

Lane, S., Lloyd, D., 2002. Current trends in research into the waterborne parasite *Giardia*. Crit. Rev. Microbiol. 28, 123-147.

Lasek-Nesselquist, E., Welch, D.M., Sogin, M.L., 2010. The identification of a new *Giardia duodenalis* assemblage in marine vertebrates and a preliminary analysis of *G. duodenalis* population biology in marine systems. Int. J. Parasitol. 40, 1063-1074.

Monis, P.T., Caccio, S.M., Thompson, R.C., 2009. Variation in *Giardia*: towards a taxonomic revision of the genus. Trends Parasitol 25, 93-100.

Morgan, U.M., Monis, P.T., Xiao, L., Limor, J., Sulaiman, I., Raidal, S., O'Donoghue, P., Gasser, R., Murray, A., Fayer, R., Blagburn, B.L., Lal, A.A., Thompson, R.C., 2001. Molecular and phylogenetic characterisation of *Cryptosporidium* from birds. Int. J. Parasitol. 31, 289-296.

Mundim, M.J., Rosa, L.A., Hortencio, S.M., Faria, E.S., Rodrigues, R.M., Cury, M.C., 2007. Prevalence of *Giardia duodenalis* and *Cryptosporidium* spp. in dogs from different living conditions in Uberlandia, Brazil. Vet. Parasitol. 144, 356-359.

O'Handley, R.M., Olson, M.E., Fraser, D., Adams, P., Thompson, R.C., 2000. Prevalence and genotypic characterisation of *Giardia* in dairy calves from Western Australia and Western Canada. Vet. Parasitol. 90, 193-200.

O'Reilly, C.E., Bowen, A.B., Perez, N.E., Sarisky, J.P., Shepherd, C.A., Miller, M.D., Hubbard, B.C., Herring, M., Buchanan, S.D., Fitzgerald, C.C., Hill, V., Arrowood, M.J., Xiao, L.X., Hoekstra, R.M., Mintz, E.D., Lynch, M.F., 2007. A waterborne outbreak of gastroenteritis with multiple etiologies among resort island visitors and residents: Ohio, 2004. Clin. Infect. Dis. 44, 506-512.

Pozio, E., 2003. Foodborne and waterborne parasites. Acta Microbiol. Pol. 52 Suppl, 83-96.

Rambozzi, L., Menzano, A., Mannelli, A., Romano, S., Isaia, M.C., 2007. Prevalence of cryptosporidian infection in cats in Turin and analysis of risk factors. J Feline Med Surg 9, 392-396.

Read, C.M., Monis, P.T., Thompson, R.C., 2004. Discrimination of all genotypes of *Giardia duodenalis* at the glutamate dehydrogenase locus using PCR-RFLP. Infect Genet Evol 4, 125-130.

Ren, X., Zhao, J., Zhang, L., Ning, C., Jian, F., Wang, R., Lv, C., Wang, Q., Arrowood, M.J., Xiao, L., 2012. *Cryptosporidium tyzzeri* n. sp. (Apicomplexa: Cryptosporidiidae) in domestic mice (*Mus musculus*). Exp. Parasitol. 130, 274-281.

Robinson, G., Wright, S., Elwin, K., Hadfield, S.J., Katzer, F., Bartley, P.M., Hunter, P.R., Nath, M., Innes, E.A., Chalmers, R.M., 2010. Re-description of *Cryptosporidium cuniculus* (Apicomplexa: Cryptosporidiidae): Morphology, biology and phylogeny. Int. J. Parasitol.

Santin, M., 2013. Clinical and subclinical infections with *Cryptosporidium* in animals. N. Z. Vet. J. 61, 1-10.

Scorza, A.V., Brewer, M.M., Lappin, M.R., 2003. Polymerase chain reaction for the detection of *Cryptosporidium* spp. in cat feces. J. Parasitol. 89, 423-426.

Scorza, A.V., Lappin, M.R., 2007. Co-infection of *Cryptosporidium* and *Giardia* in naturally infected cats. In Diagnosis and Treatment of Cryptosporidiosis and Giardiasis in Cats and Dogs in the United staes. Colorado State University, Fort Collins.

Scorza, V., Tangtrongsup, S., 2010. Update on the diagnosis and management of *Cryptosporidium* spp infections in dogs and cats. Top Companion Anim Med 25, 163-169.

Smith, A., Reacher, M., Smerdon, W., Adak, G.K., Nichols, G., Chalmers, R.M., 2006. Outbreaks of waterborne infectious intestinal disease in England and Wales, 1992-2003. Epidemiol. Infect. 134, 1141-1149.

Srisuphanunt, M., Saksirisampant, W., Karanis, P., 2011. Prevalence and genotyping of *Cryptosporidium* isolated from HIV/AIDS patients in urban areas of Thailand. Ann. Trop. Med. Parasitol. 105, 463-468.

Tangtrongsup, S., Scorza, V., 2010. Update on the diagnosis and management of *Giardia* spp infections in dogs and cats. Topics in companion animal medicine 25, 155-162.

Traub, R.J., Inpankaew, T., Reid, S.A., Sutthikornchai, C., Sukthana, Y., Robertson, I.D., Thompson, R.C., 2009. Transmission cycles of *Giardia duodenalis* in dogs and humans in Temple communities in Bangkok--a critical evaluation of its prevalence using three diagnostic tests in the field in the absence of a gold standard. Acta Trop. 111, 125-132.

Traub, R.J., Monis, P.T., Robertson, I., Irwin, P., Mencke, N., Thompson, R.C., 2004. Epidemiological and molecular evidence supports the zoonotic transmission of *Giardia* among humans and dogs living in the same community. Parasitology 128, 253-262.

Tzipori, S., Ward, H., 2002. Cryptosporidiosis: biology, pathogenesis and disease. Microbes Infect 4, 1047-1058.

Vasilopulos, R.J., Mackin, A.J., Rickard, L.G., Pharr, G.T., Huston, C.L., 2006. Prevalence and factors associated with fecal shedding of *Giardia* spp. in domestic cats. J. Am. Anim. Hosp. Assoc. 42, 424-429.

Wongstitwilairoong, B., Srijan, A., Serichantalergs, O., Fukuda, C.D., McDaniel, P., Bodhidatta, L., Mason, C.J., 2007. Intestinal parasitic infections among pre-school children in Sangkhlaburi, Thailand. Am. J. Trop. Med. Hyg. 76, 345-350.

Xiao, L., Fayer, R., 2008. Molecular characterisation of species and genotypes of *Cryptosporidium* and *Giardia* and assessment of zoonotic transmission. Int. J. Parasitol. 38, 1239-1255.

Yoder, J.S., Gargano, J.W., Wallace, R.M., Beach, M.J., Centers for Disease, C., Prevention, 2012a. Giardiasis surveillance--United States, 2009-2010. MMWR. Surveill. Summ. 61, 13-23.

Yoder, J.S., Wallace, R.M., Collier, S.A., Beach, M.J., Hlavsa, M.C., Centers for Disease, C., Prevention, 2012b. Cryptosporidiosis surveillance--United States, 2009-2010. MMWR. Surveill. Summ. 61, 1-12.

CHAPTER 4: A COMPARISON OF IFA DETECTION OF *GIARDIA* AND *CRYPTOSPORIDIUM* INFECTION AFTER TWO FECAL CONCENTRATION TECHNIQUES IN FROZEN FECAL SAMPLES[*]

Summary

The current study was conducted to determine whether a sugar concentration technique was appropriate for previously frozen fecal samples prior immunofluorescent assay (IFA) to detect *Giardia* and *Cryptosporidium* infections. Included in this study were 252 fecal samples from dogs and 61 fecal samples from cats. All fecal samples had been previously tested using fecal flotation (on fresh samples) and IFA after sugar concentration (with one freeze-thaw cycle). All fecal samples were concentrated using sedimentation technique after a second freeze-thaw cycle and IFA were applied. McNemar's test was used to determine the difference of proportion of *Giardia* and *Cryptosporidium* IFA-positive results from both concentration techniques. The agreements of IFA-positive results of *Giardia* and *Cryptosporidium* detections after sugar concentration and sedimentation were further analyzed using kappa statistics. The proportion of *Giardia*-positive results from dogs and cats were 12.3% and 13.1%, respectively, after sedimentation, compared to 9.5% and 13.1% after sugar concentration. The proportion of *Cryptosporidium*-positive results from dogs and cats after sedimentation were 10.7% and 13.1%, respectively, compared to 8.3% and 8.2% after sugar concentration. The exact McNemar's test revealed that *Giardia* and *Cryptosporidium* IFA-positive results in dogs and cats from the two

[*]Sahatchai Tangtrongsup[1], A. Valeria Scorza[2], John S. Reif[3], Lora R. Ballweber[4], Michael R. Lappin[2], Mo D. Salman[1], *[1]Animal Population Health Institute, and [2]Center for Companion Animal Studies, Department of Clinical Sciences, [3]Department of Clinical Sciences, [3]Department of Environmental and Radiological Health Sciences, and [4]Department of Microbiology, Immunology and Pathology, College of Veterinary Medicine and Biomedical Sciences, Colorado State University, Fort Collins, CO 80523,* This chapter is formatted for the requirements of Journal of Veterinary Diagnostic Investigation.

concentration techniques were not significantly different ($p>0.05$). Kappa analyses revealed substantial agreement for *Giardia* detection (kappa 0.654, $p<0.01$) and *Cryptosporidium* detection (kappa 0.632, $p<0.01$) in dogs. In cats, high agreements were observed in *Giardia* detection (kappa 0.843, $p<0.01$) and *Cryptosporidium* detection (kappa 0.920, $p<0.01$) after the two concentration techniques. Although there were no significant differences between the two concentration techniques, sedimentation is recommended for concentrating frozen fecal samples.
Keywords: Dogs; Cats; Fecal concentration; Sugar flotation; Sedimentation; Frozen feces; *Giardia*: *Cryptosporidium*

Giardia duodenalis and *Cryptosporidium* spp. are common causes of gastrointestinal diseases in humans and animals worldwide. These protozoans can be transmitted via the fecal-oral route and lead to severe disease in immunocompromised individuals, especially in young, old or HIV-infected individuals, and both are considered as potential zoonotic agents[33]. The prevalence of *Giardia* and *Cryptosporidium* infections in dogs or cats are frequently reported as 5%-15% [4,6,7,10,14-17,20,22]; however, it can be as high as 57% for *Giardia*[34] and 28% for *Cryptosporidium*[3]. Giardiasis and cryptosporidiosis can be diagnosed by the presence of *Giardia* cysts or *Cryptosporidium* oocysts in the fecal samples using light microscopy, through immunofluorescent assay (IFA), the detection of antigen using ELISAs, or DNA using the polymerase chain reaction technique. Zinc sulfate centrifugal flotation with iodine staining (for *Giardia* cysts) or modified Ziehl-Neelsen (mZN) staining (for *Cryptosporidium* oocysts) have normally been used for screening for these parasites[29,30]; however, false negative results are likely because of the small size of the *Giardia* cyst and *Cryptosporidium* oocysts. The direct immunofluorescence assay (Merifluor® *Cryptosporidium*/*Giardia* kit[a]) has been commercially available and is now widely used. This technique uses a mixture of fluorescein isothiocyanate

(FITC)-labeled monoclonal antibodies directed against cell wall antigens of *Giardia* cysts and *Cryptosporidium* oocysts. Used with human fecal samples, this test has 100% sensitivity and 99.8-100% specificity for *Giardia* detection and 93-100% sensitivity and 100% specificity for *Cryptosporidium* detection[11,13,19,38]. Because stained cysts and oocysts can be confirmed by morphology a false positive is unlikely, so this technique has been adopted as a reference test in humans[1,11,12,18]. With the high sensitivity and specificity of the IFA test in dogs and cats, some researchers have considered IFA as a gold standard test[23,27]. The nature of this test requires observing at least one oocyst of *Cryptosporidium* or a *Giardia* cyst on the testing slide. A study of human stool samples that were spiked with *Cryptosporidium* oocysts revealed that in watery stool specimens, oocysts were detected by IFA technique in 90% of specimens containing 5,000 oocysts per gram of stool, and this increased to 100% of specimens containing 10,000 oocysts per gram of stool. In formed stool specimens, the IFA detected 60% of samples with 10,000 oocysts per gram of stool and 100% of specimens seeded with 50,000 oocysts per gram of stool[37]. In cat fecal samples seeded with known numbers of *Cryptosporidium* oocysts, it was shown that 76% of fecal samples seeded with 10,000 oocysts per gram feces and 100% of samples with 100,000 oocysts were detected[28]. In a study of dogs, the detection limit for IFA was at the level of 100,000 cysts or oocysts per gram for both *Giardia* and *Cryptosporidium*[26]. It should be noted that in the human and dog study the formalin-ethyl acetate (FEA) method was used on spiked fecal samples to concentrate the feces prior to IFA tests; however, with different amounts of feces. The human fecal concentrated samples were prepared from 5 grams of feces, whereas the dog fecal concentrates were prepared from 2 grams [26,37]. For the cat study the *Cryptosporidium* oocysts detection using IFA were performed on unprocessed fecal samples[28].

Sykes et al. (1989) found that young dogs with *Giardia* infection shed an average of 2,000 cysts per gram of feces, and the mean cyst count per gram of feces for infected dogs of all ages was 705.8[31]. Another study reported that infected dogs shed between 26 and 114,486 cysts per gram of feces[5]. In cats, the shedding of *Giardia* cysts may fluctuate from undetectable to concentrations of >1,000,000 cysts per gram of feces[21]. In naturally-infected cats the mean number of *Cryptosporidium* oocysts shed in feces with and without diarrhea were 1,817 and 191 oocysts per gram, respectively[35]. Therefore, the *Giardia* and *Cryptosporidium* IFA test applied on an unprocessed fecal sample may yield a false negative result in specimens that contain low concentrations of either *Giardia* cysts or *Cryptosporidium* oocysts.

In the sugar flotation technique the ability of *Giardia* cysts and *Cryptosporidium* oocysts to float is solely dependent on their intact cyst/oocyst walls, whereas sedimentation is not affected by wall damage. The advantage of sugar flotation, however, is the cleaner final concentrated sample – less debris or fecal material – compared to the sedimentation technique, thus allowing the technician to more easily identify cysts or oocysts on the testing slides. IFA testing on fecal sediment was believed to be difficult due to masking by the remaining fecal material, but detection may actually be enhanced, as fewer organisms are lost during the concentration process. Therefore, this study was conducted on frozen fecal samples to determine whether the sugar concentration technique was appropriate to use prior to IFA detection of *Giardia* and *Cryptosporidium* as compared to the sedimentation technique. We hypothesized that IFA results after sugar concentration or sedimentation technique are comparable, and that the sugar concentration technique is appropriate to use with frozen fecal samples.

Freezing has long been used for fecal storage and is sometimes necessary for international shipment. When the freeze-thaw cycle is applied to fecal samples, however, the

Giardia cysts or *Cryptosporidium* oocysts may deteriorate[2,8]. In one study, IFA was used to detect *Giardia muris* and *Giardia lamblia* cysts in animal tissues and fecal samples that had been subjected to multiple freeze-thaw cycles[8]. After three freeze-thaw cycles, losses of approximately 22-27% of *Giardia muris* cysts in high-concentration fecal samples (>4.6 × 10^5/ml) and 70-80% at the lower cyst concentration (<9 ×10^4/ml) occurred. Given the potential loss and low starting numbers, a fecal concentration technique on samples prior to IFA testing may be recommended to increase the sensitivity of detection. Concentration methods such as the sedimentation technique[28,29] and sugar flotation techniques[25,26,36] have been studied and are in common use.

The fecal samples used in this experiment were originally collected from dogs and cats between August 2009 and February 2010 as part of an investigation of the prevalence and risk factors of *Giardia* and *Cryptosporidium* in these animals in Chiang Mai, Thailand. In that experiment, zinc sulfate flotation for parasite eggs was performed on fresh samples and the remaining feces were stored at -20°C. The frozen fecal samples were shipped on dry ice to the United States and stored at -70°C. On the day of IFA assay, the fecal samples were thawed and concentrated with the sugar concentration technique as previously described[25,36] A thin fecal smear was made on IFA slides (Merifluor® *Cryptosporidium*/*Giardia* IFA kit[a]) and the slides were processed in accordance with manufacturer's instructions. On that same day the fecal concentrates were stored at -20°C.

The original fecal samples were maintained at -70°C (9-11 months) until they were used in the experiment described here. The samples available for this study included 252 fecal samples from dogs and 61 fecal samples from cats. The fecal samples were thawed at room temperature (18°C) and were concentrated using fecal sedimentation technique. Briefly, three

grams of feces were mixed thoroughly in 4.5 ml PBS-EDTA then strained with surgical gauze. The strained mixture was transferred to a clean 15 ml tube. The mixture was centrifuged at 1200g for 10 minutes and the supernatant was discarded. The pellet was then washed with 12 ml PBS-EDTA, centrifuged at 1200g for 10 minutes and the supernatant was discarded. The pellet was washed a second time with 7 ml PBS-EDTA, centrifuged at 1200g for 10 minutes and the supernatant was discarded. The pellet was re-suspended with 1 ml PBS-EDTA. The IFA was performed on the same day of fecal concentration and the remaining fecal concentrates were stored at -20°C.

The results for *Giardia* and *Cryptosporidium* in dogs and cats for the two fecal preparations, sugar flotation and sedimentation, were analyzed using the exact McNemar's test[9]. Kappa statistics were used to determine agreement of the two different fecal preparations[9]. A P value of less than 0.05 was considered as significant. All statistical analyses were performed using the Stata statistical software release 10.1[b].

Proportions of *Giardia* and *Cryptosporidium* infections in dogs and cats are shown in Table 4.1. *Giardia* detection in dogs using IFA after sedimentation was 12.3% compared to 9.5% from IFA after sugar concentration. *Cryptosporidium* IFA results after sedimentation in dogs and cats were 10.7% and 13.1%, respectively, compared to 8.3% and 8.2% after sugar concentration. The contingency table of *Giardia* and *Cryptosporidium* detection after the two fecal concentrations are shown in Tables 4.2 and 4.3. The exact significance of McNemar's tests showed no difference in the proportion of IFA *Giardia*- and *Cryptosporidium*-positive results after sugar concentration and sedimentation in canine and feline frozen feces ($p>0.05$). For canine fecal samples, kappa statistics revealed substantial agreement between IFA results after sugar concentration and sedimentation techniques. Almost perfect agreement between IFA

results after sugar concentration and sedimentation techniques was observed on feline fecal samples.

It has been suggested that fecal samples submitted for parasitological diagnosis should not be frozen[32] as the freeze-thaw can affect the morphology, making recognition of parasitic eggs, cysts or oocysts more difficult and possibly leading to false negative results. However, freezing of samples is unavoidable in some circumstances such as the need for long-term storage or international shipment. It has been reported that freeze-thaw did not affect the IFA detection[24] of *Giardia* cysts in non-fixed long-term stored human feces; however, the freeze-thaw can deteriorate the *Giardia* cysts, and lower concentrations of *Giardia* recovered from the frozen fecal samples resulted in lower test sensitivity[8]. In the current study, we compared the detection of *Giardia* and *Cryptosporidium* using IFA after two concentration techniques, sugar flotation and sedimentation, in frozen dog and cat fecal samples. The results of IFA testing after sedimentation gave a higher proportion of detection for *Giardia* and for *Cryptosporidium* in canine fecal samples, and a lower proportion of detection for *Cryptosporidium* in feline fecal samples as compared to sugar concentration; however, there was no significant difference detected in these comparisons. Non-significant difference of two concentration techniques may have resulted from insufficient power (0.137 for *Giardia* detection and 0.116 for *Cryptosporidium* detection). In addition, it should also be noted that in the current study the fecal concentrates were prepared following different numbers of freeze-thaw cycles. Fecal concentrates using sugar technique were prepared after the first freeze-thaw cycle and the fecal concentrates using sedimentation were prepared after second freeze-thaw cycle. The study comparing the use of IFA on unprocessed feces, sugar concentrates, and sedimentation

concentrates in different freeze-thaw cycles or in different freezer storage times may be suggested.

False negative IFA results after sugar concentration and sedimentation are possible. With the sugar concentration technique, loss of *Giardia* cysts or *Cryptosporidium* oocysts may occur due to damage from freeze-thaw, and then the lower number of cysts or oocysts recovered may lead to negative results on an IFA test. In the sedimentation technique, false negative results may be from the masking of fecal material over the *Giardia* cysts or *Cryptosporidium* oocysts. False positive results, on the other hand, are unlikely as the stained *Giardia* cysts or *Cryptosporidium* oocysts can be confirmed by morphology.

If all positive samples were true positives, there were 36 canine and 11 feline positive specimens for *Giardia* and 32 canine and 8 feline positive specimens for *Cryptosporidium*. Using only the sugar concentration technique we could have missed 12/36 of *Giardia* positives and 11/32 of *Cryptosporidium* positives in dog fecal samples. In cats, when using only the sugar concentration method we could have missed 3/11 of *Giardia* positives. On the other hand if sedimentation was considered alone, 5/36 of *Giardia* positives and 5/32 *Cryptosporidium* positives in dog fecal samples and 3/11 of *Giardia* positives and 3/8 of *Cryptosporidium* positives in cat fecal samples could have been missed.

In conclusion, the IFA results for detection of *Giardia* and *Cryptosporidium* after sugar flotation and sedimentation were comparable. However, for frozen clinical samples sedimentation should be recommended instead of sugar concentration. For optimal results, however, fecal samples submitted for IFA testing for *Giardia* or *Cryptosporidium* should not be frozen.

Sources and manufacturers

a. Meridian Diagnostic Corporation, Cincinnati, OH.

b. Stata Corp., College Station, Texas, USA.

Declaration of conflicting interests

The authors declared no potential conflicts of interest with respect to the research, authorship, or publication of this article.

Table 4.1 Proportions of *Giardia*- and *Cryptosporidium*-positive samples by zinc sulfate flotation and IFA after sugar flotation and sedimentation techniques.

	Freeze-thaw cycle	% of positive *Giardia*		% of positive *Cryptosporidium*	
		Dog	Cat	Dog	Cat
Fecal Flotation	0	19.8 (50/252)	19.7 (12/61)	-	-
IFA after sugar concentration	1	9.5 (24/252)	13.1 (8/61)	8.3 (21/252)	13.1 (8/61)
IFA after sedimentation	2	12.3 (31/252)	13.1 (8/61)	10.7 (27/252)	8.2 (5/61)

Table 4.2 IFA *Giardia*-positive results in dog and cat fecal samples after sugar flotation and sedimentation techniques.

Sugar Flotation	Sedimentation					
	Dog[†] IFA positive			Cat[§] IFA positive		
IFA positive	Yes	No	Total	Yes	No	Total
Yes	19	5	24	5	3	8
No	12	216	228	3	50	53
Total	31	221	252	8	53	61

[†]Exact McNemar's p=0.14, kappa = 0.654, p<0.01
[§]Exact McNemar's p=1.00, kappa = 0.843, p<0.01

Table 4.3 IFA *Cryptosporidium*-positive results in dog and cat fecal samples after sugar flotation and sedimentation techniques.

Sugar Flotation	Sedimentation					
	Dog[†] IFA positive			Cat[§] IFA positive		
IFA positive	Yes	No	Total	Yes	No	Total
Yes	16	5	21	5	3	8
No	11	220	231	0	53	53
Total	27	225	252	5	56	61

[†]Exact McNemar's p=0.21, kappa = 0.632, p<0.01
[§]Exact McNemar's p=0.25, kappa = 0.843, p<0.01

References

1 Aldeen WE, Carroll K, Robison A, et al.: 1998, Comparison of nine commercially available enzyme-linked immunosorbent assays for detection of *Giardia lamblia* in fecal specimens. J Clin Microbiol 36:1338-1340.

2 Bachman DE, Brewer M, Hawley JR, Lappin MR: 2006, Evaluation of chromatographic immunoassays for the diagnosis of *Giardia* spp. and *Cryptosporidium* spp. of cats and dogs. *In*: Proceedings of the American College of Veterinary Internal Medicine Forum, May 31-June 3, 2006. Louisville, KY.

3 Bajer A, Bednarska M, Rodo A: 2011, Risk factors and control of intestinal parasite infections in sled dogs in Poland. Vet Parasitol 175:343-350.

4 Ballweber LR, Panuska C, Huston CL, et al.: 2009, Prevalence of and risk factors associated with shedding of *Cryptosporidium felis* in domestic cats of Mississippi and Alabama. Vet Parasitol 160:306-310.

5 Bermudez-Cruz RM, Thomas M, Alliot A, al. e: 2009, Identification of *Giardia* genotypes in dogs from kennels and veterinarian clinics in France. *In*: 3rd International *Giardia* and *Cryptosporidium* Conference. Orvieto, Italy.

6 Bugg RJ, Robertson ID, Elliot AD, Thompson RC: 1999, Gastrointestinal parasites of urban dogs in Perth, Western Australia. Vet J 157:295-301.

7 Carlin EP, Bowman DD, Scarlett JM, et al.: 2006, Prevalence of *Giardia* in symptomatic dogs and cats throughout the United States as determined by the IDEXX SNAP Giardia test. Vet Ther 7:199-206.

8 Erlandsen SL, Sherlock LA, Bemrick WJ: 1990, The detection of *Giardia muris* and *Giardia lamblia* cysts by immunofluorescence in animal tissues and fecal samples subjected to cycles of freezing and thawing. J Parasitol 76:267-271.

9 Fleiss JL: 2003, Statistical methods for rates and proportions Wiley-Interscience, Hoboken, N.J.

10 Fontanarrosa MF, Vezzani D, Basabe J, Eiras DF: 2006, An epidemiological study of gastrointestinal parasites of dogs from Southern Greater Buenos Aires (Argentina): age, gender, breed, mixed infections, and seasonal and spatial patterns. Vet Parasitol 136:283-295.

11 Garcia LS, Shimizu RY: 1997, Evaluation of nine immunoassay kits (enzyme immunoassay and direct fluorescence) for detection of *Giardia lamblia* and *Cryptosporidium parvum* in human fecal specimens. J Clin Microbiol 35:1526-1529.

12 Garcia LS, Shimizu RY, Novak S, et al.: 2003, Commercial assay for detection of *Giardia lamblia* and *Cryptosporidium parvum* antigens in human fecal specimens by rapid solid-phase qualitative immunochromatography. J Clin Microbiol 41:209-212.

13 Garcia LS, Shum AC, Bruckner DA: 1992, Evaluation of a new monoclonal antibody combination reagent for direct fluorescence detection of *Giardia* cysts and *Cryptosporidium* oocysts in human fecal specimens. J Clin Microbiol 30:3255-3257.

14 Hackett T, Lappin MR: 2003, Prevalence of enteric pathogens in dogs of north-central Colorado. J Am Anim Hosp Assoc 39:52-56.

15 Hill SL, Cheney JM, Taton-Allen GF, et al.: 2000, Prevalence of enteric zoonotic organisms in cats. J Am Vet Med Assoc 216:687-692.

16 Itoh N, Ikegami H, Takagi M, et al.: 2012, Prevalence of intestinal parasites in private-household cats in Japan. J Feline Med Surg 14:436-439.

17 Itoh N, Kanai K, Hori Y, et al.: 2009, Prevalence of *Giardia intestinalis* and other zoonotic intestinal parasites in private household dogs of the Hachinohe area in Aomori prefecture, Japan in 1997, 2002 and 2007. J Vet Sci 10:305-308.
18 Johnston SP, Ballard MM, Beach MJ, et al.: 2003, Evaluation of three commercial assays for detection of *Giardia* and *Cryptosporidium* organisms in fecal specimens. J Clin Microbiol 41:623-626.
19 Kehl KS, Cicirello H, Havens PL: 1995, Comparison of four different methods for detection of *Cryptosporidium* species. J Clin Microbiol 33:416-418.
20 Kim JT, Wee SH, Lee CG: 1998, Detection of *Cryptosporidium* oocysts in canine fecal samples by immunofluorescence assay. Korean J Parasitol 36:147-149.
21 Kirkpatrick CE, Farrell JP: 1984, Feline giardiasis: observations on natural and induced infections. Am J Vet Res 45:2182-2188.
22 Little SE, Johnson EM, Lewis D, et al.: 2009, Prevalence of intestinal parasites in pet dogs in the United States. Vet Parasitol 166:144-152.
23 Mekaru SR, Marks SL, Felley AJ, et al.: 2007, Comparison of direct immunofluorescence, immunoassays, and fecal flotation for detection of *Cryptosporidium* spp. and *Giardia* spp. in naturally exposed cats in 4 Northern California animal shelters. J Vet Intern Med 21:959-965.
24 Morimoto N, Komatsu C, Nishida M, Sugiura T: 2001, Detection of *Giardia lamblia* cysts in non-fixed long-term stored human feces by direct immunofluorescence assay. Jpn J Infect Dis 54:72-74.
25 O'Handley RM, Olson ME, Fraser D, et al.: 2000, Prevalence and genotypic characterisation of *Giardia* in dairy calves from Western Australia and Western Canada. Vet Parasitol 90:193-200.
26 Rimhanen-Finne R, Enemark HL, Kolehmainen J, et al.: 2007, Evaluation of immunofluorescence microscopy and enzyme-linked immunosorbent assay in detection of *Cryptosporidium* and *Giardia* infections in asymptomatic dogs. Vet Parasitol 145:345-348.
27 Rishniw M, Liotta J, Bellosa M, et al.: 2010, Comparison of 4 *Giardia* diagnostic tests in diagnosis of naturally acquired canine chronic subclinical giardiasis. J Vet Intern Med 24:293-297.
28 Scorza AV, Brewer MM, Lappin MR: 2003, Polymerase chain reaction for the detection of *Cryptosporidium* spp. in cat feces. J Parasitol 89:423-426.
29 Scorza AV, Lappin MR: 2012, Cryptosporidiosis. *In*: Infectious diseases of the dog and cat, ed. Greene CE, pp. 841-849. Elsevier/Saunders, St. Louis, Mo.
30 Scorza AV, Lappin MR: 2012, Giardiasis - in Enteric Protozoa infection. *In*: Infectious diseases of the dog and cat, ed. Greene CE, pp. 785-792. Elsevier/Saunders, St. Louis, Mo.
31 Sykes TJ, Fox MT: 1989, Patterns of infection with *Giardia* in dogs in London. Trans R Soc Trop Med Hyg 83:239-240.
32 Tangtrongsup S, Scorza V: 2010, Update on the diagnosis and management of *Giardia* spp infections in dogs and cats. Topics in companion animal medicine 25:155-162.
33 Thompson RC, Smith A: 2011, Zoonotic enteric protozoa. Vet Parasitol 182:70-78.
34 Traub RJ, Inpankaew T, Reid SA, et al.: 2009, Transmission cycles of *Giardia duodenalis* in dogs and humans in Temple communities in Bangkok--a critical evaluation of its

prevalence using three diagnostic tests in the field in the absence of a gold standard. Acta Trop 111:125-132.
35 Uga S, Matsumura T, Ishibashi K, et al.: 1989, Cryptosporidiosis in dogs and cats in Hyogo Prefecture, Japan. Jpn J Parasitol 38:139-143.
36 Vasilopulos RJ, Mackin AJ, Rickard LG, et al.: 2006, Prevalence and factors associated with fecal shedding of *Giardia* spp. in domestic cats. J Am Anim Hosp Assoc 42:424-429.
37 Weber R, Bryan RT, Bishop HS, et al.: 1991, Threshold of detection of *Cryptosporidium* oocysts in human stool specimens: evidence for low sensitivity of current diagnostic methods. J Clin Microbiol 29:1323-1327.
38 Zimmerman SK, Needham CA: 1995, Comparison of conventional stool concentration and preserved-smear methods with Merifluor *Cryptosporidium*/*Giardia* Direct Immunofluorescence Assay and ProSpecT *Giardia* EZ Microplate Assay for detection of *Giardia lamblia*. J Clin Microbiol 33:1942-1943.

CHAPTER 5: COMPARISON OF MOLECULAR GENOTYPING USING GLUTAMATE DEHYDROGENASE (GDH), β-GIARDIN, (BG) AND TRIOSE PHOSPHATE ISOMERASE (TPI) FOR *GIARDIA* ISOLATES AND HEAT SHOCK PROTEIN 70 KDA (HSP70) AND SMALL SUBUNIT RIBOSOMAL RNA (SSU-RRNA) FOR *CRYPTOSPORIDIUM* ISOLATES FROM DOGS AND CATS IN CHIANG MAI, THAILAND[*]

Summary

The current study was conducted to genetically characterize isolates of *Giardia duodenalis* and *Cryptosporidium* spp. from dogs and cats in Chiang Mai, Thailand using PCRs targeting *Giardia* glutamate dehydrogenase (gdh), β-giardin (bg), and triose phosphate isomerase (tpi) genes, *Cryptosporidium* heat shock protein 70 kDa (hsp70) and small subunit ribosomal RNA (SSU-rRNA). For *Cryptosporidium* SSU-rRNA gene, two PCR assays – one-step and nested PCRs – were evaluated. There were 131 canine and 28 feline fecal samples included for *Giardia* genotypic analysis and 56 canine and 12 feline fecal samples included for *Cryptosporidium* species analysis. For *Giardia* genotypic analysis, 40 canine samples were successfully amplified by gdh, bg or tpi PCR assays. Twenty-nine were assemblage D, 9 were assemblage C, and 2 were co-infected with C and D. In cats, of 8 PCR-positive samples, 4 were assemblage C, 3 were assemblage D, and 1 was assemblage A-I. For *Cryptosporidium* species analysis, of 32 canine PCR-positive samples, 23 were *C. canis*, 8 were *C. parvum*, and 1 was

[*] Sahatchai Tangtrongsup[1], A. Valeria Scorza[2], John S. Reif[3], Lora R. Ballweber[4], Michael R. Lappin[2], Mo D. Salman[1], *[1]Animal Population Health Institute, and [2]Center for Companion Animal Studies, Department of Clinical Sciences, [3]Department of Clinical Sciences, [3]Department of Environmental and Radiological Health Sciences, and [4]Department of Microbiology, Immunology and Pathology, College of Veterinary Medicine and Biomedical Sciences, Colorado State University, Fort Collins, CO 80523.* This chapter is formatted for the requirements of Journal of Veterinary Parasitology

mixed *C. canis* and *C. parvum*. In cats, of 5 PCR-positive samples, 2 were *C. canis*, 2 were *C. parvum,* and 1 was *C. felis*. PCR targeting the *Giardia* gdh gene had the highest amplification rate compared to the others (30% vs. 12% and 12%). For *Cryptosporidium*, a one-step SSU-rRNA PCR had the highest amplification rate compared to hsp70 and nested SSU-rRNA PCR assays (57% vs. 49% and 32%). In both *Giardia* and *Cryptosporidium* genetic analyses, 33% and 11% of samples were amplified by all PCR tests for each organism. Of the *Giardia* PCR-positive samples, 56% were positive by a single gene, and of the *Cryptosporidium* positive samples 73% were positive by a single PCR assay. Multilocus analysis is suggested for *Giardia* and *Cryptosporidium* for correct genotypic/species identification and also for increasing the sensitivity of detection.

Keywords: Dogs; Cats; Multilocus genotyping; *Giardia*; *Cryptosporidium*

5.1 Introduction

Giardia duodenalis and *Cryptosporidium* spp. are common causes of diarrheal disease in a wide range of mammals throughout the world including humans, domestic animals, and wildlife (Feng and Xiao, 2011). *Giardia duodenalis* is a species complex consisting of eight assemblages (A-H) (Feng and Xiao, 2011). Assemblages A and B infect humans and other species including dogs, cats and livestock. Assemblages C-H have a narrower range of hosts: assemblages C and D infect canids, assemblage E infects hoofed animals, assemblage F infects cats, assemblage G infects rats, and assemblage H infects marine mammals. Assemblages A and B have been further subclassified as A-I through A-III, B-III and B-IV based on analyses of glutamate dehydrogenase (gdh), β-giardin (bg), and triose phosphate isomerase (tpi). A-I, A-II, B-III and B-IV have a wide range of hosts and are considered zoonotic, whereas A-III has been found only in wild animals (Sprong et al., 2009). The genus *Cryptosporidium* is comprised of at

least 24 valid species (Alvarez-Pellitero et al., 2004; Alvarez-Pellitero and Sitja-Bobadilla, 2002; Fayer, 2010; Fayer and Santin, 2009; Fayer et al., 2010; Ren et al., 2012; Robinson et al., 2010). Humans can be infected by *C. parvum*, *C. hominis* and rarely by *C. canis*, *C. felis*, *C. muris*, *C. meleagridis*, and *C. cuniculus* (Chalmers et al., 2009; Gatei et al., 2002; Srisuphanunt et al., 2011; Xiao et al., 2007). Dogs and cats are mostly infected with species-specific *Cryptosporidium*, *C. canis* for dogs and *C. felis* for cats; however, they also can be infected by the zoonotic species *C. parvum*. In addition, *C. meleagridis* has been identified in dogs (Hajdusek et al., 2004) and *C. muris* has been identified in cats (Pavlasek and Ryan, 2007; Santin et al., 2006).

Dogs and cats are companion animals for people, and it has been reported that dogs and humans living in the same area, such as a remote tea-growing community in India or a temple community in Thailand, carry the same genotype of *G. duodenalis* (Traub et al., 2009; Traub et al., 2003). Therefore, dogs and cats may be considered as a potential zoonotic reservoir for human transmission (Feng and Xiao, 2011; Sprong et al., 2009).

In a clinical setting, *G. duodenalis* is traditionally diagnosed by identifying *Giardia* trophozoites or cysts, and *Cryptosporidium* spp. is diagnosed by identifying *Cryptosporidium* oocysts in fecal samples under light microscope or by using immunologic diagnostic tests. The immunologic diagnostic tests include an enzyme-linked immunosorbent assay (ELISA) or an immune fluorescent assays (IFA) (Scorza and Tangtrongsup, 2010; Tangtrongsup and Scorza, 2010). However, these tests have a known limitation, specifically that they cannot differentiate the species or genotype of these organisms. Trophozoites and cysts of all assemblages of *G. duodenalis* are morphologically alike. For *Cryptosporidium*, some species can be differentiated by the size of oocysts, but this minute difference is difficult to ascertain unless it is measured

using a micrometer. Polymerase chain reaction and DNA nucleotide analysis have been widely used to genetically identify the genotype/species of pathogens. Although various genes have been targeted for *Giardia* and *Cryptosporidium* detection, glutamate dehydrogenase (gdh), β-giardin (bg), and triose phosphate isomerase (tpi) genes for *Giardia,* and heat shocked protein 70 kDa (hsp70) and small subunit ribosomal RNA (SSU-rRNA) genes for *Cryptosporidium* are most often used and have sufficient discriminatory power to genotype these organisms (Caccio and Ryan, 2008; Caccio et al., 2005; Covacin et al., 2011; Sprong et al., 2009). Although most of the molecular studies of *Giardia* and *Cryptosporidium* in dogs and cats have been done using a single gene, multilocus genotyping of *Giardia* and *Cryptosporidium* in animals have recently been published (Abe and Makino, 2010; Lebbad et al., 2010; Scorza et al., 2012; Sprong et al., 2009; Wang et al., 2008). In multilocus studies, discordance of *Giardia* genotypes resulting from different gene analyses has been reported. In addition, not all genotypes may be amplified by a single gene PCR (Lebbad et al., 2011; Scorza et al., 2012). As a result, multilocus analysis is suggested for accurate identification of *Giardia* or *Cryptosporidium* genotype/species in suspect samples. The objectives of this study were to genetically characterize isolates of *Giardia duodenalis* and *Cryptosporidium* spp. from dogs and cats in Chiang Mai, Thailand using the gdh, bg, tpi loci for *Giardia* and the hsp70 and SSU-rRNA loci for *Cryptosporidium*.

5.2 Materials and Methods

5.2.1 Source of fecal materials

Canine and feline fecal samples available for this study were fecal samples that were collected between July and August 2008, and between August 2009 and February 2010, as part of epidemiological studies of *Giardia* and *Cryptosporidium* infections in dogs and cats in Chiang Mai, Thailand (Chapter 2 and Chapter 3). For these studies, *Giardia* infection was identified

using fecal flotation, IFA, and PCR targeting the gdh gene, and *Cryptosporidium* infection was identified using IFA and PCR targeting the hsp70 gene. Thus, fecal samples that were positive for infection with *Giardia* or *Cryptosporidium* were included in the current study.

5.2.2 Molecular analysis

Two nested PCR assays targeting for a 510-bp fragment of bg and a 511-bp fragment of tpi genes (generic and dog-specific primers) for *Giardia* molecular identification were performed as previously described (Caccio et al., 2002; Lebbad et al., 2001; Sulaiman et al., 2004). Two PCR assays for *Cryptosporidium* identification were performed. These PCR assays targeted the SSU-rRNA gene but with different primer sets: a one-step ~290-bp fragment (Morgan et al., 1997) and a ~830-bp fragment nested PCR (Xiao et al., 1999). All PCRs had several modifications from original publication; all PCR reactions were performed in 25-µl reaction using HotStarTaq Master Mix (Qiagen, Valencia, CA) with 10 pmol of each primer and 1 µl of template DNA. The PCR products were sequenced in both directions with the same set of primers using a commercially available service (Proteomics and Metabolomics Facility, Colorado State University). *Giardia* gdh and *Cryptosporidium* hsp70 sequences were obtained from the 2008 and 2009 samples for comparison (Chapter 2 and Chapter 3).

Nucleotide sequence traces were checked and manually edited using Geneious version 6.1 (Biomatter Ltd., Auckland, New Zealand, http://www.geneious.com/). When available, a consensus sequence was constructed using sequences from both forward and reverse directions. Nucleotide sequences were compared with nucleotide sequences from the NCBI GenBank database. Multiple sequence alignment was performed using MUSCLE (Edgar, 2004). Phylogenetic analyses were performed using Maximum Likelihood method based on the Kimura 2-parameter model using MEGA 5.0 (Tamura et al., 2011). The consensus tree was obtained

after bootstrap analysis with 1000 replications. For comparison, phylogenetic trees based on *Giardia* gdh and *Cryptosporidium* hsp70 were constructed using the same method.

The evolutionary divergence between PCR generated gdh sequences were estimated using the Maximum Composite likelihood model (Tamura et al., 2004). All sequences that have number of base substitutions per site less than 0.005 were group together for sub-assemblage clustering. Associations of age, diarrhea, and housing and *Giardia* assemblage (obtained from Chapter 2 and Chapter 3) were assessed using Fisher's exact test (Fleiss, 2003).

5.3 Results

5.3.1 *PCR results and sequencing analyses*

A total of 159 (131 canine and 28 feline) fecal samples were evaluated for *Giardia* gdh, bg, and tpi PCR assays, and a total of 68 (56 canine and 12 feline) fecal samples were evaluated for *Cryptosporidium* hsp70, one-step, and nested SSU-rRNA PCR assays.

Giardia duodenalis assemblage determination

A summary of *Giardia* PCR results is shown in Table 5.1. *Giardia* gdh was successfully amplified in 39 of 131 (30%) canine samples and in 8 of 28 (29%) feline samples. In dogs, 31 of 39 (79%) were identified as assemblage D and the remaining 8 isolates (21%) were identified as assemblage C. In cats, 4 of 8 (50%) were identified as assemblage C, 3 isolates as assemblage D, and one assemblage as A-I isolate.

Of 159 DNA samples, 19 (12%) were positive by *Giardia* bg locus, with 9 being assemblage C and 10 assemblage D. For the tpi locus, of 159 DNA samples, 9 (6%) were positive by tpi-generic primers and 19 (12%) of the samples were positive by tpi dog-specific primers. Of positive samples, seven were identified as assemblage C, 10 were assemblage D, and

a mixed infection of assemblages C and D was identified in 2 samples. None of the cat samples were amplified by bg or tpi PCRs.

Of the positive results, 27 samples were identified by one gene, 6 samples by 2 genes and 16 samples were identified by 3 genes (Table 5.3). In dogs, all isolates were identified as dog-adapted assemblages C or D. In cats, among 8 positive samples, 7 were dog genotype, assemblages C and D, and 1 was assemblage A-I. Two genes or more were compared in 22 samples and 3 discordant results were identified. Two discordances were assemblage C by one or two genes but D by other genes (TH08Dog17 and TH08Dog107). When interpreted with all genes, of 40 dogs 29 (73%) were infected with *G. duodenalis* assemblage D, 9 (22%) with assemblage C, and 2 (5%) were infected with assemblages C and D.

Cryptosporidium species determination

For the hsp70 locus, 18 of 68 (26%) DNA samples were successfully amplified. Of 18 PCR positive samples, *C. parvum* was identified in 7 dogs and 2 cats and *C. canis* was identified in 7 dogs and 2 cats (Table 5.2).

For SSU-rRNA PCR, 21 of 68 (31%) and 12 of 68 (21%) samples were positive by one-step and nested PCR (Table 5.2). Using one-step PCR, *C. parvum* was identified in 2 canine samples and *C. canis* was identified in 19 dog samples. Using nested PCR for SSU-RNA, 11 *C. canis* isolates were identified from dogs and one *C. felis* was identified from the cat samples.

Among the 37 positive samples, 4 samples were positive by 3 PCRs, 6 were positive by 2 PCRs and 27 were positive by a single PCR (Table 5.4). In dogs, *C. canis* was the most common, being detected in 21 of 32 (66%) *Cryptosporidium* PCR-positive samples. Eight of 32 (25%) were identified as *C. parvum*. One discordant result was *C. parvum* by hsp70 gene and *C. canis*

by one-step SSU-rRNA. In cats, of the five positives, two were identified as *C. parvum,* two as *C. canis* and one as C. *felis.*

When interpreted with all three PCR assays, of the 32 *Cryptosporidium*-infected dogs 23 (72%) were infected with *C. canis* and 9 (28%) with *C. parvum*. Of the 5 *Cryptosporidium*-infected cats, 2 (40%) had *C. parvum*, 2 (40%) had *C. canis*, and 1 was infected with *C. felis*.

5.3.2 Phylogenetic analyses

The phylogenetic analyses included 48 isolates at the gdh locus, 19 at the bg locus and 21 isolates at the tpi locus (Figure 5.1-5.3). Based on the gdh phylogenetic tree, three subgroups of assemblage D were identified. No substructuring of assemblage C isolates was observed. Based on the bg locus, no substructure was observed in either assemblage C or D. However, sample TH09Dog68 (identified as assemblage D) diverted from the main group. Based on the tpi locus, 5 subgroups of assemblage C and 6 subgroups of assemblage D were observed.

Eighteen isolates at the hsp70 locus and 33 isolates at the SSU-rRNA (21 from one-step PCR and 12 from nested PCR) were included in the phylogenetic analyses (Figure 5.4-5.6). Based on the hsp70 locus, 8 of the *C. parvum* isolates were placed together; however, a *C. parvum* isolate from one dog (TH08Dog9) was placed together with the *C. parvum* marsupial genotype. One cat isolate, which was identified as *C. canis* using BLAST analysis, diverted from the other *C. canis* isolates (Figure 5.4). Based on one-step SSU-rRNA sequences ~290-bp long, all *C. canis* isolates were placed together; however, the two isolates identified as *C. parvum* were placed together with reference sequences of *C. hominis* and *C. parvum* (Figure 5.5). This result may suggest that using one step SSU-rRNA to amplify DNA of *C. hominis* and *C. parvum* cannot discriminate the species. Based on nested PCR of SSU-rRNA with the sequence length of ~830 bps, *C. canis* and *C. felis* isolates were placed together with the reference sequences.

5.3.3. *Giardia duodenalis* sub-assemblages and risk factors (age, diarrhea and housing type)

Using the Maximum composite likelihood model, the distance of evolutionary divergence between sequences of all 47 gdh sequences were estimated. Four clusters of assemblage D and only one assemblage C cluster were identified. Table 5.5 shows a contingency table of *Giardia* sub-assemblage and age, diarrhea status, and housing type of the animals. In Table 5.6, sub-assemblages of D were collapsed and the association of risk factors was determined regarding the assemblage of the agent. There were no diarrhea in animals infected with assemblage C, whereas, 8 of 31 dogs with assemblage D have had diarrhea. All C assemblage isolates were identified from dogs age one year and over, in contrast, 41.9% of assemblage D isolates were from dogs age less than one year. All assemblage C isolates were from dogs residing in shelter/breeding setting or temples, whereas 61% of assemblage D isolates were from household dogs. Due to the small data set and zero value observed in some categories, the statistical analysis was not possible.

5.4 Discussion

Zoonotic species or genotypes of *Cryptosporidium parvum* and *Giardia duodenalis* cannot be distinguished from host-adapted organisms using morphological differentiation. However, molecular characterization using PCR assay and sequence analysis is a rapid and sensitive method to differentiate the species or genotypes of these organisms.

Worldwide, the majority of genotypes of *G. duodenalis* that infect dogs and cats are host-adapted (assemblage C or D for dogs and assemblage F for cats); however, the pattern can differ geographically (Ballweber et al., 2010; Feng and Xiao, 2011; Scorza et al., 2012). For examples, in several studies genotyping *Giardia* in subclinically infected dogs in the Western United States (Covacin et al., 2011) and in temple dogs in Bangkok Thailand (Traub et al., 2009), the majority

of *Giardia* isolates were zoonotic assemblages. In the current study, most of the *G. duodenalis* isolate assemblages detected from dogs and cats were dog-adapted assemblages (C or D), with only one zoonotic A-I assemblage identified in a cat. Whether these represent ingestion and pass-through of cysts originating from dogs, contamination of the sample during collection, or actual infection of cats by these assemblages remains to be determined. However, given that both assemblages C and D have been previously identified in cats (Sprong et al., 2009), it is likely that cats can become infected with these assemblages under certain circumstances (Scorza et al., 2012). There is no evidence of dogs have been infected by cat-adapted *G. duodenalis*. Whether the cat-adapted *G. duodenalis* infects dogs remains unclear.

In this study, 72% and 28% of *Cryptosporidium* isolates from dogs were identified as *C. canis* and *C. parvum*, respectively. In cats, of 5 *Cryptosporidium* isolates, *C. parvum* and *C. canis* were identified equally (40%) and *C. felis* was identified in one isolate (20%). The observation in dogs was supported by previous studies of *Cryptosporidium* isolates from dogs and cats worldwide. Of 41 *Cryptosporidium* species/genotypes that had been previously reported in dogs, 76% were identified as *C. canis*, 22% as *C. parvum* and 2% were identified as *C. meleagridis* (Palmer et al., 2008; Santin and Trout, 2008). In cats, previous studies reported that of 65 molecularly characterized isolates, 97% were identified as *C. felis* and 6% as *C. muris* (Ballweber et al., 2009; Palmer et al., 2008; Santin and Trout, 2008). As observed for *Giardia* genotypes, whether cats were infected by *C. canis* cross-transmission, or ingestion and pass-through of cysts originating from dogs, or contamination of the sample during collection, remains to be determined.

All PCR assays do not have the similar sensitivity for detecting *Giardia* nucleotides in fecal samples. In the current study, gdh PCR had the highest amplification rate compared to bg

and tpi genes (30% compared to 12% and 12%). This observation was similar to the study by Scorza et al. (2012) that showed the gdh PCR had higher amplification rate than bg or tpi. However, this observation was in contrast to the studies by Covacin et al. (2011) and Sprong et al. (2009) that showed that gdh PCR had the least amplification rate compared to bg, tpi and SSU-rRNA. Considering our experiences, PCR for the gdh gene may be suggested if the multilocus PCR assay is not affordable. For *Cryptosporidium* detection, SSU-rRNA one-step (amplification rate 30%) or hsp70 (amplification rate 26%) may be suggested as the nested PCR for SSU-rRNA shows a lower amplification rate (21%) in our study.

In the current study, 3 subgroups of assemblage D were identified based on the gdh gene. Based on the bg gene, 2 subgroups of assemblage D were identified. Based on the tpi gene, 5 subgroups of assemblage C and 6 subgroups of assemblage D were identified. In one study, substructuring of assemblage C (C-I to C-IV) and D (D-I to D-V) was identified when the sequences obtained from tpi genes were analyzed; however, when molecular analysis was performed on the gdh gene substructuring in assemblage D (D-I to D-IV) was observed, and substructuring of assemblage C (C-I to C-III) was only observed when bg sequences were analyzed (Lebbad et al., 2010).

The association of diarrhea and *Giardia* assemblage subgroups was not possible in this study due to the small sample size; however, several trends were observed. For example, none of assemblage C infected dogs had diarrhea whereas about a quarter of assemblage D infected dogs did. Furthermore, all assemblage C isolates were from dogs in high-density environments (breeding/shelter dogs), whereas assemblage D was identified in dogs from both sources. This suggests that assemblage D is more likely to be transmitted among household dogs. A case

control study with a larger sample size is suggested to further assess for associations among diarrhea and different *Giardia* assemblages and subgroups.

The majority of genotypes/species of *Giardia* and *Cryptosporidium* isolates from dogs and cats in this study may not reflect the majority of genotypes/species for the dog and cat population as a whole in Chiang Mai, Thailand since we made our interpretation in the light of the available nucleotide sequences. The information regarding the genotype/species for about 70% of *Giardia*-infected samples and 54% of *Cryptosporidium*-infected samples was unknown. Failure of PCR assays to amplify the organisms' target genes may be from the presence of a PCR inhibitor (da Silva et al., 1999), or degradation of DNA material in the samples which may result from international shipment or long-term storage before PCR processing. Therefore, the failure to amplify *Giardia* or *Cryptosporidium* genes in the fecal samples using PCR did not rule out *Giardia* or *Cryptosporidium* infection, thus PCR should not be used as the primary test for *Giardia* or *Cryptosporidium* diagnoses.

The current information suggests that dogs and cats in Chiang Mai may not be a primary reservoir for zoonotic transmission of *G. duodenalis*; however, these animals may be a reservoir for zoonotic transmission of *Cryptosporidium*. Further investigation using molecular analysis of *Giardia* and *Cryptosporidium* genotype/species isolated from animals and humans (pets and owners or shelter animals with the care givers) may clarify the transmission cycle of these organisms between humans and animals in the same environmental setting.

5.5 Tables

Table 5.1 *Giardia* genotypes determined by nucleotide sequence analyses of glutamate dehydrogenase (gdh), β-giardin (bg) and triose phosphate isomerase (tpi) PCR products from dog and cat samples in Chiang Mai, Thailand.

ID	gdh	bg	tpigen[a]	tpiD[b]
TH08Dog5	D	D	n/a	D
TH08Dog15	D	D	n/a	n/a
TH08Dog17	D	D	C	D
TH08Dog19	C	C	C	C
TH08Dog22	C	C	C	C
TH08Dog23	D	D	n/a	D
TH08Dog24	D[ash]	D	n/a	D
TH08Dog30	C	C	C	C
TH08Dog33	D	D	n/a	D
TH08Dog36	D[ash]	D	n/a	D
TH08Dog40	D	n/a	n/a	n/a
TH08Dog43	D	D	n/a	D
TH08Dog45	D[ash]	D	n/a	D
TH08Dog73	C	C	C	C
TH08Dog93	D	n/a	n/a	n/a
TH08Dog96	D	n/a	n/a	n/a
TH08Dog100	C	C	n/a	n/a
TH08Dog101	C	C	C	C
TH08Dog103	C	C	n/a	n/a
TH08Dog107	D[ash]	C[ash]	C[ash]	D
TH08Dog108	C[ash]	C	C	C[ash]
TH09Dog68	D	D	n/a	D
TH09Dog69	n/a	n/a	C	C
TH09Dog78	D	n/a	n/a	n/a
TH09Dog80	D	n/a	n/a	D
TH09Dog83	D	n/a	n/a	n/a
TH09Dog84	D	n/a	n/a	n/a
TH09Dog91	D	n/a	n/a	D
TH09Dog92	D	n/a	n/a	n/a
TH09Dog97	D	n/a	n/a	n/a
TH09Dog106	D	n/a	n/a	n/a
TH09Dog126	D	n/a	n/a	n/a
TH09Dog128	D	n/a	n/a	n/a
TH09Dog133	D	n/a	n/a	n/a
TH09Dog134	D	n/a	n/a	n/a
TH09Dog135	D	n/a	n/a	n/a
TH09Dog141	D	n/a	n/a	n/a
TH09Dog151	D	n/a	n/a	n/a
TH09Dog293	D	n/a	n/a	n/a

ID	gdh	bg	tpigen[a]	tpiD[b]
TH09Dog299	D	n/a	n/a	n/a
TH08Cat7	D	n/a	n/a	n/a
TH08Cat13	D	n/a	n/a	n/a
TH09Cat11	C	n/a	n/a	n/a
TH09Cat29	C	n/a	n/a	n/a
TH09Cat59	AI	n/a	n/a	n/a
TH09Cat60	C	n/a	n/a	n/a
TH09Cat63	D	n/a	n/a	n/a
TH09Cat65	C	n/a	n/a	n/a

n/a = no amplification.
ash = allelic sequence heterogeneity
[a] tpigen = PCR of tpi gene with generic primers that can detect all *G. duodenalis* assemblages except assemblage D
[b] tpiD, dog specific = PCR of tpi gene with dog-specific primers can detect both assemblages C and D

Table 5.2 *Cryptosporidium* genotypes determined by nucleotide sequence analyses of heat shock protein 70 (hsp70), one step Small Subunit-rRNA, and nested Small Subunit-rRNA PCR products from dog and cat samples in Chiang Mai, Thailand.

Sample	hsp70	One step SSU-rRNA	Nested SSU-rRNA
TH08Dog5	n/a	*C. canis*	n/a
TH08Dog7	n/a	*C. canis*	*C. canis*
TH08Dog22	n/a	*C. parvum*	n/a
TH08Dog28	n/a	*C. canis*	*C. canis*
TH08Dog 42	n/a	*C. canis*	*C. canis*
TH08Dog43	*C. parvum*	n/a	n/a
TH08Dog46	*C. canis*	*C. canis*	*C. canis*
TH08Dog54	*C. parvum*	n/a	n/a
TH08Dog55	*C. canis*	*C. canis*	*C. canis*
TH08Dog58	*C. canis*	n/a	n/a
TH08Dog61	n/a	n/a	*C. canis*
TH08Dog68	n/a	n/a	*C. canis*
TH08Dog69	n/a	*C. canis*	n/a
TH08Dog71	n/a	*C. canis*	n/a
TH08Dog76	*C. parvum*	n/a	n/a
TH08Dog86	*C. parvum*	n/a	n/a
TH08Dog87	*C. parvum*	n/a	n/a
TH08Dog92	*C. canis*	n/a	n/a
TH08Dog96	*C. canis*	n/a	n/a
TH08Dog101	*C. parvum*	n/a	n/a
TH08Dog102	n/a	*C. canis*	n/a
TH08Dog107	n/a	*C. canis*	*C. canis*
TH09Dog5	*C. canis*	*C. canis*	*C. canis*
TH09Dog9	*C. parvum*	*C. canis*	n/a
TH09Dog32	n/a	*C. parvum*	n/a
TH09Dog68	*C. canis*	*C. canis*	*C. canis*
TH09Dog203	n/a	*C. canis*	n/a
TH09Dog227	n/a	*C. canis*	*C. canis*
TH09Dog289	n/a	*C. canis*	n/a
TH09Dog290	n/a	*C. canis*	n/a
TH09Dog321	n/a	*C. canis*	n/a
TH09Dog328	n/a	*C. canis*	n/a
TH08Cat3	*C. canis*	n/a	n/a
TH08Cat6	*C. canis*	n/a	n/a
TH09Cat6	n/a	n/a	*C. felis*
TH09Cat35	*C. parvum*	n/a	n/a
TH09Cat69	*C. parvum*	n/a	n/a

n/a = no amplification.

Table 5.3 *Giardia duodenalis* assemblages in dogs and cats in Chiang Mai, Thailand by glutamate dehydrogenase (gdh), β-giardin (bg) and triose phosphate isomerase generic (tpi) genes.

# of genes amplified	Species	gdh	bg	tpigen[b]	tpiD[c]
	Dog (40)				
1 (19) [a]	18	D	n/a	n/a	n/a
	1	n/a	n/a	C	C
2 (5) [a]	2	C	C	n/a	n/a
	1	D	D	n/a	n/a
	2	D	n/a	n/a	D
3 (16) [a]	6	C	C	C	C
	8	D	D	n/a	D
	1	D	D	C	D
	1	D	C	C	D
	Cat (8)				
1 (8) [a]	1	AI	n/a	n/a	n/a
	3	D	n/a	n/a	n/a
	4	C	n/a	n/a	n/a

[a]Number of animals that tested positive for the number of genes tested
[b]tpigen = PCR of tpi gene with generic primers that can detect all *G. duodenalis* assemblages except assemblage D
[c]tpiD, dog specific = PCR of tpi gene with dog-specific primers can detect both assemblages C and D
n/a = no amplification

Table 5.4 *Cryptosporidium* species in dogs and cats in Chiang Mai, Thailand by heat shock protein 70 (hsp70), one-step and nested small subunit ribosomal RNA (SSU-rRNA) PCR assays.

# of positive PCRs	Species	hsp70	One step SSU-rRNA	Nested PCR SSU-rRNA
	Dog (32)			
1 (22) [a]	6	*C. parvum*	n/a	n/a
	3	*C. canis*	n/a	n/a
	9	n/a	*C. canis*	n/a
	2	n/a	*C. parvum*	n/a
	2	n/a	n/a	*C. canis*
2 (6) [a]	5	n/a	*C. canis*	*C. canis*
	1	*C. parvum*	*C. canis*	n/a
3 (4) [a]	4	*C. canis*	*C. canis*	*C. canis*
	Cat (5)			
1 (5) [a]	2	*C. parvum*	n/a	n/a
	2	*C. canis*	n/a	n/a
	1	n/a	n/a	*C. felis*

[a] Number of animals that tested positive for the number of PCR assays tested.
n/a = no amplification

Table 5.5 Proportion of genotype cluster of *G. duodenalis* isolates in dogs and cats in Chiang Mai, Thailand, based on nucleotide derived from glutamate dehydrogenase by diarrhea status, age and housing type.

Risk	AI % (No./Total)	C % (No./Total)	D1 % (No./Total)	D2 % (No./Total)	D3 % (No./Total)	D4 % (No./Total)
Diarrhea*						
Yes	0 (0/1)	0 (0/11)	31.8 (7/22)	0 (0/4)	16.7 (1/6)	0 (0/1)
No	100 (1/1)	100 (11/11)	68.2 (15/22)	100 (4/4)	83.3 (5/6)	100 (1/1)
Age						
<1	0 (0/1)	8.3 (1/12)	40.9 (9/22)	25 (1/4)	42.9 (3/7)	0 (0/1)
≥1	100 (1/1)	91.7 (11/12)	59.1 (13/22)	75 (3/4)	57.1 (4/7)	100 (1/1)
Housing type						
Breeder/Shelter/Temple	100 (1/1)	83.3 (10/12)	27.3 (6/22)	100 (4/4)	42.9 (3/7)	100 (1/1)
Household	0 (0/1)	16.7 (2/12)	72.7 (16/22)	0 (0/4)	57.1 (4/7)	0 (0/1)

*Contain missing value

Table 5.6 Proportion of *G. duodenalis* assemblages in dogs and cats in Chiang Mai, Thailand, based on nucleotide derived from glutamate dehydrogenase by diarrhea status, age and housing type.

Risk	A % (No./Total)	C % (No./Total)	D % (No./Total)
Dog			
Diarrhea*			
Yes		0 (0/7)	25.8 (8/31)
No		100 (7/7)	74.2 (23/31)
Age			
<1		0 (0/8)	41.9 (13/31)
≥1		100 (8/8)	58.1 (18/31)
Housing type			
Breeder/Shelter/Temple		100 (8/8)	38.7 (12/31)
Household		0 (0/8)	61.3 (19/31)
Cat			
Diarrhea			
Yes	0 (0/1)	0 (0/4)	0 (0/2)
No	100 (1/1)	100 (4/4)	100 (2/2)
Age†			
<1	0 (0/1)	25 (1/4)	0 (0/3)
≥1	100 (1/1)	75 (3/4)	100 (3/3)
Housing type			
Breeder/Shelter/Temple	100 (1/1)	50 (2/4)	66.7 (2/3)
Household	0 (0/1)	50 (2/4)	33.3 (1/3)

*Contain missing value

5.6 Figures

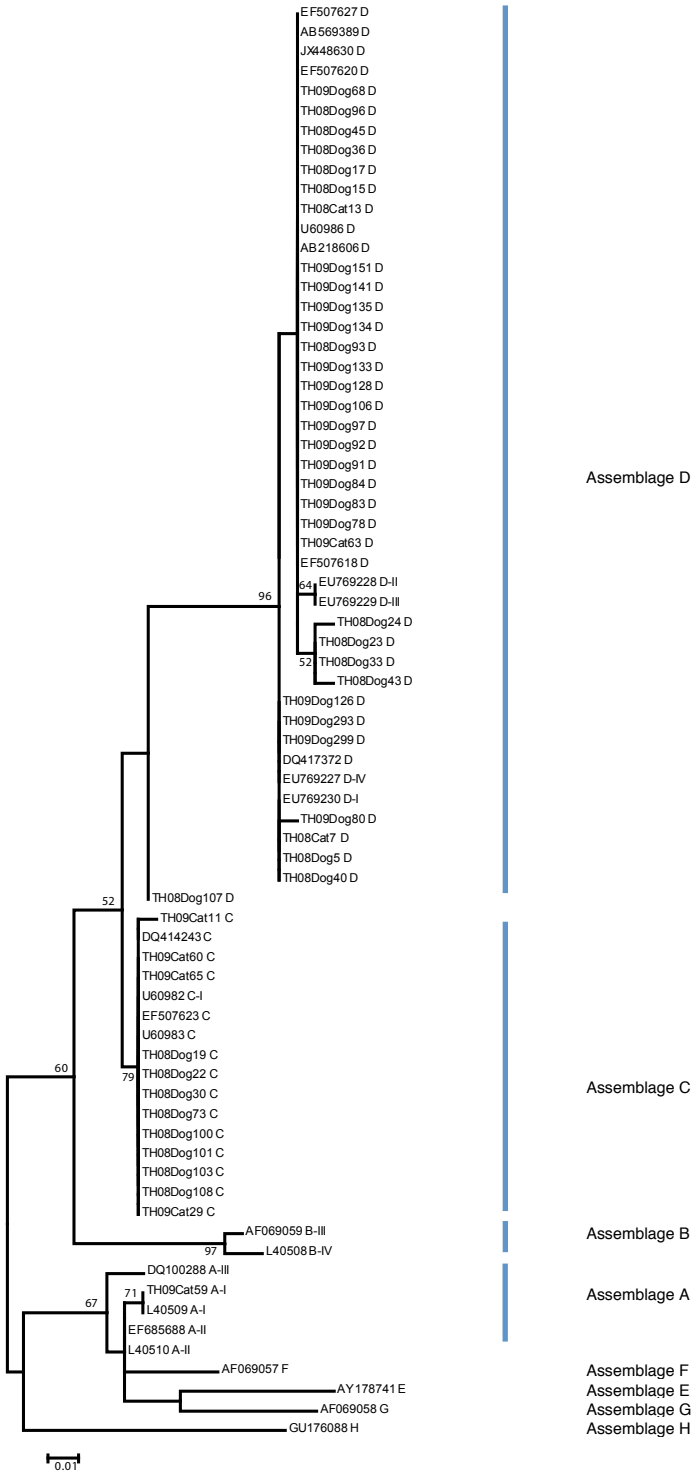


Figure 5.1 Phylogenetic relationships of the *Giardia* isolates from dogs and cats in Chiang Mai, Thailand based on the glutamate dehydrogenase (gdh) gene. The numbers on the branches are the percentage bootstrap support values from 1000 replicates. Only values greater than 50% are included to the left of each node. Reference sequences were labeled as its accession number and assemblage. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site.

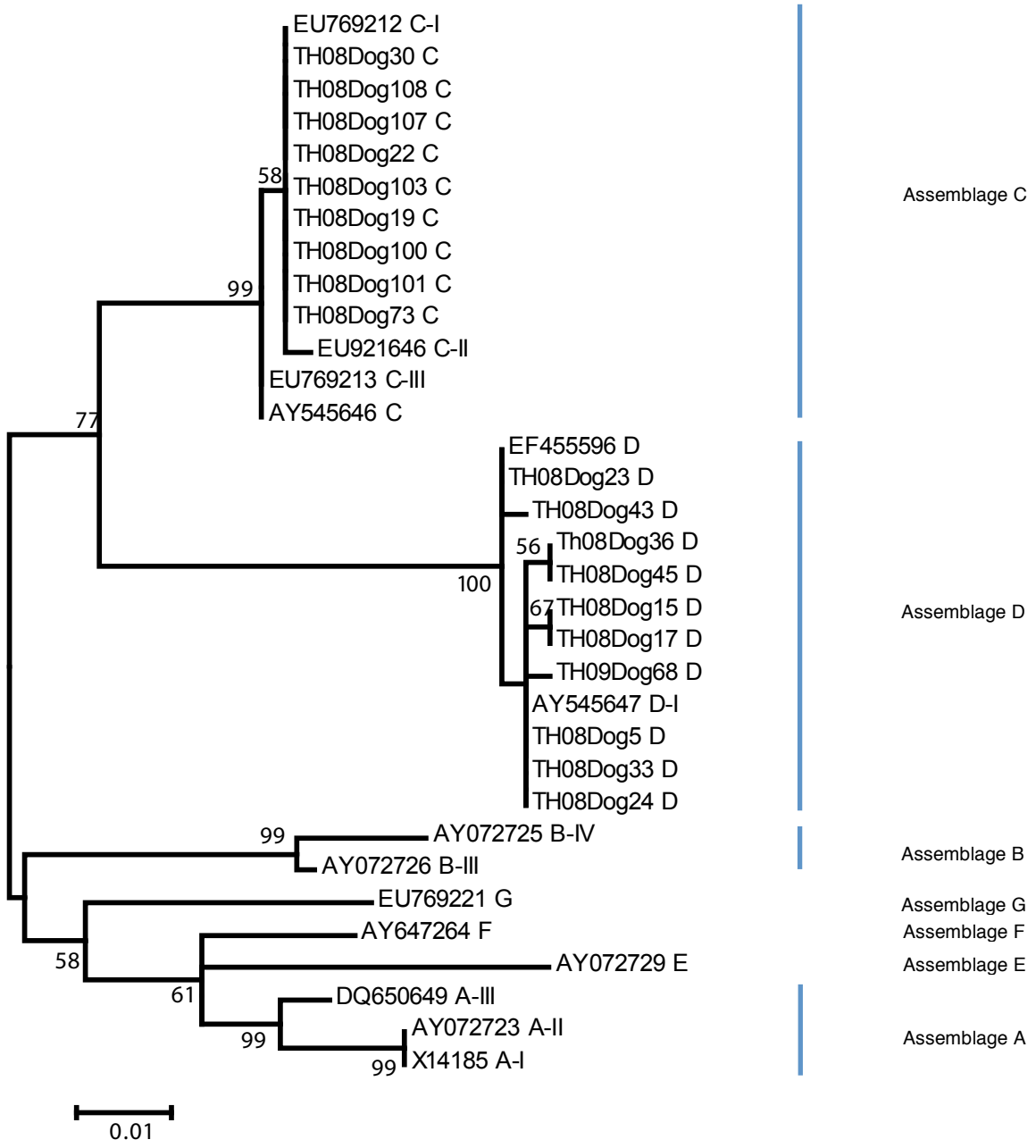


Figure 5.2 Phylogenetic relationships of the *Giardia* isolates from dogs and cats in Chiang Mai, Thailand based on β-giardin gene (bg). The numbers on branches are the percentage bootstrap support values from 1000 replicates. Only values greater than 50% are included to the left of each node. Reference sequences were labeled as its accession number and assemblage. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site.

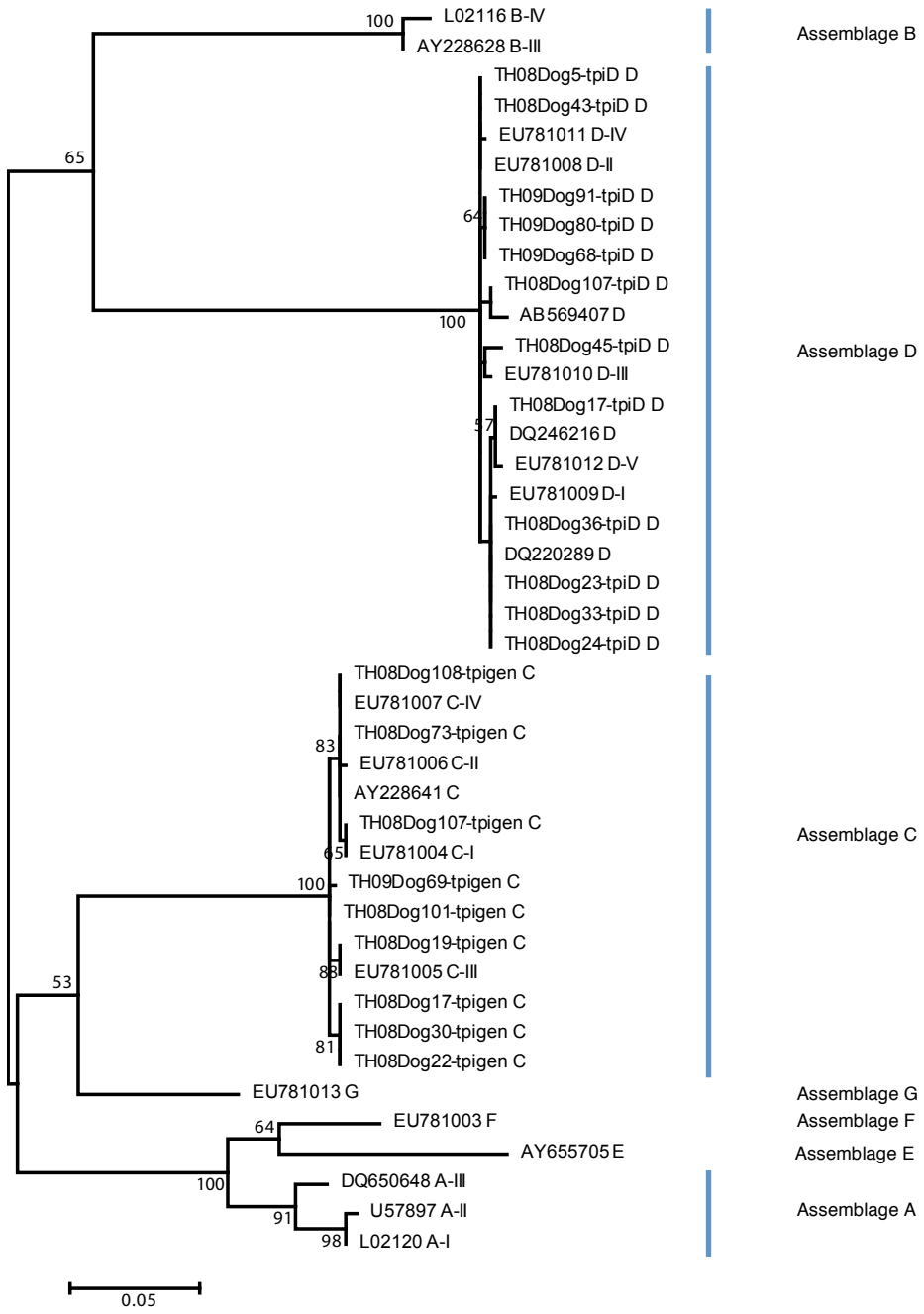


Figure 5.3 Phylogenetic relationships of the *Giardia* isolates from dogs and cats in Chiang Mai, Thailand based on the triose phosphate isomerase (tpi) gene. The numbers on branches are the percentage bootstrap support values from 1000 replicates. Only values greater than 50% are included to the left of each node. Reference sequences were labeled as its accession number and assemblage. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. tpigen = tpi sequences amplified by generic primers, tpiD = tpi sequences amplified by dog genotype specific primers.

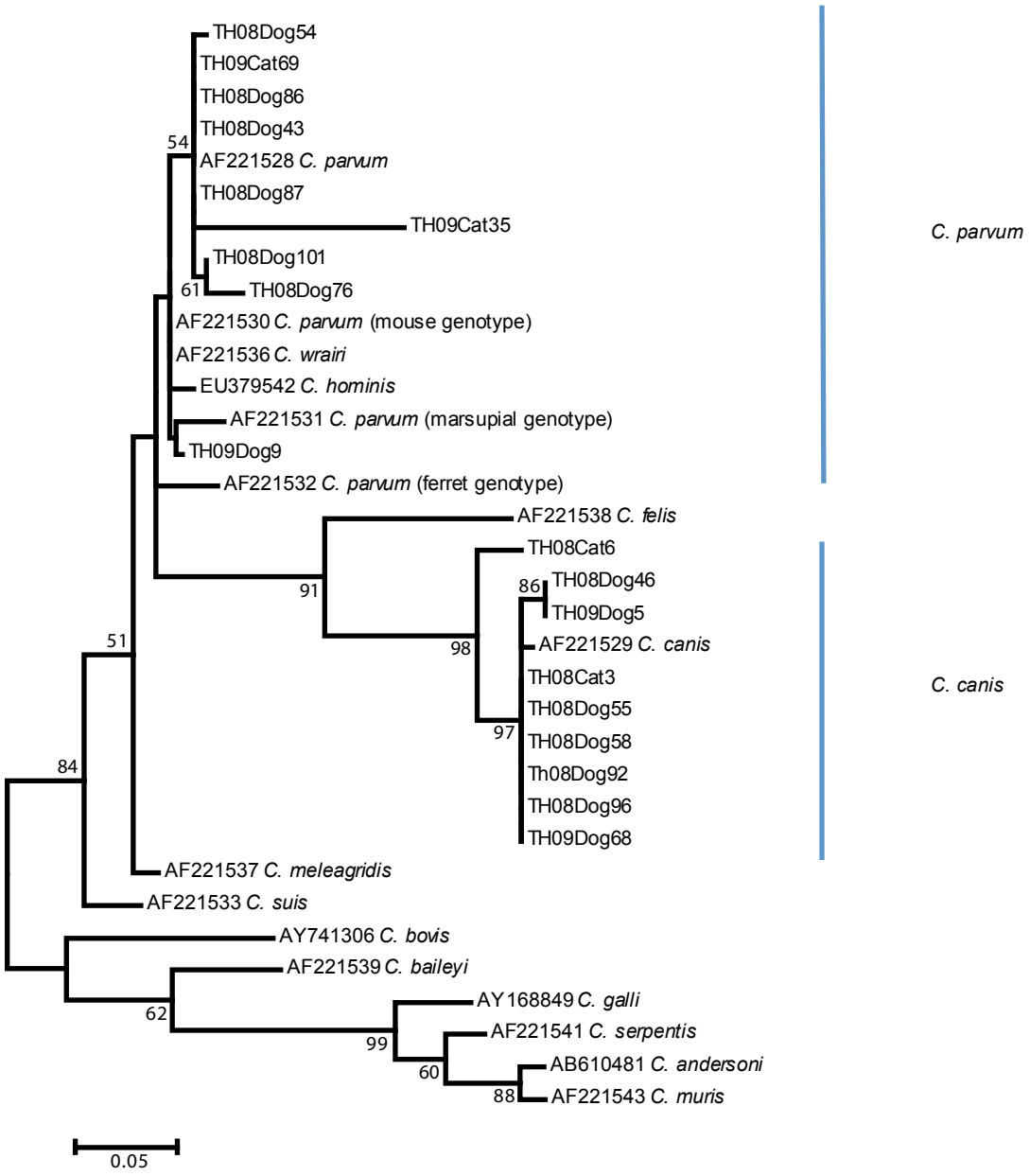


Figure 5.4 Phylogenetic relationships of the *Cryptosporidium* isolates from dogs and cats in Chiang Mai, Thailand based on the heat shock protein 70 (hsp70) gene. The numbers on branches are the percentage bootstrap support values from 1000 replicates. Only values greater than 50% are included to the left of each node. Reference sequences were labeled as its accession number and identified species. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site.

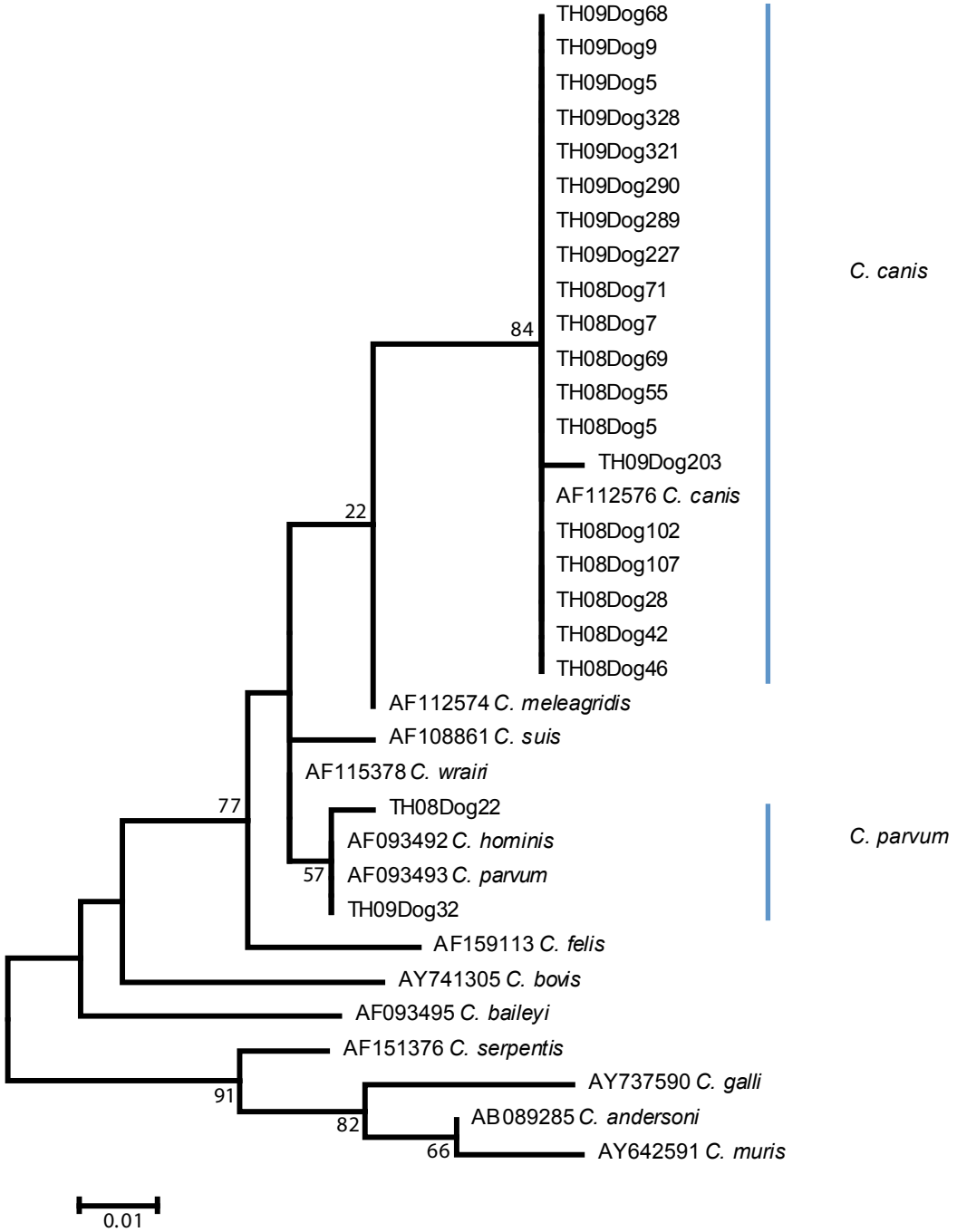


Figure 5.5 Phylogenetic relationships of the *Cryptosporidium* isolates from dogs and cats in Chiang Mai, Thailand based on the ~290-bps fragment from one step PCR of small subunit RNA (SSU-rRNA) gene. The numbers on branches are the percentage bootstrap support values from 1000 replicates. Only values greater than 50% are included to the left of each node. Reference sequences were labeled as its accession number and identified species. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site.

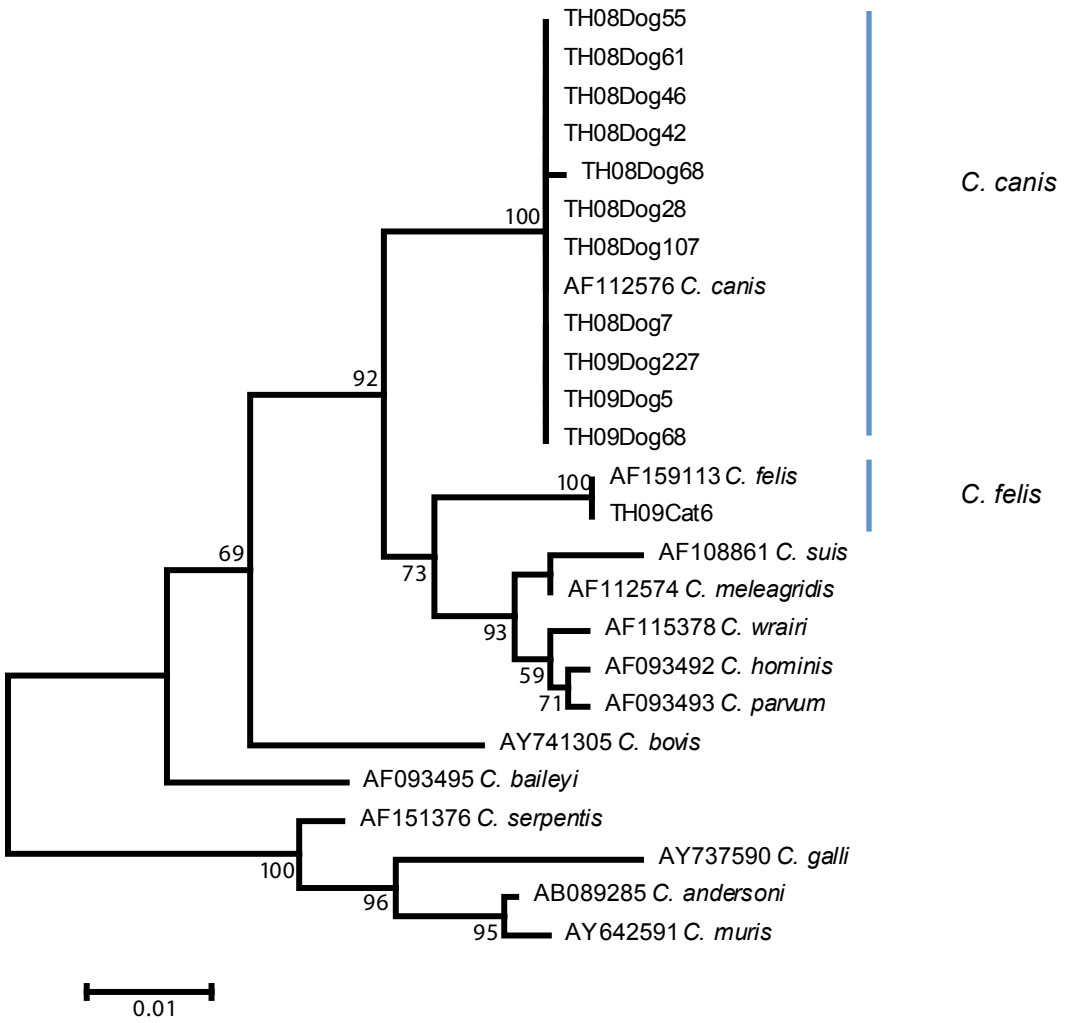


Figure 5.6 Phylogenetic tree of the *Cryptosporidium* isolates from dogs and cats in Chiang Mai, Thailand based on the ~ 820-bps fragment from a nested PCR of small subunit RNA (SSU-rRNA) gene. The numbers on branches are the percentage bootstrap support values from 1000 replicates. Only values greater than 50% are included to the left of each node. Reference sequences were labeled as its accession number and identified species. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site.

5.7 References

Abe, N., Makino, I., 2010. Multilocus genotypic analysis of *Cryptosporidium* isolates from cockatiels, Japan. Parasitol. Res. 106, 1491-1497.

Alvarez-Pellitero, P., Quiroga, M.I., Sitja-Bobadilla, A., Redondo, M.J., Palenzuela, O., Padros, F., Vazquez, S., Nieto, J.M., 2004. *Cryptosporidium scophthalmi* n. sp. (Apicomplexa: Cryptosporidiidae) from cultured turbot *Scophthalmus maximus*. Light and electron microscope description and histopathological study. Dis. Aquat. Organ. 62, 133-145.

Alvarez-Pellitero, P., Sitja-Bobadilla, A., 2002. *Cryptosporidium molnari* n. sp. (Apicomplexa: Cryptosporidiidae) infecting two marine fish species, *Sparus aurata* L. and *Dicentrarchus labrax* L. Int. J. Parasitol. 32, 1007-1021.

Ballweber, L.R., Panuska, C., Huston, C.L., Vasilopulos, R., Pharr, G.T., Mackin, A., 2009. Prevalence of and risk factors associated with shedding of *Cryptosporidium felis* in domestic cats of Mississippi and Alabama. Vet. Parasitol. 160, 306-310.

Ballweber, L.R., Xiao, L., Bowman, D.D., Kahn, G., Cama, V.A., 2010. Giardiasis in dogs and cats: update on epidemiology and public health significance. Trends Parasitol 26, 180-189.

Caccio, S.M., De Giacomo, M., Pozio, E., 2002. Sequence analysis of the beta-giardin gene and development of a polymerase chain reaction-restriction fragment length polymorphism assay to genotype *Giardia duodenalis* cysts from human faecal samples. Int. J. Parasitol. 32, 1023-1030.

Caccio, S.M., Ryan, U., 2008. Molecular epidemiology of giardiasis. Mol. Biochem. Parasitol. 160, 75-80.

Caccio, S.M., Thompson, R.C., McLauchlin, J., Smith, H.V., 2005. Unravelling *Cryptosporidium* and *Giardia* epidemiology. Trends Parasitol 21, 430-437.

Chalmers, R.M., Robinson, G., Elwin, K., Hadfield, S.J., Xiao, L., Ryan, U., Modha, D., Mallaghan, C., 2009. *Cryptosporidium* sp. rabbit genotype, a newly identified human pathogen. Emerg. Infect. Dis. 15, 829-830.

Covacin, C., Aucoin, D.P., Elliot, A., Thompson, R.C., 2011. Genotypic characterisation of *Giardia* from domestic dogs in the USA. Vet. Parasitol. 177, 28-32.

da Silva, A.J., Bornay-Llinares, F.J., Moura, I.N., Slemenda, S.B., Tuttle, J.L., Pieniazek, N.J., 1999. Fast and reliable extraction of protozoan parasite DNA from fecal specimens. Mol. Diagn. 4, 57-64.

Edgar, R.C., 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Res 32, 1792-1797.

Fayer, R., 2010. Taxonomy and species delimitation in *Cryptosporidium*. Exp. Parasitol. 124, 90-97.

Fayer, R., Santin, M., 2009. *Cryptosporidium xiaoi* n. sp. (Apicomplexa: Cryptosporidiidae) in sheep (*Ovis aries*). Vet. Parasitol. 164, 192-200.

Fayer, R., Santin, M., Macarisin, D., 2010. *Cryptosporidium ubiquitum* n. sp. in animals and humans. Vet. Parasitol. 172, 23-32.

Feng, Y., Xiao, L., 2011. Zoonotic potential and molecular epidemiology of *Giardia* species and giardiasis. Clin. Microbiol. Rev. 24, 110-140.

Fleiss, J.L., 2003. Statistical methods for rates and proportions. Wiley-Interscience, Hoboken, N.J.

Gatei, W., Suputtamongkol, Y., Waywa, D., Ashford, R.W., Bailey, J.W., Greensill, J., Beeching, N.J., Hart, C.A., 2002. Zoonotic species of *Cryptosporidium* are as prevalent as the anthroponotic in HIV-infected patients in Thailand. Ann. Trop. Med. Parasitol. 96, 797-802.

Hajdusek, O., Ditrich, O., Slapeta, J., 2004. Molecular identification of *Cryptosporidium* spp. in animal and human hosts from the Czech Republic. Vet. Parasitol. 122, 183-192.

Lebbad, M., Mattsson, J.G., Christensson, B., Ljungstrom, B., Backhans, A., Andersson, J.O., Svard, S.G., 2010. From mouse to moose: multilocus genotyping of *Giardia* isolates from various animal species. Vet. Parasitol. 168, 231-239.

Lebbad, M., Norrgren, H., Naucler, A., Dias, F., Andersson, S., Linder, E., 2001. Intestinal parasites in HIV-2 associated AIDS cases with chronic diarrhoea in Guinea-Bissau. Acta Trop. 80, 45-49.

Lebbad, M., Petersson, I., Karlsson, L., Botero-Kleiven, S., Andersson, J.O., Svenungsson, B., Svard, S.G., 2011. Multilocus genotyping of human *Giardia* isolates suggests limited zoonotic transmission and association between assemblage B and flatulence in children. PLoS Negl Trop Dis 5, e1262.

Palmer, C.S., Traub, R.J., Robertson, I.D., Devlin, G., Rees, R., Thompson, R.C., 2008. Determining the zoonotic significance of *Giardia* and *Cryptosporidium* in Australian dogs and cats. Vet. Parasitol. 154, 142-147.

Pavlasek, I., Ryan, U., 2007. The first finding of a natural infection of *Cryptosporidium muris* in a cat. Vet. Parasitol. 144, 349-352.

Ren, X., Zhao, J., Zhang, L., Ning, C., Jian, F., Wang, R., Lv, C., Wang, Q., Arrowood, M.J., Xiao, L., 2012. *Cryptosporidium tyzzeri* n. sp. (Apicomplexa: Cryptosporidiidae) in domestic mice (*Mus musculus*). Exp. Parasitol. 130, 274-281.

Robinson, G., Wright, S., Elwin, K., Hadfield, S.J., Katzer, F., Bartley, P.M., Hunter, P.R., Nath, M., Innes, E.A., Chalmers, R.M., 2010. Re-description of *Cryptosporidium cuniculus* (Apicomplexa: Cryptosporidiidae): Morphology, biology and phylogeny. Int. J. Parasitol.

Santin, M., Trout, J.M., 2008. Companion animals, In: Fayer, R., Xiao, L. (Eds.) *Cryptosporidium* and cryptosporidiosis. CRC Press, Boca Raton, FL, pp. 437-449.

Santin, M., Trout, J.M., Vecino, J.A., Dubey, J.P., Fayer, R., 2006. *Cryptosporidium*, *Giardia* and *Enterocytozoon bieneusi* in cats from Bogota (Colombia) and genotyping of isolates. Vet. Parasitol. 141, 334-339.

Scorza, A.V., Ballweber, L.R., Tangtrongsup, S., Panuska, C., Lappin, M.R., 2012. Comparisons of mammalian *Giardia duodenalis* assemblages based on the beta-giardin, glutamate dehydrogenase and triose phosphate isomerase genes. Vet. Parasitol.

Scorza, V., Tangtrongsup, S., 2010. Update on the diagnosis and management of *Cryptosporidium* spp infections in dogs and cats. Top Companion Anim Med 25, 163-169.

Sprong, H., Caccio, S.M., van der Giessen, J.W., 2009. Identification of zoonotic genotypes of *Giardia duodenalis*. PLoS Negl Trop Dis 3, e558.

Srisuphanunt, M., Saksirisampant, W., Karanis, P., 2011. Prevalence and genotyping of *Cryptosporidium* isolated from HIV/AIDS patients in urban areas of Thailand. Ann. Trop. Med. Parasitol. 105, 463-468.

Sulaiman, I.M., Jiang, J., Singh, A., Xiao, L., 2004. Distribution of *Giardia duodenalis* genotypes and subgenotypes in raw urban wastewater in Milwaukee, Wisconsin. Appl. Environ. Microbiol. 70, 3776-3780.

Tamura, K., Nei, M., Kumar, S., 2004. Prospects for inferring very large phylogenies by using the neighbor-joining method. Proc. Natl. Acad. Sci. U. S. A. 101, 11030-11035.

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M., Kumar, S., 2011. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol. Biol. Evol. 28, 2731-2739.

Tangtrongsup, S., Scorza, V., 2010. Update on the diagnosis and management of *Giardia* spp infections in dogs and cats. Topics in companion animal medicine 25, 155-162.

Traub, R.J., Inpankaew, T., Reid, S.A., Sutthikornchai, C., Sukthana, Y., Robertson, I.D., Thompson, R.C., 2009. Transmission cycles of *Giardia duodenalis* in dogs and humans in Temple communities in Bangkok--a critical evaluation of its prevalence using three diagnostic tests in the field in the absence of a gold standard. Acta Trop. 111, 125-132.

Traub, R.J., Robertson, I.D., Irwin, P., Mencke, N., Monis, P., Thompson, R.C., 2003. Humans, dogs and parasitic zoonoses--unravelling the relationships in a remote endemic community in northeast India using molecular tools. Parasitol. Res. 90 Suppl 3, S156-157.

Wang, R., Zhang, L., Ning, C., Feng, Y., Jian, F., Xiao, L., Lu, B., Ai, W., Dong, H., 2008. Multilocus phylogenetic analysis of *Cryptosporidium andersoni* (Apicomplexa) isolated from a bactrian camel (*Camelus bactrianus*) in China. Parasitol. Res. 102, 915-920.

Xiao, L., Cama, V.A., Cabrera, L., Ortega, Y., Pearson, J., Gilman, R.H., 2007. Possible transmission of *Cryptosporidium canis* among children and a dog in a household. J. Clin. Microbiol. 45, 2014-2016.

Xiao, L., Morgan, U.M., Limor, J., Escalante, A., Arrowood, M., Shulaw, W., Thompson, R.C., Fayer, R., Lal, A.A., 1999. Genetic diversity within *Cryptosporidium parvum* and related *Cryptosporidium* species. Appl. Environ. Microbiol. 65, 3386-3391.

CHAPTER 6: CONCLUSIONS AND FUTURE DIRECTIONS

6.1 Conclusions

Giardia and *Cryptosporidium* are leading infectious causes of diarrhea in humans, domestic animals, and wildlife throughout the world. These infections can lead to significant morbidity and mortality in developing and developed countries. The genera *Giardia* and *Cryptosporidium* include both host-specific and zoonotic species/genotypes, and dogs and cats can be infected with zoonotic genotypes and potentially serve as a reservoir for zoonotic transmission. Understanding the prevalence and risk factors associated with *Giardia* and *Cryptosporidium* infections as well as the genotypes/species of the organisms that infect dogs and cats could aid veterinarians in the control and prevention of these infections and reduce the potential for zoonotic transmission. The major goals of this dissertation were to estimate the prevalence of *Giardia* spp. and *Cryptosporidium* spp. infections in dogs and cats in Chiang Mai, Thailand, to determine risk factors associated with these infections including seasonality, and to molecularly characterize the genotype/species of *Giardia* and *Cryptosporidium* isolates from these animals to determine whether infected dogs and cats can be a potential reservoir for zoonotic transmission.

This dissertation describes epidemiological studies of *Giardia* and *Cryptosporidium* in dog and cat populations in Chiang Mai, Thailand. The results suggest that *Giardia* and *Cryptosporidium* infections in dogs and cats are common. Significant risk factors associated with *Giardia* and *Cryptosporidium* infections were young age, having diarrhea or chronic diarrhea, and living in crowded settings. The prevalence of *Giardia* infection in the rainy season is higher than during the dry winter season. The prevalence of *Cryptosporidium* was higher in winter than in the rainy season. Multilocus analyses of *Giardia duodenalis* genotypes and *Cryptosporidium*

species suggested that dogs and cats in this area were infected by a canine-specific *G. duodenalis* and that *C. parvum* was present in about 1/3 of the tested samples. This finding documents transmission of C. parvum to dogs, likely from ingesting materials contaminated with bovine feces and suggests that people may also be commonly exposed to this agent. However, the work as designed does not address whether or not dogs are associated with transmission of *C. parvum* to people. The results also suggested that using multiple loci is better than targeting a single gene, as a polymerase chain reaction (PCR) assay on one target gene may be able to detect the presence of organisms where other assays do not. However, if testing must be limited to single gene detection, *Giardia* gdh PCR and one-step *Cryptosporidium* SSU-rRNA are recommended.

In the studies presented in this dissertation, sugar flotation was used to concentrate the fecal samples to enhance the detection of *Giardia* cysts and *Cryptosporidium* oocysts by immunofluorescent assay (IFA). In some situations, the freezing of samples is unavoidable, such as when international shipment or long-term storage is required. A comparison of the sugar and sedimentation concentration techniques was performed to assure that using sugar technique was appropriate. Although there were no significant differences between the two tests, for best results I recommend using the sedimentation concentration technique for *Giardia* cysts or *Cryptosporidium* oocysts to prepare frozen samples for IFA.

6.2 Future direction

6.2.1 National study

The results presented in this dissertation show that *Giardia* and *Cryptosporidium* infections in dogs and cats are common; however, in previous reports the prevalence, risk factors and genotype/species of these organisms varied depending on geographical area. Therefore, further study on a national or region level should be designed to better understand the nature of

these diseases in Thailand. The prevalence of *Giardia* and *Cryptosporidium* infections in dogs and cats reported in this dissertation could be used for sample size estimation in future studies.

6.2.2 Determining the potential for *Giardia* and *Cryptosporidium* zoonotic transmission from dogs and cats to their owners.

Although most of the *Giardia duodenalis* assemblages and *Cryptosporidium* species in this study were dog-adapted, 30% of *Cryptosporidium* isolates found in our study were the zoonotic species. In addition, *C canis and C. felis* have been isolated from HIV+ patients. Therefore, the potential zoonotic transmission of these two organisms from dogs and cats to humans is still of interest. In addition, in Chiang Mai, many dogs and cats are allowed to run free. This behavior could increase the risk of *Giardia* and *Cryptosporidium* infection by direct contact with the infected animals. Especially, in the dairy farm or other types of livestock, dogs and cats could serve as a potential reservoir for zoonotic transmission. Further studies based on molecular analyses of *Giardia* and *Cryptosporidium* isolates from dogs and cats and their owners and/or livestock could establish whether a zoonotic link exists from dogs and cats to humans.

APPENDIX: QUESTIONNAIRE

Questionnaire

"Prevalence and molecular analysis of *Giardia* and *Cryptosporidium* spp. isolates from dogs and cats in Chiang Mai, Thailand"

General information

Species: __ Dog __Cat
Age: ___ year(s)___month(s)
Sex: __Male __Female __Intact __spayed/neutered
Breed:_____
Area: subdistrict _____, District_____, Postcode_____

Risk information

1. How many dogs/cats do you own?
   ____ dog(s) ____ cat(s)

2. Where did you get this dog/cat?
   __ bought from pet shop/farm __ in house bred
   __ adopt from strayed dog/cat __ other (please indicate)_____

3. Please indicate the type of the household?
   __ home (owned dog) __ breeding farm __ animal shelter
   __ temple __ strayed dog __ other (please indicate) _____

4. How do you keep your dogs/cats?
   __in house ___ hours __ out side ___ hours __free roaming

5. Do you cage your dogs/cats?
   __No __ Yes __sometimes

6. Do you allow your dogs/cats as a free roaming animal?
   __ Never let animal out of the house area
   __ Yes, always allow free roaming outside.
   __ Restrict free roaming time ____ hours every day

7. Where does your dog/cat defecate?
   __ in household area __ out of the household area
   __ in the litter box

8. After you dog/cat defecate, do you
   __ pick up/clean the area immediately. __ did not pick up/clean the area immediately.

9. Your dog/cat drinking water is from
   __ tap water __bottle water __roof harvested water __Well
   __ underground water __river_ _other (please indicate) _____

10. The water was boiled before given to dog/cat?
    __no __yes __sometimes

11. Food type:
    __home made __ commercial food __mixed

12. Have you ever let your dog/cat eat raw meat?
    __no __yes
    If yes: you give __pork __beef __poultry (chicken/duck) __fish
    __other (please indicate)_____

13. Within past month have you dog/cat had chronic diarrhea (7-10 days)?
    __no __yes

14. Have your dog/cat had regular deworming program?
    ___no
    __ yes __ every year
    __ every 6 month
    __ every 3 month
    __ other_____

15. Latest deworming was _____ .

16. Your dog/cat has concurrent disease?
    __no __yes, what is it?_____

17. Your dog/cat is on immunosuppressant drug/corticosteroid?
    __no __yes __ I don't know

18. Within past month have your dog/cat had any clinical signs

Clinical signs	No	Yes
Fever		
Anorexia		
Lethargy		
Vomiting		
Dehydrate		
Bloody diarrhea		
Watery diarrhea		
Pale		

19. Have you had any another animal?

__ no __yes

Plase indicate.

Animal	Number	**Animal**	Number
☐ Pig		☐ Rodent	
☐ Cow		☐ Rabbit	
☐ Buffalo		☐ Chicken	
☐ Goat		☐ Duck	
☐ Sheep		☐ Other……	

20. Within past month, have these animals had diarrhea?

__no

__yes had diarrhea for___ days

__don't know

21. Is your household located near any of the below water resources?

Water resources	Distance					
	Don't know	< 0.5 km.	0.5 – 1 km.	1.01 - 2.5 km.	2.51 – 5 km.	> 5 km.
1. River						
2. Irrigation canal						
3. Swamp						
4. Dam						
5. Other.....						

22. How often you take your dog/cat for water recreation?

Water resources	Frequency					
	Never	< 1-2 times/week	1-2 times/week	3-4 times/week	5-6 times/week	everyday
1. River						
2. Irrigation canals						
3. Swamp						
4. Dam						
5. Other.....						

23. Is your household located near agricultural activity?

Agriculture	Distance					
	Don't know	< 0.5 km.	0.5 – 1 km.	1.01 - 2.5 km.	2.51 – 5 km.	> 5 km.
1. Dairy farm						
2. Feedlot						
3. Poultry						
4. Rice field						
5. Orchard						
6. Fishery						
7. Other.....						

24. How many members in household?, Have anyone had diarrhea and been taking antibiotics?

Member (Indicate only gender)	Age (year)	Diarrhea	Duration (day)	Receiving Antibiotics
1.Female (example)		☐ no ☐yes ☐don'tknow		☐ no ☐yes ☐don'tknow
2.Male		☐ no ☐yes ☐don'tknow		☐ no ☐yes ☐don'tknow
3.		☐ no ☐yes ☐don'tknow		☐ no ☐yes ☐don'tknow
4.		☐ no ☐yes ☐don'tknow		☐ no ☐yes ☐don'tknow
5.		☐ no ☐yes ☐don'tknow		☐ no ☐yes ☐don'tknow
6.		☐ no ☐yes ☐don'tknow		☐ no ☐yes ☐don'tknow

(Please use space on backside, if the table is not enough.)

25. Have your household every had flooding problem?

__never
__within past months __within 1-3 months
__within 4-6 months __within 6-12 months
__more than 1 year
__don't know

Owner's knowledge

1. Diarrhea in dog/cat can be from
   __ fungi/mold __bacteria __virus __parasite __protozoa __don't know
   __other _____

2. Do you know Giardia/cryptosporidium?
   __ no
   __ know *Giardia*
   __ know *Cryptosporidium*

3. Do you know if you have zoonottic *Giardia* or *Cryptosporidium* infection you can transmit the protozoa to your pet?
   __no __yes

4. Do you know if your dog/cat has zoonottic *Giardia* or *Cryptosporidium* infection, it can be transmitted to you?

   __no __yes

5. What is your occupation

☐Government officer	☐Office worker	☐Retired
☐Medical service	☐working in day car/nursery	☐Freelance
☐Agriculture(animal)	☐Agriculture (plant)	☐Self-employed (business)
☐Student	☐Academic	☐Other .....

6. What is your highest education?

☐Elementary School	☐Bachelor degree or equivalence
☐Junior high school	☐Master degree or equivalence
☐High school or equivalence	☐PhD
☐Diploma	☐Other.....

7. Your income?
   - ☐< 5,000 Baht
   - ☐5,001-10,000 Baht
   - ☐10,001-25,000 Baht
   - ☐25,001-50,000 Baht
   - ☐> 50000 Baht
8. Your household income?
   - ☐< 5,000 Baht
   - ☐5,001-10,000 Baht
   - ☐10,001-25,000 Baht
   - ☐25,001-50,000 Baht
   - ☐> 50000 Baht