DISSERTATION

A STUDY OF FACTORS ASSOCIATED WITH *GIARDIA* AND *CRYPTOSPORIDIUM* INFECTIONS IN HUMANS, DOGS AND CATS IN THE USA

Submitted by

Hanaa Thigeel

Department of Clinical Sciences

In partial fulfillment of the requirements

For the Degree of Doctor of Philosophy

Colorado State University

Fort Collins, Colorado

Fall 2016

Doctoral Committee:

Advisor: Michael Lappin
Co-Advisor: Francisco Olea-Popelka

David Twedt
Doreene Hyatt

Copyright by Hanaa Thigeel 2016

All Rights Reserved

ABSTRACT

A STUDY OF FACTORS ASSOCIATED WITH *GIARDIA* AND *CRYPTOSPORIDIUM* INFECTIONS IN HUMANS, DOGS AND CATS IN THE USA

Giardia spp. and *Cryptosporidium* spp. are two of the leading causal agents of parasitic diarrhea in humans, dogs and cats. The two pathogens contain both host-adapted and zoonotic strains and dogs and cats can harbor both strains. There is critical need to understand factors potentially associated with the risk and prevalence of infection due to *Giardia* spp. and *Cryptosporidium* spp. in dogs, cats and humans. This will ultimately aid in disease management and control. Furthermore, molecular characterization of the human, dog or cat isolates may identify zoonotic genotypes and may provide further information concerning the transmission routes between humans, dogs and cats.

In Chapter 1, a review of literature regarding *Giardia duodenalis* and *Cryptosporidium* spp. in humans and companion animals (dogs and cats) was conducted. The review involves a brief description of the two pathogens' current taxonomy, epidemiology, and diagnostic methods.

Chapter 2 presents a retrospective study designed to analyze results from dog and cat polymerase chain reaction (PCR) panels from the commercial laboratory, ANTECH Diagnostics. The main purpose of this study was to evaluate associations between the probability of testing positive to *Giardia* spp. and *Cryptosporidium* spp. and risk factors such as animal's age, sex, region, and season. The results of this study showed that age (younger animals) was significantly associated with the risk of PCR positive results for *Giardia* spp. and *Cryptosporidium* spp. in

both dogs and cats. Region was significantly associated with *Cryptosporidium* spp. in both dogs and cats, whereas season was only associated with *Giardia* spp. in dogs.

Chapter 3 describes the validation and optimization a previously published 60 kDa glycoprotein (gp60) gene-based PCR assay. The objective of this study was to use the assay to subtype *C. parvum* and *C. hominis* isolated from human fecal samples. The analytical sensitivity of this PCR assay was determined by assaying serial dilutions of *C. parvum* oocysts and *C. hominis* DNA. The analytic specificity was determined by assaying *Cryptosporidium* and non-*Cryptosporidium* spp. DNA. The gp60 PCR assay consistently detected DNA of *C. parvum* if oocysts were present at 10^4/mL. The assay was detected the DNA of *C. hominis* in the lowest concentration.

In Chapter 4, a prospective study was conducted to assess the risk of factors potentially associated with *Giardia duodenalis* and *Cryptosporidium* spp. infections and estimate the prevalence of these two pathogens in senior veterinary students and their pet dogs and cats. A structured questionnaire was developed to assess a baseline exposure of the students to large and small animals. In addition, a single voluntary sample was requested from students and their dogs or cats that live within the household. *Giardia duodenalis* and *Cryptosporidium* spp. were detected by the PCR and immunofluorescence (IFA) assays in students and their dogs and cats. As a result of the recruitment, 51 surveys, 42 human fecal samples, 31 dog fecal samples, and 17 cat fecal samples were collected. Clinical rotation, track preference, gender, pet ownership and farm exposure were factors selected to be evaluated for the risk of both pathogens in senior veterinary students. As a result of this evaluation, none of these factors selected was statistically associated with the risk of infection due to *G. duodenalis* or *Cryptosporidium* spp. All *Giardia*

isolated from dogs were host-adapted assemblages. However, a zoonotic *Cryptosporidium* genotype (*C. parvum* subtype family IIa) was identified in one human sample.

The analysis conducted in this dissertation provided an evaluation of potential risk factors associated with giardiasis and cryptosporidiosis in pet dogs and cats. The results of this research enhanced the understanding of the disease prevalence of *Giardia* spp. and *Cryptosporidium* spp. among senior veterinary students and their dogs and cats. The survey collected valuable and novel information on the students' characteristics, student health status, their pets' health status and activities that may have led to an increased risk of infection during their clinical rotations or intense handling of small or large animals. The analysis of the survey provided an evaluation of potential risk factors associated with the risk of infection in senior veterinary students. Molecular analysis of isolates of human, dog and cat origin helped in differentiating between *G. duodenalis* assemblages and *Cryptosporidium* spp. genotypes.

Future directions may include an evaluation for associations of positive test results with clinical findings and further studies determining the likelihood dogs or cats are carrying zoonotic *Giardia* spp. or *Cryptosporidium* spp.. National research is recommended to be conducted to identify risk factors in veterinary students from different states in the United States. Additionally, a larger study should be performed to determine the baseline exposure of veterinary school faculty, specifically, those who work on large animal rotations and collect fecal samples from their pet dogs and cats to for genotyping to detail whether zoonotic infections with these two protozoans occur.

ACKNOWLEDGMENTS

I must offer my sincere thanks and appreciation to my advisors, Dr. Michael Lappin and Dr. Francisco Olea–Popelka, for their support and encouragement to complete my research and pursue my interests. Dr. Lappin has been supportive and has provided valuable guidance throughout my training. I highly appreciate and give special thanks to Dr. Olea–Popelka for his patience and support both academically and morally over this journey. Without my advisors, I would never have reached this point of my study.

Thanks to my committee members, Dr. Doreene Hyatt and Dr. David Twedt, for supporting me in the first phase of this program, particularly, in my preliminary examination.

Special thanks to Dr. Valeria Scorza for her patience, support, and laboratory training. Beyond providing guidance as a Research Scientist, Dr. Scorza became a valuable friend who supported me personally, through good and bad times over the last three years of my study.

Special thanks go to Dr. Lappin's laboratory personnel in the Center for Companion Animal Studies for allowing me to use the laboratories and for their guidance and teaching. Thanks to Ms. Jennifer Hawley for taking care of the student financial compensation which was part of my study.

Special thanks to the Animal Population Health Institute, which I am a member of, for the financial support in the first two years of this Ph.D. program. Thank you to Dr. Lyndsey Linke, a Research Scientist and the APHI Laboratory Coordinator, for being a good, supportive friend and for her guidance in the laboratory diagnostic part of this project.

Thanks to ANTECH® Diagnostics, particularly Dr. David Aucoin for facilitating and providing the dataset that was used in chapter 2 of this dissertation.

My thanks and appreciation to Dr. Paula Cowen for her support both morally and financially. I thank her for being an aspiration to me. She taught me to never give up and always start again. I thank her family for being my best friends and family. Thank you to Dr. John Belfrage, and the boys, Brice, Seth and Sean Belfrage.

Last but not least, I would like to thank my friends in the USA and outside the country. Special thanks to Ms. Loretta Scott in Greeley, Colorado for her continuous encouragement to pursue my degree. Special thanks go to my friends: Huda Tawir in Fort Collins, Colorado; Estabraq Abdulhadi in Detroit, Michigan and Hanaa Salih in Plymouth, England for their emotional support, much like a sister.

My heartfelt thanks go to my family in Iraq: my wonderful, loving husband Bahaa Abosaooda and my two brothers: Ali and Raeid Thigeel for their support and love.

A portion of this project was funded by the Research Council of the College of Veterinary Medicine and Biomedical Sciences (CRC) and the Young Investigator Grant program in the Center for Companion Animal Studies at Colorado State University.

DEDICATION

This dissertation is dedicated to my mother. May God rest her soul in peace

TABLE OF CONTENTS

CHAPTER 1: *GIARDIA DUODENALIS* AND *CRYPTOSPORIDIUM* SPP.*:* A REVIEW

Introduction to Internal Parasites:

Most internal parasites that infect dogs and cats and colonize in the gastrointestinal tract are worms such as hookworms, roundworms, whipworms and tapeworms and single-celled organisms such as coccidia and *Giardia*.[1] In this chapter, *G. duodenalis,* and *Cryptosporidium* spp. will be addressed in humans, dogs and cats.

1.1 *Giardia duodenalis*

1.1.1 Taxonomy

Based on the new systematic data for the taxonomical classification of the parasite, *Giardia duodenalis* belongs to the kingdom: Protista; subkingdom: Protozoa; phylum: Metamonada; subphylum: Trichozoa; class: Trepomonadea; subclass: Diplozoa; order: Giardiida and Family Giardiidae.[2]

Giardia was first described in 1859 by Lambl.[3] He described a flagellate in the human intestine that he named *Cercomonas intestinalis*. In 1875, Davaine described a strain of *Giardia* that infected a rabbit and called it *Hexamita duodenalis*.[3] Even though the generic name described by Davaine was incorrect, Filice (1952) proposed that this name is valid and the species name (*duodenalis*) has priority over (*intestinalis*) according to the Rules of Zoological Nomenclature.[4] The latter generic name of *Giardia* was accepted by several scientists to be used to name isolates from humans and animals. Filice suggested in a later evaluation that the taxonomy of *Giardia* would be more meaningful if it was based on the organism morphology not the host due to the lack of reliable experimental evidence.[3,4] Thus, Filice divided *Giardia* into three morphologically distinct groups based on the shape of the trophozoites and the median bodies. In short, the current scientific name of *Giardia* is *Giardia duodenalis*. The use of *G.*

lamblia or *G. intestinalis* interchangeably is based on personal preference and has no taxonomic justification.[3,4]

Giardia duodenalis trophozoites, which was the first group of organisms classified by Filice, are pyriform-shaped and have a distinctive "claw-hammer" median body and adhesive disc as well as four pairs of flagella[5] (Figure 1.1). The second group, *G. muris* species trophozoites have rounded median bodies and a rounder trophozoite shape. The third group is *G. agilis* whose trophozoites have long narrow bodies and long club-shaped median bodies. Three more species were later identified including *G. psittaci*, *G. ardeae* and *G. microti* (Table 1.1).[6-8,10]

Giardia spp. are single celled organisms that share many biological characteristics with anaerobic prokaryotes.[6,9] Laboratory classification of *G. duodenalis* strains defined all species using the ribosomal RNA gene sequencing.[11,12] In fact, *G. duodenalis* genotypes are named after identifying substantial sequence differences in the glutamate dehydrogenase (GDH), triose-phosphate isomerase (TPI) and β-giardin (βG) genes using phylogenetic analysis.[11,12] The closely related genotypes are grouped into assemblages and sub-assemblages. Recent genotypic classification of *G. duodenalis* isolates has identified a change in the rule of sub-assemblage grouping that is based on three different loci not genes. Due to the inconsistency of the usage of genotype, assemblage, sub-assemblage, and sub-genotype in international literature, and because sub-genotype means under the category genotype, one study suggested avoiding the use of sub-genotype and recommended using genotype, assemblage, and sub-assemblage terms only.[11,12]

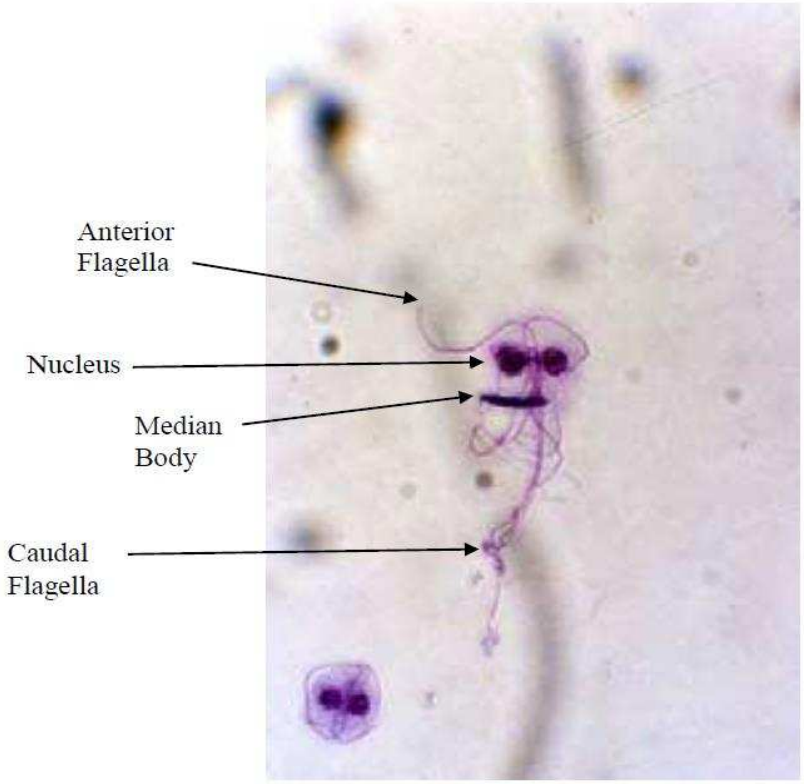


Figure 1.1 Giemsa-stained trophozoite of *Giardia duodenalis* showing multiple flagella, nuclei and median bodies[a]
[a]Adapted from Thompson, RC. The zoonotic significance and molecular epidemiology of *Giardia* and giardiasis. *Vet Parasitol.* 2004; 126:15–35. doi: 10.1016/j.vetpar.2004.09.008.[11]

A proposed nomenclature for *Giardia duodenalis* A-G Assemblages has been recently published. In that proposal, assemblage A is called *G. duodenalis*, assemblage B is *G. enterica*, assemblage C and D are *G. canis*, assemblage E is *G. bovis*, assemblage F is *G. cati*, and assemblage G is *G. simmondis.*[13]

1.1.2 Epidemiology

Giardiasis was recently added to the World Health Organization's Neglected Disease Initiative. [14] This initiative includes a spectrum of parasitic, bacterial, and viral diseases that are widespread in developing countries. Major risk factors that influence the occurrence of the disease in such countries can include poverty, climate, and lack of access to medical services. These factors, when considered together, lead to an increase in the disease global burden.[14] Multiple factors contribute to the variability in the prevalence estimates of *Giardia* infection. First, giardiasis is only reportable in some countries. Second, the diagnostic methods used have variable analytical sensitivity and specificity. Third, many infected people in endemic areas are asymptomatic and have no access to the medical care nor they do not seek out medical treatment.[15] Regardless, *G. duodenalis* is the most common causes of protozoal diarrhea in most countries in the world.[15] In a meta-analytic study of giardiasis and cryptosporidiosis in European countries, it has been estimated that the prevalence of *Giardia* is 3.0% in asymptomatic human patients and 6.0% in symptomatic ones.[16] In a review article published evaluating 33 different studies, it was found that prevalence estimates varied drastically and the risk was higher in rural areas, among poor communities, in males, among college students, in the elderly, in HIV-positive patients, and in patients with gastric carcinoma.[17] It also has been reported that co-infections with other parasites frequently occur in patients with giardiasis.[18] A mix of prospective and retrospective studies in the Netherlands was used to determine the incidence of gastroenteritis due to parasitic agents. The target population ages ranged from infants to 65 years old. The authors determined the incidence of giardiasis was 3.3% in asymptomatic people compared to 5.4% in the symptomatic ones. [19,20] In several European countries, the overall incidence rate is about 58 cases per 100,000 persons.[19]

The incidence rates of giardiasis in people in the United States ranged from 1-30 cases per 100,000 during the period from 2003-2005. The number of annual reported cases per 100,000 people in the United States is higher in the northern states than that in the southern states.[18]

Giardia outbreaks vary in their occurrence due to the season. For instance, a marked seasonality in the onset of illness was described in early summer through early fall.[18] Also, the odds of disease transmission through summer time is two times higher than other seasons due to the increased outdoor activities and swimming.[15]

Giardia outbreaks are most frequently waterborne, i.e. the consumption of contaminated drinking water).[22,23] Before 2007, more than 100 water-related *Giardia* outbreaks were reported worldwide.[24] It was reported that *G. duodenalis* was responsible for about 132 out of 325 waterborne outbreaks recorded from World War I until 2003. Of these 132 *Giardia* outbreaks, 103 (78%) were associated with contaminated drinking water systems.[24] In 2010, a large *Giardia* outbreak in Belgium was caused by consumption of drinking water contaminated with river water.[25] Generally, waterborne giardiasis is more important than foodborne giardiasis[26] because of the large water bodies such as rivers or lakes that serve as water supplies for a large communities; thus, if water is contaminated with viable *Giardia* cysts and distributed, a large number of individuals who drink this contaminated water can become infected. Epidemiologically this is called a point-source outbreak. Transmission in this type of outbreak is terminated when the source of contamination is eradicated. Interestingly, the majority of waterborne outbreaks were reported in the United States where improved contamination detection and monitoring systems for drinking water supply are more likely to be in place. In the underdeveloped countries, giardiasis is considered an endemic disease where water supply

detection and monitoring systems are usually not in place which puts these countries at an increased risk of giardiasis.[27] However, in recent years, it was reported that outbreaks associated with drinking water have been significantly reduced due to the establishment of water treatment and regulations.[26]

Giardia duodenalis also has been identified as the cause of recreational water outbreaks.[28-30] These waters include swimming and wading pools, thermal and other natural springs, fresh and marine waters, water parks, interactive fountains, and any other places where water contact occurs. Contamination of water bodies occurs due to urban and non-urban run-off, industrial pollution, storm waters, and human or animal fecal matter. Contamination of swimming pools is often associated with accidental fecal contamination, poor pumping, poor filtration systems, and insufficient use of disinfectants.[28-30]

In terms of sporadic cases, the routes of transmission usually are unknown. Some studies have identified potential risk factors for giardiasis that include: person to person transmission, travel to endemic areas, interaction with livestock and consumption of potable and recreational fresh water.[15] In addition to the risk factors of sporadic giardiasis mentioned above, other studies have determined the cause of sporadic cases is because of consumption of contaminated lettuce, drinking treated tap water, and consumption of green salads on a daily basis.[31,32]

Foodborne giardiasis has received relatively little attention compared to waterborne outbreaks and few outbreaks have been reported. These outbreaks have implicated mainly food handlers, but direct contamination of foodstuffs is possible. Regardless, foodborne *Giardia* outbreaks are underreported due to the low number of cases identified.[27]

1.1.2.1 Agent

Giardia cysts are relatively small in size (8-12 µm).[33] One feature that influences *Giardia*'s infectivity is that the cysts are readily infectious when excreted in feces into the environment. Furthermore, the cysts are extraordinarily stable in the environment and can survive from weeks to months.[15] The life cycle of *Giardia duodenalis* consists of two key stages: a trophozoite stage and a cyst stage.[34,35] An infectious *Giardia* cyst excysts in the upper part of the small intestine to release the two trophozoites. After ingestion by the host, the trophozoites will attach to the intestinal epithelial wall and reproduce asexually by the binary fission. The trophozoite encysts again after an exposure to the biliary salts to produce immediately infectious cysts when passed via feces to the environment. This life cycle is completed within 72 hours after ingestion.[34,35]

1.1.2.2 Host

Giardia duodenalis is distributed worldwide and it causes diarrhea in variety of hosts including humans. *Giardia duodenalis* is transmitted via ingestion of the cysts, commonly called the fecal – oral route, either directly or indirectly.[12] Giardiasis is usually reported in younger populations that range in age from 1 to 9 years old and younger adults from 35 to 39 years. Older hosts can develop adaptive immunity which results in less cyst excretion; however, they also can be considered as a source of infection.[36] Research has been conducted to study the adverse health effects of *Giardia* infection in children from underdeveloped countries. The research involved investigating whether the health effects followed by *Giardia* infection are temporary or cause long-term health problem such as poor cognitive function or death. However, the possibility that *Giardia* causes long term health problems determined from such research have been controversial.[15] A study conducted in Guatemala concluded that the reduced age effect observed

in diseased children compared to non-diseased was confounded with co-infections with other endo-parasites.[37] The parents of infected children, especially mothers, are more prone to infection with *Giardia* compared to the general public and outbreaks of giardiasis have been reported in daycare centers.[15] Additionally, children who have the voluntary consumption behavior that tend to eat mud or sand (also known as geophagia or 'pica') are more susceptible to giardiasis.[38] The host immune system can be suppressed due to malnutrition, HIV infection, cancer, and immune-suppressive therapy. Immunity can influence the severity of infection due to giardiasis in the host. For example, giardiasis is more frequently reported in immune-compromised individuals compared to immune-competent individuals.[39] However, the severity of giardiasis in HIV positive individuals is not significantly different from HIV negative individuals.[39] Also, in immune-compromised individuals' giardiasis tend to become a chronic infection.[40]

Physiopathology of Giardiasis

People can be infected with *G. duodenalis* without exhibiting any symptoms and can be considered healthy carriers.[12] The mechanism of pathogenesis that causes some individuals to develop clinical signs and others to remain asymptomatic still is not fully understood (Figure 1.2). Personal risk factors such as the immune status, nutritional status and age of the host can be potential risk factors for acquiring the disease. Also, environment-related factors can be responsible for differences in the severity of infection.[11] Infection with *Giardia* produce alterations in villus and microvillus which can cause decreased crypt/villus ratio, shortening of the microvillus brush border and brush border enzyme deficiencies. This outcome is due to trophozoite/epithelium interaction and host immune reaction. Also, pathogenesis of giardiasis can involve enterocyte apoptosis along with cytoskeletal re-organization induced by trophozoite

toxic products which result in an increased epithelial permeability and local disruption of tight-junctional proteins. [41-43] The toxins excreted by the trophozoites along with the T-cell activation lead to a diffuse shortening of brush border microvilli and a decreased activity of the small intestinal brush border enzymes, especially lipase, some proteases and the disaccharidases lactase, and maltase[44] Malabsorption due to giardiasis is associated with an increased number of intraepithelial lymphocytes and a decreased villus to crypt ratio. The malabsorptive diarrhea can lead to a lower weight gain.[45] In addition, the presence of the mucous in diarrheal stool is due to the reduced activity of lipase and the increased production of mucine by goblet cells.[45] Giardiasis can lead to decreased transit time of food in the gut and an increase in gut contractility. The increased contractility may explain the abdominal cramps that frequently are reported in giardiasis.[46]

1.1.2.3 Environmental risk factors

Generally, surface water can become contaminated with *Giardia* cysts through the discharge of untreated human sewage and/or from urban or rural land drainage containing animal fecal waste, especially livestock feces. In fact, the presence of cattle and livestock around water resources is considered a risk factor for water contamination with *Giardia* cysts, especially if these animals are infected with this protozoan.[12] Usually large rivers and lakes receive agricultural runoff and both treated and untreated local wastewater.[47] Additionally, aquatic rodents such as muskrats, beaver, nutria and wild otter play an important role in water contamination with *Giardia* spp. cysts.[12]

One of the risk factors associated with *Giardia* contamination of the environment is that *Giardia* spp. cysts are readily infectious to the new host once they are excreted in the environment and do not require sporulation.[48] These cysts are hardy and can survive in surface

water and soil for 2-3 months, but the cysts do not persist well in cold environments.[49] Another factor is that a large number of cysts are shed to the environment from infected animals. The prolonged excretion period of infectious cysts to the environment can be another factor that go hand in hand with the high excretion rate from infected individuals that contribute to a significant environmental contamination.[27]

Several challenges can contribute to the persistent of *Giardia* in the environment. According to previous studies, *Giardia* cysts may remain viable or infective for at least one month at low temperatures and in the absence of freeze-thaw cycles.[50,51] The survival of the cysts is due to their filamentous cyst wall. This wall contains an even ratio of carbohydrate to protein. The filament has a unique type of carbohydrates that is not degradative by any enzyme.[52] Second, *Giardia* cysts are resistant to many water treatment procedures which means treated water can be contaminated with *Giardia* cysts. Third, some *Giardia* assemblages can be zoonotic. This increases the potential for ongoing environmental spread of contamination by many mammals.[27] Fourth, the spread of disease by transport hosts such as wild and aquatic birds and insects can facilitate environmental contamination by *Giardia*.[53-55]

1.1.3 *Giardia* in Humans

The infective dose of *G. duodenalis* for symptomatic humans is relatively low and is thought to be about 10 to 100.[15] Infected humans shed *Giardia* cysts intermittently[12] up to 2 x 10^6/gram of feces.[56] Human giardiasis can range from mild or self-limiting illness to severe or life threatening. Common clinical symptoms include abdominal cramps and diarrhea, bloating and flatulence, nausea and weight loss.[12] The illness usually lasts from 1-2 weeks, but in some cases it can extend to up to seven weeks. Giardiasis can become chronic illness in malnourished children.[57]

Giardia duodenalis usually inhabits the upper part of the small intestine.[58] This can be attributable to agent factors such as the virulence of the infective strains or the infective dose (i.e. number of cysts ingested) or host factors such as age, the status of the immune system at the time of infection.[15] Human giardiasis can be classified into acute and chronic phases. The acute phase is usually short, characterized by flatulence and abdominal distension with cramps, small bowel watery diarrhea that becomes greasy, and bulky with mal odors. The chronic phase of giardiasis includes malaise, weight loss, and other features of malabsorption. Diarrhea in the chronic phase is characterized as pale or yellow, frequent and small in volume.[39]

1.1.4 *Giardia* in Companion Animals

The *G. duodenalis* assemblages responsible of infection in dogs are C and D whereas assemblage F is responsible for infection in cats. However, assemblages A and B can also infect both dogs and cats.[59] The estimated prevalence of *G. duodenalis* in companion animals varies depending on the region of the study, the diagnostic method used and the general health condition of the host. In the USA, the prevalence estimated for 38 kennel/shelter dogs using microscopic examination was 39.0%, whereas the prevalence estimate for 79 household dogs using the same method of diagnosis was 34.0%.[60] In another study conducted in the USA, the estimate of the prevalence was 15.6% for 16,064 clinically affected dogs using a immunochromatography (ICG) method[61] whereas the prevalence estimate was only 4.0% for 1,119,293 clinically affected dogs in a study in the USA using microscopic examination.[62] The prevalence estimates of *Giardia* in 211,105 cats in a study conducted using microscopic examination was relatively low (0.58%) compared to dogs.[63]

Young animals tend to shed more cysts than adult animals do, and this can be attributed to the slow development of adaptive immune system by the host.[64] Thus, young animals may be

more likely to transmit the agent directly to other susceptible hosts as well as contribute to the environmental contamination with *Giardia* cysts.[59]

1.1.5 Zoonotic Consideration of *Giardia duodenalis*

Giardia duodenalis was characterized as zoonotic agent by the World Health Organization because of the waterborne outbreaks in people by infected beavers.[65] Zoonosis means disease can be transmitted from animals to humans.[66,14] Reverse zoonosis (i.e. infection from humans to animals) can also occur with giardiasis.[67] The clinical impact of zoonotic transmission due to *Giardia* has not been explained yet. Among *G. duodenalis* eight assemblages that include assemblage A-H, assemblages' A and B are isolated in both humans and animals.[67] Assemblage A has four subgroups (AI, AII, AIII, and AIV). These subgroups were described by the analysis of 10 isolates at 23 genetic loci in a study. The subgroup of assemblage A normally isolated from humans is AII. Subgroups AIII and AIV are frequently isolated from animals. The subgroup AI is isolated from humans and animals' isolates; thus, it is the only subgroup that has a zoonotic potential.[68] Additionally this study supported the existence of four subgroups of assemblage B (BI, BII, BIII and BIV).[68] The zoonotic potential among the subgroups is minimal if it exists.[68] In this study, one human isolate characterized in subgroup BIII also was close to subgroups BI and BII. Assemblage B subgroups BIII and BIV are commonly isolated from humans. Animal isolates belong to subgroups BI and BII. Assemblage A and B can be maintained by direct transmission between humans. In addition, these two assemblages can infect companion animals, livestock and wildlife.[3]

Giardia isolates in assemblage A have a greater zoonotic risk than isolates in assemblage B.[69] However, some studies have reported the zoonotic potential for assemblage A only.[70,71] For example, a prospective study conducted in daycare centers in Western Australia concluded that

the odds of diarrhea in children infected with assemblage A were 26 times higher than with assemblage B of *Giardia* isolates.[72] Furthermore, in a study conducted in Bangladesh, patients infected with assemblage A (genotype AII) isolates had the highest probability of developing diarrhea compared to patients infected with assemblage B.[72-74] However, several studies have shown that assemblage B patients can also develop persistent diarrhea.[75,76] These studies reveal evidence of genetic variation between and within the genotypes[3,71,77]

In most case-control studies, companion animals appeared to be negatively associated with risk to their owners.[47] In fact, the issue of whether there is potential zoonotic transfer of *Giardia* spp. infection between companion animals and their owners is controversial. Numerous studies conducted in different parts of the world demonstrated that dogs or cats can be infected with host-adapted and/or zoonotic *Giardia* assemblages.[78,79] However, there is a lack of data that support the frequency of zoonotic transmission in dogs or cats to humans.[11,78] A study conducted in northern India indicated that there is potential that dogs that live in close contact with humans can transmit *Giardia* to humans, but the molecular data of the study was rather unconvincing.[80] In contrast, a study conducted in Bangkok, Thailand has provided more epidemiological evidence supporting the role of dogs in disease transmission to humans.[81]

1.1.6 Diagnosis

The diagnosis of *Giardia* infections is difficult in that the clinical signs are not specific to the disease. Therefore, the clinical diagnosis is confirmed by detecting the parasite in fecal samples. The diagnostic assays for giardiasis include: microscopic examination after fecal flotation, immunofluorescence antibody assay (IFA), fecal antigen tests or polymerase chain reaction (PCR). The intermittent shedding of *Giardia* cysts requires collecting multiple samples from the same animals to increase the clinical sensitivity of the test. Also, sampling in young

animals should be performed routinely at 2-4 weeks of age where the peak excretion is reached even if they do not exhibit clinical signs.[59]

1.1.6.1 Microscopic Examination

Giardia trophozoites can only be detected by fecal smear on a fresh sample. *Giardia* cysts can be detected by microscopic examination, either directly (fecal smear) or after concentration with sucrose, zinc sulfate or sodium nitrate. Frequently, the cysts are preferred for diagnosis over the trophozoites because the latter requires the fecal samples to be fresh.[59] The cysts can be stained using common stains such as iodine and trichrome.[82,83] The main advantage of microscopic examination is the lower cost associated with the test and the main disadvantages are the need for an experienced and skilled microscopist, the lower sensitivity of the test and that the assay is time consuming.[59] The test also is less specific because pseudoparasites or other particles can be diagnosed as *Giardia* cysts.[84]

1.1.6.2 Antigen Detection

Antigen detection tests include commercially available assays such as immunofluorescence assays (IFA), enzyme-linked immunosorbent assays (ELISA) and rapid solid-phase qualitative immunochromatography assays. These assays were developed and evaluated for use in human and animal stool samples. [85-87] Even though the IFA assay is relatively sensitive,[88] there is a higher cost associated with the technique compared to microscopy, it is time consuming and requires experienced personnel.[59]

The immunochromatography assay uses monoclonal antibodies directed against specific cyst wall proteins. This assay enables on-site diagnosis within 15 minutes. The commercial antigen detection assays in human medicine include dip-sticks and rapid membrane assays. Similarly, in veterinary medicine the SNAP *Giardia* test (IDEXX Laboratories Inc., Westbrook,

Maine, USA) has been approved to be used with dog and cat samples.[88] Commercial fecal ELISA kits are effective for dogs and cats.[59]

1.1.6.3 Molecular Typing

The lack of morphological differentiation among *Giardia* spp. isolates is a limitation in understanding the taxonomy, epidemiology and public health impact of this important pathogen.[14] However, molecular tools have expanded the understanding of the variation among isolates of this parasite.[36] The PCR- based procedures allow for direct characterization of the parasite isolates from fecal and environment samples which eliminates the need for laboratory culture. Multilocus genotyping has improved the species level taxonomy for *Giardia* spp. and obviates the disagreement of the results when comparing genotypes.[89,90] Advanced molecular analysis such as multiplexing, real-time PCR and melting curve analysis also facilitate genotype and multiple species detection. The value of sub-genotyping or strain characterization tools is to help define the map of transmission in an outbreak analysis.[47] In some cases, the detection limit of the PCR is one cyst which substantially improves the diagnostic sensitivity.[91] One of the disadvantages of this assay is the presence of PCR inhibitors which are known to occur in DNA extracted from fecal samples.[92] The other disadvantage is that it can be too expensive and labor intensive for some veterinary diagnostic laboratories.[92]

1.2 *Cryptosporidium* spp.

1.2.1 Taxonomy

Cryptosporidium spp. belong to the kingdom: Protozoa, phylum: Apicomplexa, class: Coccidea, order: Eucoccidiorida, family: Cryptosporidiida.[93,94] *Cryptosporidium* spp. are rather divergent from other Coccidea by several characteristics, both genomic and biochemical.[95,96] In

addition, *Cryptosporidium* spp. are closely related to Gregarines, which are a diverse group of apicomplexan parasites that inhabit vertebrates and invertebrates hosts.[97,98]

Ernest Edward Tyzzer, a British physician, was first to describe the genus *Cryptosporidium* and recognize its multispecies nature.[90] During this period, the pathogens *C. muris* and *C. parvum* were identified and named by Tyzzer.[98] A few years after Tyzzer, *C. meleagridis* was described by Slavin.[94,99] Since Tyzzer's discovery of *Cryptosporidium* in 1907, more than 40 species and over 40 genotypes have been reported. However, only 25 species have been confirmed by the International Code for Zoological Nomenclature (ICZN) in the genus *Cryptosporidium* (Table 1.2). [94,100]

Molecular tools and phylogenetic analysis (that have been widely used to characterize *Cryptosporidium*) have provided insights about the biology, epidemiology and the public health significance of this pathogen.[90,101] Because hosts can be naturally infected with multiple species of *Cryptosporidium*, differentiating those species based on morphology alone is not sufficient. Instead, genetic differences between species that have been identified by PCR and DNA sequence analysis have been used to determine the identity of the organism.[90] *Cryptosporidium* spp. genes such as 18S ribosomal RNA (rRNA) and 60-kDa glycoprotein (gp60) have been widely used as genetic markers to identify different species and help determine the mode of transmission.[102-104] For instance, the gp60 gene character has tandem repeats and extensive sequence differences in the non-repeat regions, that characterize *C. parvum* and *C. hominis* each to several subtype families.[102]

1.2.2 Epidemiology in Humans

Like *Giardia* spp., *Cryptosporidium* spp. were included in the "Neglected Diseases Initiative" in 2004.[14] Cryptosporidiosis is a reportable disease in the USA[105] and the first full year

of reporting was 1995.[106] The transmission route of *Cryptosporidium* spp. is fecal-oral. This means the pathogen is excreted from the gastro-intestinal tract of an infected person to the environment and enter another person's gut via mouth.[107]

Cryptosporidiosis can occur through several modes of exposure. Contaminated water (either drinking, recreational or surface water) is one mode of *Cryptosporidium* exposure and likely the most common one. In the USA, the first waterborne outbreak due to *Cryptosporidium* spp. was reported in 1984 in Braun Station, a suburb of San Antonio, Texas.[108] Two years later, another outbreak due to *Cryptosporidium* spp. among college students was identified in Carroll County in Georgia, USA.[109] Since the 1980's, approximately 43 waterborne outbreaks due to *Cryptosporidium* spp. have been reported worldwide.[110] Exposure to reactional water led to nine waterborne outbreaks due to *Cryptosporidium* spp. worldwide during the period from 1987-1996.[110] In 1993, *Cryptosporidium* spp. caused the largest documented waterborne outbreak in Milwaukee, Wisconsin.[111] *Cryptosporidium* was considered the main causative agent of all waterborne outbreaks that occurred during 2001-2010 in the USA.[112] Each year, an estimated 748,000 human cases occur in the USA caused by *Cryptosporidium* spp.[113] However, this number of cases can be an underestimate because less than 2.0% of cases are reported to health authorities.[114] Human cryptosporidiosis can result in hospitalizations that can cost an estimate $45.8 million annually.[115]

Another mode of *Cryptosporidium* spp. transmission is person-to-person either directly or indirectly. Studies suggest that *Cryptosporidium* spp. can be transmitted sexually.[116] Data also has shown that the risk of acquiring cryptosporidiosis is higher in homosexual men compared to intravenous drug users and HIV positive individuals.[116] Another example of person to person contact of cryptosporidiosis was in one study that reported 19.0% of family members that have

children with cryptosporidiosis in Brazil developed clinical signs of the disease.[117] Additionally, 5.4% of Milwaukee residents developed diarrhea due to contact with infected family members during the *Cryptosporidium* epidemic in 1993.[118] *Cryptosporidium* infections due to contact with infected individuals also can occur in hospitals and daycare facilities if sanitation is inadequate.[117,119]

Cryptosporidium spp. can be transmitted via animal to-human or animal-to-animal contact. In one study, dairy farmers had a 44% *Cryptosporidium* seroprevalence compared to 24% seroprevalence in individuals that are not exposed to cattle.[120] Dogs and cats infected with host adapted *Cryptosporidium*, also can be infected with *C. parvum* and *C. meleagridis* that mainly infect cattle and birds, respectively.[121,122]

Another mode of transmission for *Cryptosporidium* spp. infection in humans can be via food. Even though waterborne cryptosporidiosis is of greater public health significance than foodborne cryptosporidiosis,[123] it has been recognized that food may play a more significant role in the transmission of cryptosporidiosis than formerly believed.[124] *Cryptosporidium* spp. oocysts have been detected in shellfish such as oysters, clams, and mussels.[125-127] Other foods such as raw vegetables[128-130], milk and chicken salad have been implicated in causing cases of cryptosporidiosis.[131,132]

Although uncommon, airborne transmission of *Cryptosporidium* spp. have been suggested in numerous papers.[116,133] Generally, the epidemiologic triad of *Cryptosporidium* spp. is described in the flowing sections.

1.2.2.1 Agent

Cryptosporidium spp. oocysts are subspherical in shape. The dimensions of the oocysts slightly vary among species of *Cryptosporidium*, but in general, the length ranges from 4.5 to 7.5

µm and the width ranges from 4.2 to 5.7 µm.[93] The sporozoites of the pathogen are 4.5 to 7.5 µm long and 1.2 to 1.8 µm wide. The pathogen completes its sexual and asexual life cycle in one host. The ingestion of the sporulated oocysts by the host results in *Cryptosporidium* spp. infection. This pathogen does not reproduce outside the host.[105] Additionally, all stages of *Cryptosporidium* spp. life cycle occur within one host[93] (Figure 1.3). After ingestion, the oocysts excyst in the epithelial cells of the gastrointestinal tract or other tissue such as the respiratory tract. This process (excystation) releases the infective sporozoites which become trophozoites. These trophozoites asexually proliferate by merogony to produce two types of meronts: type I meronts (contain eight merozoites) invade other epithelial cells where they develop into more type I meronts or type II meronts. The latter contains four merozoites. Type II meronts do not undergo merogony but produce sexual reproductive stages (gamonts). The zygotes formed by sexual reproduction (gametogony between male microgamonts and female macrogamonts) form either thick-walled or thin-walled oocysts, each containing four sporozoites. Thick walled oocysts then are passed in the infected host feces.[134] The oocysts which are shed in the feces are readily infectious and highly resistant to environmental conditions.[93] This pathogen can cause infection with a very low dose. For instance, some studies have demonstrated that healthy individuals can be develop cryptosporidiosis with as low as 10 oocysts for *C. hominis* or *C. parvum.*[135,136] It has been reported that infected persons can shed 10^7–10^8 oocysts in a single bowel movement[137] and continue to excrete the oocysts for up to 60 days after recovering from the clinical signs of the disease.[137]

It has been reported that *C. parvum* oocysts can remain viable in the environment for months.[110] The oocysts can resist moderate temperatures at 20˚C and remain infectious to suckling mice.[138] In experimental conditions, the oocysts lose their infectivity at 55˚C and

59.7˚C[139,140] and are killed at 71.7˚C.[141] Furthermore, the oocysts remain infectious at -5˚C for up to two months. Additionally, oocysts survived at -20˚C or -10˚C for a week and 8 hours, respectively.[138,142] Extreme low temperature like -70˚C resulted in immediate killing of the oocysts.[143,144] Oocysts are killed in extremely dry conditions or desiccation. Only 3.0% of oocysts were viable after 2 hours of desiccation and 100% killing was reported at 4 hours.[143,144]

1.2.2.2 Host

Cryptosporidium spp. infect the small intestine of a wide range of vertebrate hosts, including humans. Clinical signs vary from self-limiting to acute or life threatening depending on the immune status of the infected host.[90] For instance, *Cryptosporidium* spp. infection is more common in children, elderly, and immunosuppressed individuals, and the prognosis of cryptosporidiosis can be more severe in patients that are immunocompromised such as HIV+ individuals.[105,145-147] Cryptosporidiosis occurs more often in children under five years of age[114] (in the USA and developing countries) and clinical signs develop in children younger than two years of age in developing countries.[148-150] Overall, cryptosporidiosis rates were higher among females than males. For specific age groups, rates were higher among males than females aged <15 years and higher among females than males aged ≥15 years.[105] Even though it is unclear why the risk of cryptosporidiosis is elevated in females at this age, females aged ≥15 years are more likely to fill caregiver roles for young children, which is considered a risk factor for *Cryptosporidium* infection.[151]

Physiopathology

The mechanism of diarrhea due to *Cryptosporidium* spp. is not fully understood. It has been suggested that diarrhea occurs due to the disruption of microvillus surface area, the presence of an enterotoxin, or adhesion factors affecting parasite attachment to host cells.[152]

Generally, diarrhea due to *Cryptosporidium* spp. is profuse, watery, and non-bloody. Other symptoms are non-specific and can include weight loss, abdominal pain, anorexia, fatigue, cramps, headache, fever, and vomiting.[153] However, diarrhea does not develop in the majority of infections and are classified as asymptomatic infections.[154-157] Recurrence of symptoms after apparent recovery has been reported. Regardless, illness is self-limiting, and symptoms typically resolve completely within 2–3 weeks in immunocompetent persons.[158]

1.2.2.3 Environment

The pathogenic oocysts enter the environment in feces from both human and other hosts.[159] One of the challenges presented by *Cryptosporidium* spp. is that it is ubiquitous in the environment. This characteristic allows for several transmission routes of *Cryptosporidium* infectious to humans and other animals.[160]

It has been reported that livestock manure production is approximately 5.45 billion metric tons per year.[161] In developed countries, most of this manure is deposited to the soil with little or no treatment which can result in an accumulation of numerous number of pathogens. Also, *Cryptosporidium* spp. oocysts have been detected in the soil.[161]

Other sources that play a role in *Cryptosporidium* spp. contamination to the environment are wild animals and insects. Examples of wild animals are Canadian geese, Peking ducks, bears, marsupials, mountain gorillas, and red deer.[110,162] Furthermore, insects such as flies, and cockroaches have been reported to serve as reservoirs for *C. parvum* oocysts and capable of transmitting of the agent into the environment.[110,163]

1.2.3 *Cryptosporidium* spp. in Companion Animals

Cryptosporidium canis is a *Cryptosporidium* genotype that infects dogs.[164] This genotype was first identified as a dog genotype in 1999[165]and classified as a species in 2001 on the basis

that *C. canis* oocysts were genetically distinct from all other species and were not infectious to mice.[164] *Cryptosporidium canis* and its sub-genotypes (*C. canis* fox genotype and *C. canis* coyote genotype) have been reported in dogs, foxes and coyotes.[101] *Cryptosporidium canis* also has been reported worldwide in humans. [101,166,167]

Canine cryptosporidiosis is more prevalent in pups compared to adult dogs and the risk of the disease increases when coinfections with other pathogens are present.[168-173] Commonly reported clinical signs in dogs include: diarrhea, anorexia, and weight loss. The diarrhea is small bowel and is characterized as watery, non–mucoid or non-bloody. Vomiting is uncommon unless other abnormalities exist. The small intestines may feel slightly thickened when the abdomen is palpated.[174]

Cats become infected with *C. felis* which were first described in 1979.[175] When the oocysts from a cat were fed to mice, rats, guinea pigs and dogs, and infection was induced only in cats. [175,176] *Cryptosporidium felis* also has been detected in cattle and in human cases.[166,167,177-181] Immunocompetent cats may not develop diarrhea due to cryptosporidiosis and sub-clinically infected cats can shed *Cryptosporidium* oocysts.[175,176,182]

Clinical signs of cats with cryptosporidiosis include diarrhea, anorexia and weight loss. Factors such as weakened immune system, pre-existing diseases in the intestinal tract or coinfection with other infectious or non-infectious causes can lead to the risk of developing clinical signs.[183-185] Coinfection with pathogens such as *Cystoisospora* spp., *Toxocara cati*, coronavirus, and *Campylobacter* have been documented in cats with cryptosporidiosis.[184,186-189]

1.2.4 Zoonotic Consideration of *Cryptosporidium* spp.

Infection with *Cryptosporidium* spp. is believed to be primarily zoonotic.[47] Cryptosporidiosis has been reported among veterinarians and farm workers. In these populations,

it was found that the oocysts were transmitted from cattle infected with *C. parvum*.[190,191] In the late 1990's, genetic analysis of *C. parvum* identified type I which is considered human exclusive (now named *C. hominis*) and type II *C. parvum* that infects both humans and cattle.[47,192,193]

Even though some species are considered host adapted, such as *C. canis* and *C. felis* have been detected in humans,[194-197] the pet's role in transmitting the infection to their owners has not been defined.[47]

1.2.5 Laboratory Diagnosis

To diagnose *Cryptosporidium* spp. disease or infection in humans and animals, several techniques have been employed. These techniques include: ultrastructural examination of biopsy material for life cycle stages, microscopic examinations, and molecular based techniques. Types of specimens that are submitted for *Cryptosporidium* detection can include: feces, sputum, bile, mucoid secretions and tissue biopsies. Fecal specimens are the primary type of samples examined for *Cryptosporidium*.[198] In this section, microscopic examination of the oocysts, *Cryptosporidium* spp. antibody and antigen tests, and molecular testing for *Cryptosporidium* spp. DNA from fecal specimens of humans and companion animals will be discussed.

1.2.5.1 Microscopic Examination

Detection of *Cryptosporidium* spp. oocysts using microscopy has been widely used in the diagnostic workup of diarrhea.[199] This examination can be performed by several methods.

Concentration Techniques

Prior to the microscopic examination of fecal specimens, it is recommended to use concentration procedures for detection of the oocysts. These procedures are useful in maximizing the recovery of the oocysts.[198] Sheather's sucrose flotation, zinc sulfate flotation, saturated sodium chlorine methods,[200] discontinuous sucrose, isopropynic Percoll, discontinuous Percoll,

or cesium chloride gradient centrifugation[201,202] are used as concentration methods for fecal specimens.

Staining Methods

Two types of stains are used to stain the *Cryptosporidium* spp. oocysts. These include: acid-fast staining and non-acid-fast staining. The acid fast Ziehl-Neelsen stain is widely used in the *Cryptosporidium* spp. oocysts staining procedure for fecal smears.[203] This stain permits a better differentiation from fecal matter after counter-staining with malachite green or methylene blue.[204] The oocysts have distinct walls and stain from light pink to bright red color. This stain is used for oocyst detection.[205] The acid-fast carbol-fuchsine also may be used to stain the oocysts.[204] The oocysts in this type of stain appear ringlike (4-6 µm in diameter) and exhibit a characteristic bright fluorescence (brilliant green) against a dark red background.[198] Other acid-fast staining methods include safranin-methylene blue,[206] Trichrome,[205] and Kinyoun[207] stains. In the staining procedures using safranin and trichrome stains, the detection of the oocysts can be accomplished, but these techniques are not adequate for the confirmation.[205] The Kinyoun stain is preferable to the enzyme immunoassays.[207] This assay is considered the gold standard assay by many laboratories for *Cryptosporidium* spp. oocyst detection.[205] The non-acid-fast staining techniques include: negative stains such as light–green or mercuramine, and fluorescent stains such as phenol auramine. The advantage of these techniques is that they allow a fast screening of the specimen, but they require a fluorescent microscope.[204]

Monoclonal antibody based immunofluorescence staining of *Cryptosporidium* spp. oocysts is a type of staining that involves detection of an immunologic reaction between antibody and antigen. The IFA detects antigen using a fluorescent antibody performed either directly or indirectly. The direct IFA method involves the conjugation of a specific monoclonal

immunoglobulin (antibody) with a fluorescent dye and this complex is added to a concentrated fecal specimen. The product then combines with specific antigen and results in an antigen-antibody complex. This complex is visualized using a fluorescent microscope. The indirect IFA involves adding an unlabeled immunoglobulin (antibody) to the fecal specimen. The antigen-antibody complex then is labeled with fluorescein-conjugated anti-immunoglobulin antibody with the resulting triple complex visualized with a fluorescent microscope.[208] Studies have found no significant difference in the limit of detection of stool samples from asymptomatic carriers when this assay was compared to the acid-fast staining techniques.[204]

Wet Mount

This type of examination is used for screening of oocysts. It is useful when the specimen contains a high number of oocysts. Fresh or concentrated fecal specimens can be examined, using either conventional bright light, phase contrast or differential interference contrast microscopy without staining of the sample.[209] In this method, the oocysts appear as small spherical structures (on average 5μm), but misdiagnoses can occur if yeast is present.[205]

1.2.5.2 Immunological Methods

Enzyme-Linked Immunosorbent Assay (ELISA)

The enzyme-linked immunosorbent assay (ELISA) has been used to detect both the antibody and antigen of *C. parvum*. *Cryptosporidium* spp. such as *C. parvum* induce mucosal infection and *C. parvum* IgA antibody is released in the feces. Thus, ELISA has been developed to detect this antibody in the fecal samples.[210] Fecal anti-*C. parvum* IgA, IgM, and IgG were monitored by ELISA in calves that were experimentally and naturally infected with *C. parvum*.[210] In this experiment, even though experimentally infected calves had high level of colostral antibodies in their feces, they were all infected with *C. parvum* and three of five died.

Calves naturally infected with *C. parvum*, had only diarrhea. Experimental infection was followed by a rise in local anti-*C. parvum* IgM levels. In naturally infected calves, serum anti-*C. parvum* IgG levels rose during maximal oocyst excretion, whereas serum anti-*C. parvum* IgA levels peaked later than did local IgA levels.[210] In addition, ELISA has been used to detect the free fecal antigen of *Cryptosporidium* spp. in feces.[134] This approach has been widely applied in laboratories that do not have a fluorescent microscope, and in situations when processing batch specimens may be crucial due to its increased sensitivity compared to microscopy for *Cryptosporidium* spp. diagnosis.[134] Seven commercial ELISA kits were evaluated for their sensitivity using the IFA as a reference test. The sensitivity of five kits ranged from 94.5% - 100% which was equivalent to the sensitivity of the IFA assay.[204] However, two ELISA kits performed poorly with sensitivity ranged from 29%-93%.[204] The sensitivity of a fecal ELISA kit was compared to carbol fuchsine stain (microscopy) in the detection of *Cryptosporidium* in canine and feline samples in a study.[211] Twenty-six of 270 dog samples (9.5%) tested positive for *Cryptosporidium* by microscopy. However, only eight of 270 (2.95%) tested positive by the fecal ELISA. Whereas none of the 100 cats tested positive for *Cryptosporidium* by microscopy, but 22 of 100 (22.4%) tested positive by the fecal ELISA, in the same study.[211]

1.2.5.3 Molecular Techniques

Molecular techniques or DNA – based methods include polymerase chain reaction (PCR) assays. The PCR assays are characterized by the increased sensitivity, specificity and reproducibility. In addition, the PCR product results are easy to interpret.[134] It has been reported that PCR has increased sensitivity of detection in comparison to microscopic and immunological-based techniques for clinical samples.[92,212] Several PCR protocols have been developed to differentiate *Cryptosporidium* spp. using 18srRNA, HSP70,[162] and GP60[213] genes.

Molecular techniques also can include sub-genotyping tools. These tools are helpful in the epidemiological investigation of outbreak situations because they provide more accurate identification of the causative agent and hopefully the mode of transmission.[214] Sub-genotyping tools available include: DNA sequence analysis of microsatellites,[215,216] HSP-70 gene,[217] GP60 gene,[218,219] and a double stranded (ds) RNA.[220] Molecular tools based the oocyst wall protein (COWP) have limited usefulness in genotyping *Cryptosporidium* spp. of animals because of their narrow specificity.[221]

There are several benefits in using subtyping tools, especially in understanding the epidemiology of *Cryptosporidium* spp. for instance, these tools provide understanding of the complexity of human cryptosporidiosis at the genotype and sub-genotype levels.[221] Furthermore, subtyping provides understanding of the transmission routes of *Cryptosporidium* in developing and developed countries and has improved the understanding of infection sources in humans.[221]

1.2.6 Prevention and Control of Giardiasis and Cryptosporidiosis

In humans, the prevention of giardiasis can be accomplished by practicing good hygiene such as handwashing and avoid direct contact with animal's feces, especially young animals. Because giardiasis is more frequently reported in young children, those with diarrhea should not be sent to the daycare until the disease has resolved. Also, hands must be washed after changing diapers. Contaminated food or water with *Giardia* cysts or *Cryptosporidium* oocysts should be avoided. Disinfection of public drinking water is not effective in inactivating *Giardia* cysts. However, *Giardia* cysts can be inactivated by thorough steam cleaning or using effective detergent regimes.[222,223]

Routine diagnosis of *Cryptosporidium* cases and reporting the disease in humans to local and national surveillance organizations is an important measure for disease prevention.

Furthermore, implementing of water treatment procedures and enforce better regulations can be useful in controlling the spread of *Cryptosporidium* contamination to the environment, yet this protocol can be challenging, especially in swimming pools. Additionally, as a preventative measure, travel-related causes should be examined and controlled.[224]

Prevention measures for *Giardia* transmission include treating water collected from the environment by either filtration or boiling. For premises contaminated with feces containing *Giardia* cysts, steam cleaning or quaternary ammonium compounds should be used. To control the spread of the pathogen in infected animals, treatment or bathing of all animals as well as prompt removal of feces from infected animals should be implemented. Even though *Cryptosporidium* spp. oocysts are environmentally resistant, they can be controlled by steam cleaning. This application can rupture the oocysts wall. Best prevention measures for cryptosporidiosis are avoiding contaminated water or food.[225]

1.3 Tables

Table 1.1 *Giardia* species based on original taxonomic description and new nomenclature in different hosts[a]

Species	Assemblages[b]	Hosts
Giardia duodenalis	Assemblage[c] A	Humans and other primates, dogs, cats, livestock, rodents, wild mammals
	Assemblage[d] B	Humans and other primates, dogs, cats, wild mammals
	Assemblage[e] C/D	Dogs, canids
	Assemblage[f] E	Cattle, hoofed livestock
	Assemblage[g] F	Cats
	Assemblage[h] G	Rats
	Assemblage[i] H	Pinnipeds
	Assemblage[i] --	Marsupial (Quenda, bandicoot)
G. muris		Rodents
G. microti		Rodents
G. psittaci		Birds
G. ardeae		Birds
G. agilis		Amphibians

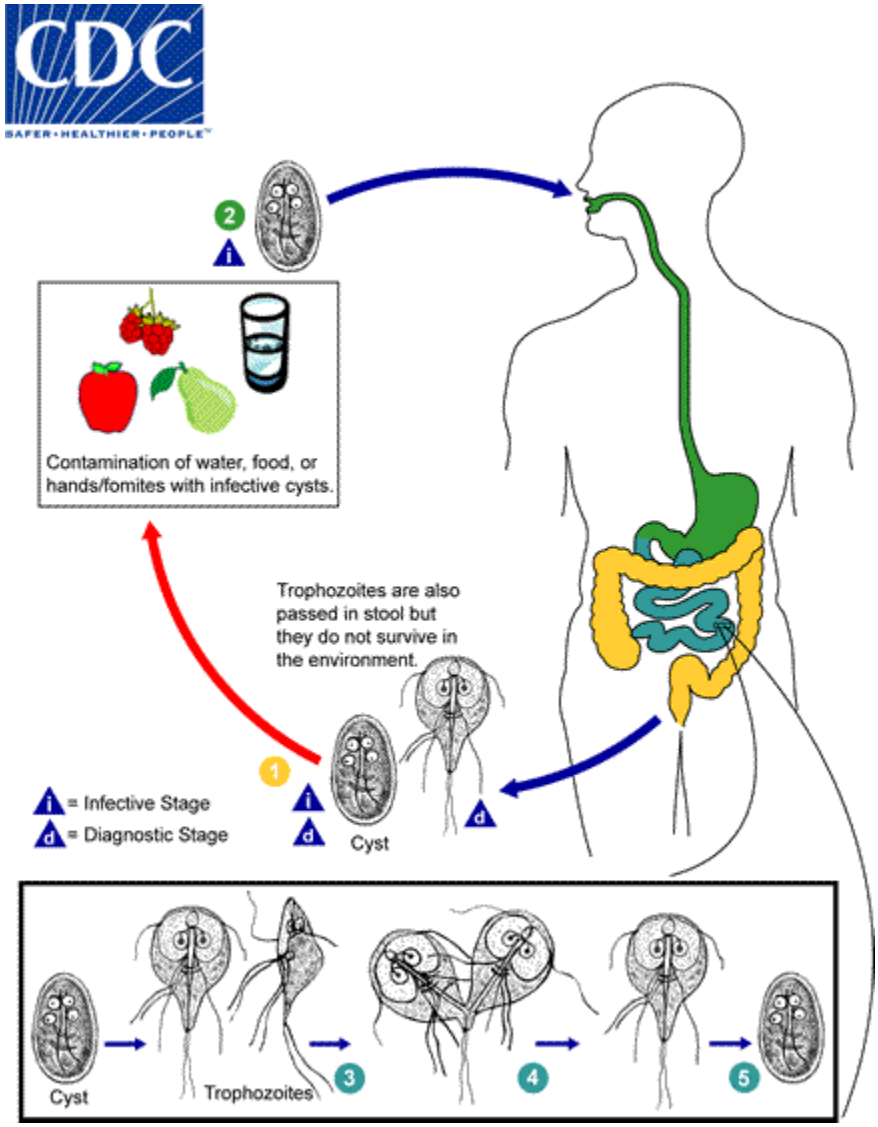
[b] New proposed nomenclature
[c] *G. duodenalis*
[d] *G. enterica*
[e] *G. canis*
[f] *G. bovis*
[g] *G. cati*
[h] *G. simondi*
[i] Novel lineages or likely new species of *Giardia* that have not been formally described yet
[a]Modified from Thompson RCA, Monis P. *Giardia* – from genome to proteome. *Adv Parasitol.* 2012;78:57-95. doi: 10.1016/B978-0-12-394303-3.00003-7.[226]

Table 1.2 *Cryptosporidium* species in different hosts[a]

Species	**Host**	**Reference**
C. hominis	Humans	227
C. viatorum	Humans	228
C. parvum	Cattle, sheep, humans, whiting (fish), barramundi (fish)	229,230,231
C. parvum like	whiting (fish), barramundi (fish)	229,231
C. bovis	Cattle	232
C. ryanae	Cattle	233
C. andersoni	Cattle	234
C. xiaoi	Sheep, whiting (fish), barramundi (fish)	229,231,235
C. ubiquitum	Sheep/ wildlife	236
C. felis	Cats	175
C. canis	Dogs	164
C. muris	Rodents, (ringed, harbor, hooded) seals	98,237,238
C. tyzzeri	Mice	239
C. suis	Pigs,	240
C. scrofarum	Pigs, whiting (fish), barramundi (fish)	229,231,241
C. wrairi	Guinea pigs	242
C. cuniculus	Rabbits	243,244
C. fayeri	Marsupials	245
C. macropodum	Marsupials	246
C. meleagridis	Turkey, Indian ring-necked parrot, red-legged partridge, cockatiels, Bohemian waxwing, rufousturle dove, fan-tailed pigeon, chicken, quails, Pekin ducks	99,247-257

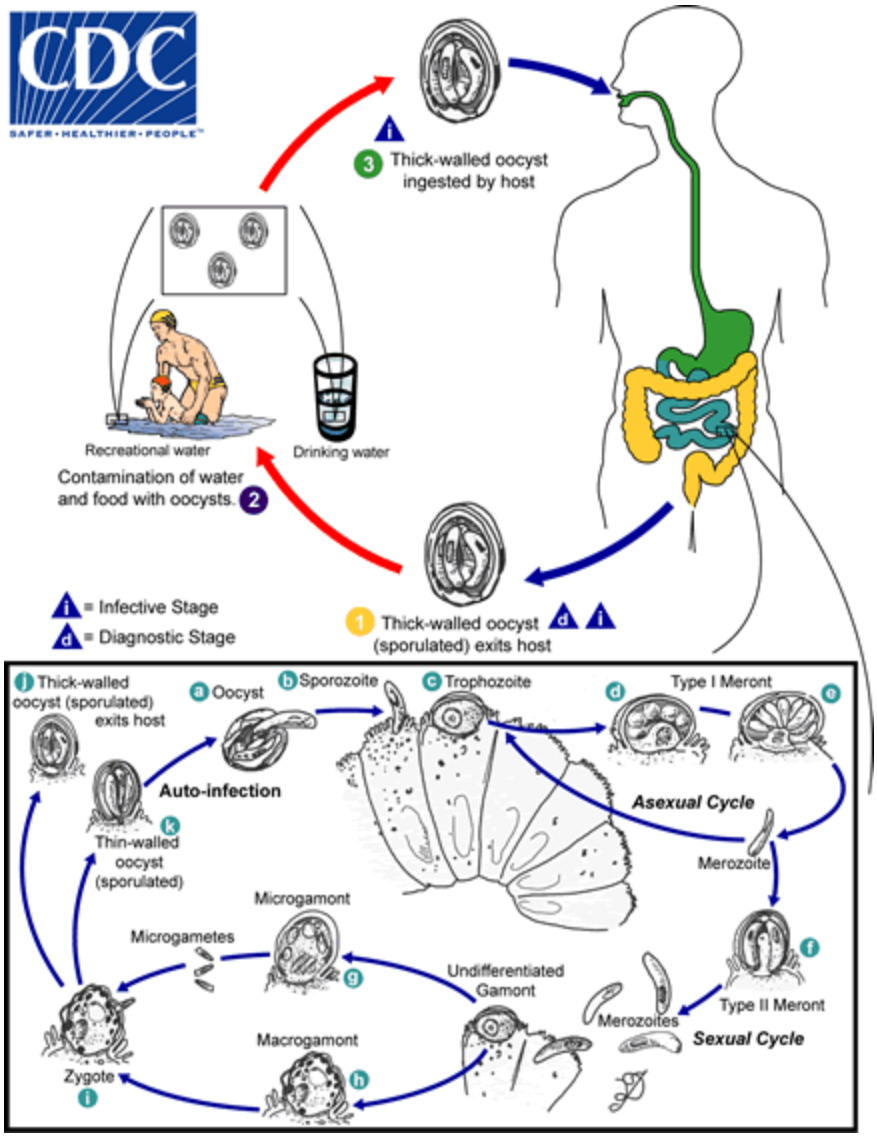
[a]Adapted from Ryan U, Xiao L. Taxonomy and molecular taxonomy. In: Cacciò SM, Widmer G, ed. *Cryptosporidium: Parasite and Disease*. New York, NY: Springer; 2014: 3-41. doi 10.1007/978-3-7091-1562-6.[100]

1.4 Figures



[a]From http://www.cdc.gov/parasites/giardia/pathogen.html [258]

Figure 1.2 Illustration of the *Giardia duodenalis* life cycle in humans[a]



[a]From http://www.cdc.gov/parasites/crypto/pathogen.html[259]
Figure 1.3 Illustration of the *Cryptosporidium parvum* life cycle in humans[a]

REFERENCES

1. Internal parasites in cats & dogs. Zoonotic Risk. https://ebusiness.avma.org/files/productdownloads/internalparasites_brochure.pdf. Accessed March 14, 2016.

2. Cavalier-Smith T. Protist phylogeny and the high-level classification of Protozoa. *European J Protist.* 2003;39:338–348. doi: 10.1078/0932-4739-00002.

3. Thompson RC, Monis PT. Taxonomy of *Giardia* species. In: Lujan HD, Svärd S, ed. *Giardia A Model Organism*. Wien, Australia: Springer; 2011:3-15.

4. Filice FP. Studies on the cytology and life history of a *Giardia* from the laboratory rat. Berkeley and Los Angeles, CA: University of California Press; 1952.

5. Thompson RC, Monis PT. Taxonomy of *Giardia* species. In: Lujan HD, Svärd S, ed. *Giardia A Model Organism*. Wien, Australia: Springer; 2011:3-15.

6. Monis PT, Caccio SM, Thompson RC. Variation in *Giardia*: towards a taxonomic revision of the genus. *Trends Parasitol.* 2009;25:93–100. doi: 10.1016/j.pt.2008.11.006.

7. Erlandsen SL, Bemrick WL. SEM evidence for a new species, *Giardia psittaci. J Parasitol.* 1987;73:623–629. doi: 10.2307/3282146.

8. Erlandsen SL, Bemrick WJ, Wells CL, et al. Axenic culture and characterization of *Giardia ardeae* from the great blue heron (*Ardea herodias*). *J Parasitol.* 1990;76:717–724. doi: 10.2307/3282988.

9. Marti M, Regös A, Li Y, et al. An ancestral secretory apparatus in the protozoan parasite *Giardia intestinalis*. *J Biol Chem.* 2003;278:24837–24848. doi: 10.1074/jbc.M302082200.

10. Feely DE. Morphology of the cyst of *Giardia microti* by light and electron microscopy. *J Protozool*. 1988;35:52–54.

11. Thompson, RC. The zoonotic significance and molecular epidemiology of *Giardia* and giardiasis. *Vet Parasitol.* 2004;126:15–35. doi: 10.1016/j.vetpar.2004.09.008.

12. Plutzer J, Ongerth J, Karanis, P. *Giardia* taxonomy, phylogeny and epidemiology: Facts and open questions. *Int J Hyg Environ Health.* 2010;213:321-333. doi:10.1016/j.ijheh.2010.06.005.

13. Scorza AV, Lappin MR. Giardiosis. In: Beugnet F, Halos L, ed. *Parasitoses and Vector Borne Diseases of Cats*. Lyon, France: Merial S.A.S.; 2015:67-73.

14. Savioli L, Smith H, Thompson A. *Giardia* and *Cryptosporidium* join the 'Neglected Diseases Initiative'. *Trends Parasitol.* 2006;22:203-208. doi:10.1016/j.pt.2006.02.015.

15. Cacciò SM, Sprong H. Epidemiology of giardiasis in humans. In: Lujan HD, Svärd S, ed. *Giardia A Model Organism*. Wien, Australia: Springer; 2011:18-28.

16. Hörman A, Korpela H, Sutinen J, et al. Meta-analysis in assessment of the prevalence and annual incidence of *Giardia spp.* and *Cryptosporidium spp.* infections in humans in the Nordic countries. *Int J Parasitol.* 2004;34:1337–1346. doi: 10.1016/j.ijpara.2004.08.009.

17. Dib HH, Lu SQ, Wen SF. Prevalence of *Giardia lamblia* with or without diarrhea in South East, South East Asia and the Far East. *Parasitol Res.* 2008;103:239–251. doi 10.1007/s00436-008-0968-6.

18. Yoder JS, Beach MJ. Giardiasis surveillance – United States, 2003–2005. *MMWR Surveill Summ.* 2007;56:11–18.

19. Hoque E, Hope V, Scragg R. A descriptive epidemiology of giardiasis in New Zealand and gaps in surveillance data. *N Z Med J.* 2004;117:1-13.

20. de Wit MA, Koopmans MP, Kortbeek LM, et al. Sensor, a population-based cohort study on gastroenteritis in the Netherlands: incidence and etiology. *Am J Epidemiol.* 2001;154:666–674. doi: 10.1093/aje/154.7.666.

21. de Wit MA, Koopmans MP, Kortbeek LM, et al. Gastroenteritis in sentinel general practices, The Netherlands. *Emerg Infect Dis.* 2001;7:82–91. doi: 10.3201/eid0701.700082.

22. Adam RD.Biology of *Giardia lamblia*. *Clin Microbiol Rev*. 2001;14(3):447–475. doi: 10.1128/CMR.14.3.447-475.2001.

23. Eisenberg JN, Brookhart MA, Rice G, Brown M, Colford JM Jr. Disease transmission models for public health decision making: analysis of epidemic and endemic conditions caused by waterborne pathogens. *Environ Health Perspect.* 2002;110(8):783–790.

24. Karanis P, Kourenti C, Smith H. Water-borne transmission of protozoan parasites: a review of world-wide outbreaks and lessons learnt. *J Water Health.* 2007;5:1–38. doi: 10.2166/wh.2006.002.

25. Braeye T, de Schrijver K, Wollants E, van Ranst M, Verhaegen J. A large community outbreak of gastroenteritis associated with consumption of drinking water contaminated by river water, Belgium, 2010. *Epidemiol Infect.* 2014;1-9. doi:10.1017/S0950268814001629.

26. Escobedo AA, Almirall P, Robertson LJ, et al. Giardiasis: the ever-present threat of a neglected disease. *Infect Disord Drug Targets.* 2010;10:329-348. doi: 10.2174/187152610793180821.

27. Robertson LJ, Lim YA. Waterborne and environmentally-borne giardiasis. In: Lujan HD, Svärd S, ed. *Giardia A Model Organism*. Wien, Australia: Springer; 2011:29-69.

28. Smith HV, Robertson LJ, Ongerth JE. Cryptosporidiosis and giardiasis: the impact of waterborne transmission. *J Wat Suppl: Res Technol – AQUA*. 1995;44:258–274.

29. Kramer MH, Sorhage FE, Goldstein ST, Dalley E, Wahlquist SP, Herwaldt BL. First reported outbreak in the United States of cryptosporidiosis associated with a recreational lake. *Clin Infect Dis.* 1998;26:27–33. doi: 10.1086/516271.

30. Joce RE, Bruce J, Kiely D, et al. An outbreak of cryptosporidiosis associated with a swimming pool. *Epidemiol Infect.* 1991;107(3):497–508. http://www.jstor.org/stable/3864135

31. Stuart JM, Orr HJ, Warburton FG, et al. Risk Factors for sporadic giardiasis: a case-control study in Southwestern England. *Emerg Infect Dis.* 2003;9:229–233. doi: 10.3201/eid0902.010488.

32. Breathnach AS, McHugh TD, Butcher PD. Prevalence and clinical correlations of genetic subtypes of *Giardia lamblia* in an urban setting. *Epidemiol Infect.* 2010;13:1459–1467. doi:10.1017/S0950268810000208.

33. Plutzer J, Takó MH, Márialigeti K, Törökné A, Karanis P. First investigations into the prevalence of *Cryptosporidium* and *Giardia* spp. in Hungarian drinking water. *J Water Health*. 2007;5(4):573–584. doi:10.2166/wh.2007.007.

34. Meloni BP, Lymbery AJ, Thompson RC. Characterization of *Giardia* isolates using a non-radiolabeled DNA probe, and correlation with the results of isoenzyme analysis. *Am J Trop Med Hygiene*. 1989;40(6):629–637.

35. Thompson RC, Reynoldson JA, Mendis AH. *Giardia* and giardiasis. *Adv Parasitol*. 1993;32:71–160. http://dx.doi.org/10.1016/S0065-308X(08)60207-9.

36. Cacciò SM, Thompson RC, McLauchlin J, Smith HV. Unravelling *Cryptosporidium* and *Giardia* epidemiology. *Trends Parasitol.* 2005;21:430–437. doi:10.1016/j.pt.2005.06.013.

37. Farthing MJ, Mata L, Urrutia JJ, Kronmal RA. Natural history of *Giardia* infection of infants and children in rural Guatemala and its impact on physical growth. *Am J Clin Nutr*. 1986;43: 395–405. http://ajcn.nutrition.org/content/43/3/395.long

38. Mumtaz S, Siddiqui H, Ashfaq T. Frequency and risk factors for intestinal parasitic infection in children under five years age at a tertiary care hospital in Karachi. *J Pak Med Assoc*. 2009;59(4):216–219.

39. Faubert G. Immune response to *Giardia duodenalis*. *Clin Microbiol Rev.* 2000;13(1):35–54.

40. Dwivedi KK, Prasad G, Saini S, Mahajan S, Lal S, Baveja UK. Enteric opportunistic parasites among HIV infected individuals: associated risk factors and immune status. *Jpn J Infect Dis.* 2007;60(2-3):76–81.

41. Chin AC, Teoh DA, Scott KG, Meddings JB, Macnaughton WK, Buret AG. Strain-dependent induction of enterocyte apoptosis by *Giardia lamblia* disrupts epithelial barrier function in a caspase-3-dependent manner. *Inf Imm.* 2002;70:3673–3680. doi: 10.1128/IAI.70.7.3673-3680.2002.

42. Buret AG, Mitchell K, Muench DG, Scott KG. *Giardia lamblia* disrupts tight junctional ZO-1 and increases permeability in non-transformed human small intestinal epithelial monolayers: effects of epidermal growth factor. *Parasitology*. 2002;125:11–19. doi: 10.1017/S003118200200185.

43. Scott KG, Meddings JB, Kirk DR, Lees-Miller SP, Buret AG. Intestinal infection with *Giardia* spp. reduces epithelial barrier function in a myosin light chain kinase dependent fashion. *Gastroenterology*. 2002;123:1179–1190. doi: 10.1053/gast.2002.36002.

44. Scott KG, Logan MR, Klammer GM, Teoh DA, Buret AG. Jejunal brush border microvillus alterations in *Giardia muris*-infected mice: role of T lymphocytes and interleukin-6. *Inf Imm.* 2000;68:3412–3418. doi: 10.1128/IAI.68.6.3412-3418.2000.

45. Koudela B, Vitovec J. Experimental giardiosis in goat kids. *Vet Parasitol*. 1998;74: 9–18. doi: 10.1016/S0304-4017(97)00146-5.

46. Moncada DM, Kammanadiminti SJ, Chadee K. Mucin and Toll-like receptors in host defense against intestinal parasites. *Trends Parasitol.* 2003;19:305–311. doi:10.1016/S1471-4922(03)00122-3.

47. Hunter PR, Thompson RC. The zoonotic transmission of *Giardia* and *Cryptosporidium*. *Int J Parasitol.* 2005;35:1181–1190. doi:10.1016/j.ijpara.2005.07.009.

48. Svärd SG, Hagblom P, Palm JE. *Giardia lamblia*—a model organism for eukaryotic cell differentiation. *FEMS Microbiol Lett.* 2003;218:3–7.

49. Robertson LJ, Gjerde BK. Effects of the Norwegian winter environment on *Giardia* cysts and *Cryptosporidium* oocysts. *Microb Ecol.* 2004;47:359–365. doi: 10.1007/s00248-003-0003-5.

50. De Regnier DP, Cole L, Schupp DG, Erlandsen SL. Viability of *Giardia* cysts suspended in lake, river, and tap water. *Appl Environ Microbiol.* 1989;55(5):1223–1229.

51. Robertson LJ, Gjerde BK. Fate of *Cryptosporidium* oocysts and *Giardia* cysts in the Norwegian aquatic environment over winter. *Microb Ecol.* 2006; 52: 597–602. doi:10.1007/s00248-006-9005.

52. Gerwig GJ, van Kuik JA, Leeflang BR, et al. The *Giardia intestinalis* filamentous cyst wall contains a novel beta (1–3)-N-acetyl-D-galactosamine polymer: a structural and conformational study. *Glycobiology.* 2002;12(8):499–505. doi: 10.1093/glycob/cwf059.

53. Plutzer J, Tomor B. The role of aquatic birds in the environmental dissemination of human pathogenic *Giardia duodenalis* cysts and *Cryptosporidium* oocysts in Hungary. *Parasitol Int.* 2009;58:227–231. doi:10.1016/j.parint.2009.05.004.

54. Graczyk TK, Knight R, Tamang L. Mechanical transmission of human protozoan parasites by insects. *Clin Microbiol Rev.* 2005;18:128–132. doi:10.1128/CMR.18.1.128–132.2005.

55. Conn DB, Weaver J, Tamang L, Graczyk TK. Synanthropic flies as vectors of *Cryptosporidium* and *Giardia* among livestock and wildlife in a multispecies agricultural complex. *Vector Borne Zoonotic Dis*. 2007;7:643–651. doi: 10.1089/vbz.2006.0652.

56. Geurden T, Vandenhoute E, Pohle H. The effect of a fenbendazole treatment on cyst excretion and weight gain in calves experimentally infected with *Giardia duodenalis*. *Vet Parasitol.* 2010;169:18–23. doi:10.1016/j.vetpar.2009.12.027.

57. Lebwohl B, Deckelbaum RJ, Green PH. Giardiasis. *Gastrointest Endosc*. 2003;57:906–913. doi:10.1067/mge.2003.245.

58. Halliez MC, Buret AG. Extra-intestinal and long term consequences of *Giardia duodenalis* infections. *World J Gastroenterol*. 2013;19:8974-8985. doi:10.3748/wjg.v19.i47.8974.

59. Geurden T, Olson M. *Giardia* in pets and farm animals, and their zoonotic potential. In: Lujan HD, Svärd S, ed. *Giardia A Model Organism*. Wien, Australia: Springer; 2011:71-92.

60. Hahn NE, Glaser CA, Hird DW, Hirsh DC. Prevalence of *Giardia* in the feces of pups. *J Am Vet Med Assoc*. 1988;192(10):1428–1429.

61. Carlin EP, Bowman DD, Scarlett JM, Garrett J, Lorentzen L. 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):199–206.

62. Little SE, Johnson EM, Lewis D, et al. Prevalence of intestinal parasites in pet dogs in the United States. *Vet Parasitol*. 2009;166:144–152. doi: 10.1016/j.vetpar.2009.07.044.

63. De Santis-Kerr AC, Raghavan M, Glickman NW, et al. Prevalence and risk factors for Giardia and Coccidia species of pet cats in 2003–2004. *J Feline Med Surg*. 2006;8:292–301. doi:10.1016/j.jfms.2006.02.005.

64. O'Handley RM, Ceri H, Anette C, Olson ME. Passive immunity and serological immune response in dairy calves associated with natural *Giardia duodenalis* infections. *Vet Parasitol*. 2003;113:89–98. doi: 10.1016/S0304-4017(03)00059-1.

65. Parasitic Zoonoses. Report of a WHO Expert Committee with the Participation of FAO. World Health Organization Web site. http://www.who.int/iris/handle/10665/41353#sthash.uYO3zSl9.dpuf. Published on 1979. Updated 2014. Accessed September 29, 2014.

66. Lalle M, Pozio E, Capelli G, Bruschi F, Crotti D, Caccio SM. 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;35:207–213. doi: 10.1016/j.ijpara.2004.10.022.

67. Ryan U, Cacciò SM. Zoonotic potential of *Giardia*. *Int J Parasitol.* 2013;43:943–956. doi: 10.1016/j.ijpara.06.001.

68. Monis PT, Andrews RH, Mayrhofer G, Ey PL. Genetic diversity within the morphological species *Giardia intestinalis* and its relationship to host origin. *Infect Genet Evol.* 2003;3:29–38. doi: 10.1016/S1567-1348(02)00149-1.

69. Thompson RC. Giardiasis as a re-emerging infectious disease and its zoonotic potential. *Int J Parasitol.* 2000;30:1259–1267. doi: 10.1016/S0020-7519(00)00127-2.

70. Lebbad M, Mattsson JG, Christensson B. From mouse to moose: multilocus genotyping of *Giardia* isolates from various animal species. *Vet Parasitol.* 2010;168:231–239. doi: 10.1016/j.vetpar.2009.11.003.

71. Sprong H, Cacciò SM, van der Giessen, et al. Identification of zoonotic genotypes of *Giardia duodenalis*. *PLoS Negl Trop Dis.* 2009;3:1-12. doi:10.1371/journal.pntd.0000558.

72. Read C, Walters J, Robertson ID, Thompson RC. Correlation between genotype of *Giardia duodenalis* and diarrhoea. *Int J Parasitol*. 2002;32:229–231. doi: 10.1016/S0020-7519(01)00340-X.

73. Haque R, Roy S, Kabir M, Stroup SE, Mondal D, Houpt ER. *Giardia* assemblage A infection and diarrhea in Bangladesh. *J Infect Dis.* 2005;192:2171–2173. doi: 10.1086/498169.

74. Sahagun J, Clavel A, Goni P, et al. Correlation between the presence of symptoms and the *Giardia duodenalis* genotype. *Eur J Clin Microbiol Infect Dis*. 2008;27:81–83. doi 10.1007/s10096-007-0404-3.

75. Gelanew T, Lalle M, Hailu A, Pozio E, Cacciò SM. Molecular characterization of human isolates of *Giardia* duodenalis from Ethiopia. *Acta Trop*. 2007;102: 92–99. doi: 10.1016/j.actatropica.2007.04.003.

76. Homan WL, Mank TG. Human giardiasis: genotype linked differences in clinical symptomatology. *Int J Parasitol.* 2001; 31:822–826. doi: 10.1016/S0020-7519(01)00183-7.

77. Cacciò SM, Ryan U. Molecular epidemiology of giardiasis. *Mol Biochem Parasitol*. 2008; 160:75–80. doi: 10.1016/j.molbiopara.2008.04.006.

78. Leonhard S, Pfister K, Beelitz P, Wielinga C, Thompson RCA. The molecular characterization of *Giardia* from dogs in Southern Germany. *Vet Parasitol.* 2007;150:33–38. doi: 10.1016/j.vetpar.2007.08.034.

79. Thompson RC, Traub RJ, Parameswaran N. Molecular epidemiology of foodborne parasitic zoonoses. In: Murrell KD, Fried B, ed. *Food-Borne Parasitic Zoonoses*. New York, United States: Springer; 2007:383–415.

80. Traub RJ, Monis P, Robertson I, Irwin P, Mencke N, Thompson RC. Epidemiological and molecular evidence support the zoonotic transmission of *Giardia* among humans and dogs living in the same community. Parasitology. 2004;128:53–262. doi: 10.1017/S0031182003004505.

81. Traub RJ, Inpankaew T, Reid SA, 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; 111:125–132. doi: 10.1016/j.actatropica.2009.03.006.

82. Zajac A. Giardiosis. *Comp Contin Ed Practic Vet*. 1992;14:604–609.

83. Addiss DG, Mathews HM, Stewart JM, et al. Evaluation of a commercially available enzyme-linked immunosorbent assay for *Giardia lamblia* antigen in stool. *J Clin Microbiol.* 1991;29:1137–1142.

84. Dryden MW, Payne PA, Smith V. Accurate diagnosis of *Giardia* spp. and proper fecal examination procedures. *Vet Therap.* 2006;7:4–14.

85. Xiao L, Herd RP. Quantitation of *Giardia* cysts and *Cryptosporidium* oocysts in fecal samples by direct immunofluorescence assay. *J Clin Microbiol.* 1993;31:2944–2946.

86. Boone JH, Wilkins TD, Nash TE, et al. TechLab and Alexon *Giardia* enzyme-linked immunosorbent assay kits detect cyst wall protein 1. *J Clin Microbiol.* 1999;37:611–614.

87. Garcia LS, Shimizu RY, Novak S. Commercial assay for detection of *Giardia lamblia* and *Cryptosporidium parvum* antigens in human fecal specimens by rapid solid-phase qualitative immunochromatography. *J Clin Microbiol*. 2003;41:209–212. doi: 10.1128/JCM.41.1.209-212.2003.

88. Geurden T, Berkvens D, Casaert S, Vercruysse J, Claerebout E. A Bayesian evaluation of three diagnostic assays for the detection of *Giardia duodenalis* in symptomatic and asymptomatic dogs. *Vet Parasitol*. 2008;157:14–20. doi: 10.1016/j.vetpar.2008.07.002.

89. Thompson RC, Monis PT. Variation in *Giardia*: implications for taxonomy and epidemiology. *Adv Parasitol.* 2004;58:69–137. doi: 10.1016/S0065-308X(04)58002-8.

90. Xiao L, Fayer R, Ryan U, Upton SJ. *Cryptosporidium* taxonomy: recent advances and implications for public health. *Clin Microbiol Rev.* 2004;17:72–97. doi: 10.1128/CMR.17.1.72–97.2004.

91. Amar CFL, Dear PH, Pedraza-Diaz S, Looker N, Linnane E, McLauchlin J. Sensitive PCR-restriction fragment length polymorphism assay for detection and genotyping of *Giardia duodenalis* in human feces. *J Clin Microbiol.* 2002;40:446–452. doi: 10.1128/JCM.40.2.446–452.2002.

92. da Silva AJ, Bornay-Llinares FJ, Moura INS,Slemenda SB, Tuttle JL, Pleniazek NJ. Fast and reliable extraction of protozoan parasite DNA from fecal specimens. *Mol Diagn.* 1999;4:57–64.

93. Marquardt WC, Demaree RS, Grieve RB. *Parasitology and Vector Biology*. San Diego, CA: Academic Press;2000.

94. Fayer R. General biology. In: Fayer R, Xiao L, ed. *Cryptosporidium* and *Cryptosporidiosis*. Boca Raton, FL: CRC Press;2008;1-42.

95. Zhu G, Keithly JS, Philippe H. What is the phylogenetic position of *Cryptosporidium*? *Int J Syst Evol Microbiol*. 2000;50:1673–1681. doi: 10.1099/00207713-50-4-1673.

96. Abrahamsen MS, Templeton TJ, Enomotoet S. Complete genome sequence of the apicomplexan, *Cryptosporidium parvum*. *Science*. 2004;304:441–445. doi: 10.1126/science.1094786.

97. Barta JR, Thompson RC. What is *Cryptosporidium*? Reappraising its biology and phylogenetic affinities. *Trends Parasitol*. 2006;22:463–468. doi:10.1016/j.pt.2006.08.001.

98. Tyzzer EE. A sporozoan found in the peptic glands of the common mouse. *Exp Biol Med.* 1907;5:12-13. doi: 10.3181/00379727-5-5.

99. Slavin D. *Cryptosporidium meleagridis* (sp. nov.). *J Comp Pathol*. 1955;65(3):262-266. doi:10.1016/S0368-1742(55)80025-2.

100. Ryan U, Xiao L. Taxonomy and molecular taxonomy. In: Cacciò SM, Widmer G, ed. *Cryptosporidium: Parasite and Disease*. New York, NY: Springer;2014:3-41. doi 10.1007/978-3-7091-1562-6.

101. Fayer R. Taxonomy and species delimitation in *Cryptosporidium*. *Exp Parasitol.* 2010; 124:90–97. doi:10.1016/j.exppara.2009.03.005.

102. Xiao L. Molecular epidemiology of cryptosporidiosis: an update. *Exp Parasitol.* 2010; 124:80–89. doi:10.1016/j.exppara.2009.03.018.

103. Plutzer J, Karanis P. Genetic polymorphism in *Cryptosporidium* species: an update. *Vet Parasitol*. 2009;165:187–199. doi:10.1016/j.vetpar.2009.07.003.

104. Ng J, Yang R, McCarthy S, Gordon C, Hijawi N, Ryan U. Molecular characterization of *Cryptosporidium* and *Giardia* in pre-weaned calves in Western Australia and New South Wales. *Vet Parasitol.* 2011;176:145–150. doi: 10.1016/j.vetpar.2010.10.056.

105. Painter JE, Hlavsa MC, Collier SA, Yoder JS. Cryptosporidiosis surveillance — United States, 2011–2012. Giardiasis surveillance — United States, 2011–2012. *Morb Mortal Wkly Rep.* 2015;64(3):1-28.

106. Dietz VJ, Roberts JM. National surveillance for infection with *Cryptosporidium parvum*, 1995–1998: what have we learned? *Public Health Rep.* 2000;115:358–63.

107. Checchi F. Principles of Infectious Disease Transmission. 2009.

108. D'Antonio RG, Winn RE, Taylor JP, et al. A waterborne outbreak of cryptosporidiosis in normal hosts. *Ann Intern Med.* 1985;103:886-888.

109. Hayes EB, Matte TD, O'Brien TR, et al. Large community outbreak of cryptosporidiosis due to contamination of a filtered public water supply. *N Engl J Med.* 1989;320:1372-1376. doi: 10.1056/NEJM198905253202103.

110. Fayer R, Morgan U, Upton SJ. Epidemiology of *Cryptosporidium*: transmission, detection and identification. *Int J Parasitol.* 2000;30(12-13):1305-1322. doi:10.1016/S0020-7519(00)00135-1.

111. MacKenzie WR, Hoxie NJ, Proctor ME, et al. A massive outbreak in Milwaukee of *Cryptosporidium* infection transmitted through the public water supply. *N Engl J Med.* 1994;331(3):161-167. doi: 10.1056/NEJM199407213310304.

112. Hlavsa MC, Roberts VA, Kahler AM, et al. Recreational water-associated disease—United States, 2009–2010. *Morb Mortal Wkly Rep.* 2014;63:6–10.

113. Scallan E, Hoekstra RM, Angulo FJ, et al. Foodborne illness acquired in the United States—major pathogens. *Emerg Infect Dis*. 2011;17:7–15. doi: 10.3201/eid1701.P11101.

114. Yoder JS, Wallace RM, Collier SA, Beach MJ, Hlavsa MC. Cryptosporidiosis surveillance—United States, 2009–2010. *Morb Mortal Wkly Rep.* 2012;61:1–12.

115. Collier SA, Stockman LJ, Hicks LA, Garrison LE, Zhou FJ, Beach MJ. Direct healthcare costs of selected diseases primarily or partially transmitted by water. *Epidemiol Infect.* 2012;11:1–11. doi: 10.1017/S0950268811002858.

116. Griffiths JK. Human cryptosporidiosis: epidemiology, transmission, clinical disease, treatment and diagnosis. In: Baker JR, Muller R, Rollinson D, Tzipori S, ed. *Opportunistic Protozoa in Humans*. New York: Academic Press;1999:38-85.

117. Dillingham RA, Lima AA, Guerrant RL. Cryptosporidiosis: epidemiology and impact. *Microbes Infect*. 2002;4:1059-1066. doi:10.1016/S1286-4579(02)01630-1.

118. MacKenzie WR, Schell WL, Blair KA, 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):57-62. doi: 10.1093/clinids/21.1.57.

119. Koch KL, Phillips DJ, Aber RC, Current WL. Current cryptosporidiosis in hospital personnel. *Ann Intern Med.* 1985;102:593-596. doi:10.7326/0003-4819-102-5-593.

120. Casemore DP. Cryptosporidiosis in human and animal epidemiology. In: Fayer R, Xiao L, ed. *Cryptosporidium* and *Cryptosporidiosis*. Boca Raton, FL: CRC Press;1997:66-92.

121. Hajdusek O, Ditrich O, Slapeta J. Molecular identification of *Cryptosporidium* spp. in animal and human hosts from the Czech Republic. *Vet Parasitol.* 2004;122:183-192. doi:10.1016/j.vetpar.2004.04.005.

122. Zhou L, Fayer R, Trout JM, Ryan UM, Schaefer FW, Xiao L. Genotypes of *Cryptosporidium* species infecting fur-bearing mammals differ from those of species infecting humans. *Appl Environ Microbiol.* 2004;70:7574-7577. doi: 10.1128/AEM.70.12.7574–7577.2004.

123. Robertson LJ. *Cryptosporidium* as a Foodborne Pathogen. New York, NY: Springer; 2014. doi:10.1007/978-1-4614-9378-5.

124. Duffy G, Moriarty EM. *Cryptosporidium* and its potential as a food-borne pathogen. *Anim Health Res Rev*. 2003;4(2):95-107. doi: http://dx.doi.org/10.1079/AHRR200357.

125. Fayer R, Lewis EJ, Trout JM, et al. *Cryptosporidium parvum* in oysters from commercial harvesting sites in the Chesapeake Bay. *Emerg Infect Dis*. 1999;5(5):706-710. doi: 10.3201/eid0505.990513.

126. Graczyk TK, Fayer R, Lewis EJ, Trout JM, Farley CA. *Cryptosporidium* oocysts in Bent Mussels (*Ischadium recurvum*) in the Chesapeake Bay. *Parasitol Res.* 1999;85:518-520.

127. Chalmers RM, Sturdee AP, Mellors P, et al. *Cryptosporidium parvum* in environmental samples in the Sligo area, Republic of Ireland: A preliminary report. *Lett Appl Microbiol*.1997;25:380-384. doi: 10.1046/j.1472-765X.1997.00248.x.

128. Monge R, Chinchilla M. Presence of *Cryptosporidium* oocysts in fresh vegetables. *J Food Protect*. 1996;59:202-203.

129. Ortega YR, Sheehy RR, Cama VA, Oishi KK, Sterling CR. Restriction fragment length polymorphism analysis of *Cryptosporidium parvum* isolates of bovine and human origin. *J Protozool.* 1991;38(Suppl):40S-401S.

130. Millard P, Gensheimer K, Addiss D, et al. An outbreak of cryptosporidiosis from fresh pressed apple cider. *J Am Med Assoc*. 1994;272:1592-1596.

131. Gelletlie R, Stuart J, Soltanpoor N, Armstrong R, Nichols G. Cryptosporidiosis associated with school milk. *Lancet*. 1997;350:1005-1006. doi: http://dx.doi.org/10.1016/S0140-6736(05)64071-8.

132. Besser-Wiek JW, Forfang J, Hedberg CW, et al. Foodborne outbreak of diarrheal illness associated with *Cryptosporidium parvum* - Minnesota 1995. *Morbid Mortal Wkly Rep.* 1996;45:783.

133. Hojlyng N, Holten-Andersen W, Jepsen S. Cryptosporidiosis: A case of airborne transmission. *Lancet.* 1987;2:271-272. doi: http://dx.doi.org/10.1016/S0140-6736(87)90851-8.

134. Scorza AV. Diagnosis And Treatment Of Cryptosporidiosis And Giardiasis In Cats And Dogs In The United States [doctoral dissertation]. United States: Colorado, Colorado State University;2007.

135. Chappell CL, Okhuysen PC, Langer-Curry R, et al. *Cryptosporidium hominis*: experimental challenge of healthy adults. *Am J Trop Med Hyg*. 2006;75:851–857.

136. Okhuysen PC, Chappell CL, Crabb JH, Sterling CR, DuPont HL. Virulence of three distinct *Cryptosporidium parvum* isolates for healthy adults. *J Infect Dis*. 1999;180:1275–1281. doi: 10.1086/315033.

137. Goodgame RW, Genta RM, White AC, Chappell CL. Intensity of infection in AIDS-associated cryptosporidiosis. *J Infect Dis*.1993;167:704–709. doi: 10.1093/infdis/167.3.704.

138. Fayer R, Trout JM, Jenkins MC. Infectivity of *Cryptosporidium parvum* oocysts stored in water at environmental temperatures. *J Parasitol.* 1998;84:1165-1169. doi: 10.2307/3284666.

139. Anderson BC. Effect of drying on the infectivity of Cryptosporidia-laden calf feces for 3- to 7-day old mice. *Am J Vet Res.* 1986;47:2272-2273.

140. Fayer R. Effect of high temperature on infectivity of Cryptosporidium parvum oocysts in water. *Appl Environ Microbiol.* 1994;60:2732-2735.

141. Harp JA, Fayer R, Pesch BA, Jackson GJ. Effect of pasteurization on infectivity of *Cryptosporidium parvum* oocysts in water and milk. *Appl Environ Microbiol.* 1996;62:2866-2868.

142. Fayer R, Nerad T. Effects of low temperatures on viability of *Cryptosporidium parvum* oocysts. *Appl Environ Microbiol.* 1996;62:1431-1433.

143. Robertson LJ, Campbell AT, Smith HV. Survival of *Cryptosporidium parvum* oocysts under various environmental pressures. *Appl Environ Microbiol.* 1992;58:3494-3500.

144. Anderson BC. Moist heat inactivation of *Cryptosporidium* sp. *Am J Pub Health.* 1985;75:1433-1434.

145. Navin TR., Weber R, Vugia DJ, et al. Declining CD4+ T-lymphocyte counts are associated with increased risk of enteric parasitosis and chronic diarrhea: results of a 3-year longitudinal study. *J Acquir Immune Defic. Synd.* 1999;20:154–159.

146. Pozio E, Rezza G, Boschini A, et al. Clinical cryptosporidiosis and human immunodeficiency virus (HIV)-induced immunosuppression: findings from a longitudinal study of HIV positive and HIV-negative former injection drug users. *J Infect Dis.* 1997;176:969–975. doi: 10.1086/516498.

147. Sorvillo F, Beall G, Turner PA, et al. Seasonality and factors associated with cryptosporidiosis among individuals with HIV infection. *Epidemiol Infect.* 1998;121:197–204.

148. Bern C, Hernandez B, Lopez M B, Arrowood MJ, De Merida AM, Klein RE. The contrasting epidemiology of *Cyclospora* and *Cryptosporidium* among outpatients in Guatemala. *Am J Trop Med Hyg.* 2000;63:231–235.

149. Bhattacharya MK, Teka T, Faruque AS Fuchs GJ. *Cryptosporidium* infection in children in urban Bangladesh. *J Trop Pediatr.* 1997;43:282–286. doi: 10.1093/tropej/43.5.282.

150. Newman RD, Sears CL, Moore SR, et al. Longitudinal study of *Cryptosporidium* infection in children in northeastern Brazil. *J Infect Dis*. 1999;180:167–175. doi: 10.1086/314820.

151. Cordell RL, Addiss DG. Cryptosporidiosis in child care settings: a review of the literature and recommendations for prevention and control. *Pediatr Infect Dis J*. 1994;13:310–7.

152. Carey CM, Lee H, Trevors JT. Biology, persistence and detection of *Cryptosporidium parvum* and *Cryptosporidium hominis* oocysts. *Water Res.* 2004;38:818-862. doi:10.1016/j.watres.2003.10.012.

153. Warren C, Guerrant R. Clinical Disease and Pathology. In: Fayer R, Xiao L, ed. *Cryptosporidium and Cryptosporidiosis*. 2nd ed. Boca Raton, FL: CDC Press;2008;235–54.

154. Hellard ME, Sinclair MI, Hogg GG, Fairley CK. Prevalence of enteric pathogens among community based asymptomatic individuals. *J Gastroenterol Hepatol.* 2000;15:290–293. doi: 10.1046/j.1440-1746.2000.02089.x.

155. Pettoello-Mantovani M, Di Martino L, Dettori G, et al. Asymptomatic carriage of intestinal *Cryptosporidium* in immunocompetent and immunodeficient children: a prospective study. *Pediatr Infect Dis J.* 1995;14:1042–1047.

156. Davies AP, Campbell B, Evans MR, Bone A, Roche A, Chalmers RM. Asymptomatic carriage of protozoan parasites in children in day care centers in the United Kingdom. *Pediatr Infect Dis J.* 2009;28:838–40. doi: 10.1097/INF.0b013e31819d646d.

157. Horman A, Korpela H, Sutinen J, et al. Meta-analysis in assessment of the prevalence and annual incidence of *Giardia* spp. and *Cryptosporidium* spp. infections in humans in the Nordic countries. *Int J Parasitol.* 2004;34:1337–1346. doi: 10.1016/j.ijpara.2004.08.009.

158. Hunter PR, Hughes S, Woodhouse S, et al. Health sequelae of human cryptosporidiosis in immunocompetent patients. *Clin Infect Dis.* 2004;39(4):504–510. doi:10.10861422649.

159. Fayer R, Dubey JP, Lindsay DS. Zoonotic protozoa: from land to sea. *Trends Parasitol.* 2004:20(11):531-536. doi: 10.1016/j.pt.2004.08.008.

160. Nazemalhosseini-Mojarad E, Feng Y, Xiao L. The importance of subtype analysis of Cryptosporidium spp. in epidemiological investigations of human cryptosporidiosis in Iran and other Mideast countries. *Gastroenterol Hepatol Bed Bench*. 2012;5(2):67-70.

161. Anguish LJ, Ghiorse WC. Computer assisted scanning and video microscopy for analysis of *Cryptosporidium parvum* oocysts in soil, sediment, and feces. *Appl Environ Microbiol.* 1997;63:724-733.

162. Xiao L, Bern C, Sulaiman IM, Lal AA. Molecular epidemiology of human cryptosporidiosis. In: Thompson RC, Armson A, Ryan UM, ed. *Cryptosporidium*: *From Molecules to Disease*. Amsterdam, The Netherlands: Elsevier;2003:121-146.

163. Graczyk TK, Cranfield MR, Fayer R, Bixler H. House flies (*Musca domestica*) as transport host of *Cryptosporidium parvum. Am J Trop Med Hyg.* 1999;61(3):500-504.

164. Fayer R, Trout J, Xiao L, Morgan UM, Lai AA, Dubey JP. *Cryptosporidium canis* n. sp from domestic dogs. *J Parasitol.* 2001;87(6):1415-1422. doi: http://dx.doi.org/10.1645/0022-3395(2001)087[1415:CCNSFD]2.0.CO;2

165. Xiao L, Escalante H, Yang C, et al. Phylogenetic analysis of *Cryptosporidium* parasites based on the small-subunit rRNA gene locus. *Appl Environ Microbiol.* 1999;65:1578–1583.

166. Lucio-Forster A, Griffiths JK, Cama VA, Xiao L, Bowman DD. Minimal zoonotic risk of cryptosporidiosis from pet dogs and cats. *Trends Parasitol.* 2010; 26:174–179. doi: 10.1016/j.pt.2010.01.004.

167. Elwin K, Hadfield SJ, Robinson G, Chalmers RM. The epidemiology of sporadic human infections with unusual *Cryptosporidia* detected during routine typing in England and Wales, 2000–2008. *Epidemiol Infect.* 2012; 140:673–683. doi: 10.1017/S0950268811000860.

168. Miller DL, Liggett A, Radi ZA, Branch LO. Gastrointestinal cryptosporidiosis in a puppy. *Vet Parasitol*. 2003;115(3):199-204. http://dx.doi.org/10.1016/S0304-4017(03)00237-1.

169. Turnwald GH, Barta O, Taylor HW, Kreeger J, Coleman SU, Pourciau SS. Cryptosporidiosis associated with immunosuppression attributable to distemper in a pup. *J Am Vet Med Assoc*. 1988;192(1):79-81.

170. Fukushima K, Helman RG. Cryptosporidiosis in a pup with distemper. *Vet Pathol.* 1984;21:247-248. doi: 10.1177/030098588402100218.

171. Willard MD, Bouly D. Cryptosporidiosis, coccidiosis and total colonic mucosal collapse in an immunosuppressed puppy. *J Am Anim Hosp Assoc.* 1999;35(5):405-409. doi: 10.5326/15473317-35-5-405.

172. Denholm KM, Haitjema H, Gwynne BJ, Morgan UM, Irwin PJ. Concurrent *Cryptosporidium* and parvovirus infections in a puppy. *Aust Vet J.* 2001;79:98-101. doi: 10.1111/j.1751-0813.2001.tb10708.x.

173. Aydin Y, Guüvenç T, Beyaz L, Sancak A. Intestinal cryptosporidiosis associated with distemper in a dog. *Ankara Univ Vet Fak Derg.* 2004;51(3):233-235 (Abstract).

174. Scorza V, Tangtrongsup S. Update on the diagnosis and management of *Cryptosporidium* spp. infections in dogs and cats. *Top Companion Anim Med.* 2010;25(3):163-169. doi:10.1053/j.tcam.2010.07.007.

175. Iseki M. *Cryptosporidium felis* sp. from the domestic cat. *Jpn J Parasitol.* 1979;28:13–35.

176. Asahi H, Koyama T, Funakoshi Y, et al. Biological nature of *Cryptosporidium* sp. isolated from a cat. *Parasitol Res.* 1991;77:237–240. doi: 10.1007/BF00930864.

177. Bornay-Llinares FJ, da Silva AJ, Moura IN, et al. Identification of *Cryptosporidium felis* in a cow by morphologic and molecular methods. *Appl Environ Microbiol.* 1999;65:1455–1458.

178. Raccurt CP. Worldwide human zoonotic cryptosporidiosis caused by *Cryptosporidium felis*. *Parasite.* 2007;14:15–20.

179. Cieloszyk J, Goñi P, García A, Clavel A. Two cases of zoonotic cryptosporidiosis in Spain by the unusual species *Cryptosporidium ubiquitum* and *Cryptosporidium felis*. *Enferm Infecc Microbiol Clin*. 2012;30:549–551. doi: 10.1016/j.eimc.2012.04.011

180. Gherasim A, Lebbad M, Insulanderet M, et al. Two geographically separated food-borne outbreaks in Sweden linked by an unusual *Cryptosporidium parvum* subtype, October 2010. *Euro Surveill*. 2012;17(46):20318.

181. Insulander M, Silverlås C, Lebbad M, Karlsson L, Mattsson JG, Svenungsson B. Molecular epidemiology and clinical manifestations of human cryptosporidiosis in Sweden. *Epidemiol Infect*. 2012;141:1009–1020. doi: 10.1017/S0950268812001665.

182. Arai H, Fukuda Y, Hara T, Koyama T. Prevalence of *Cryptosporidium* infection among domestic cats in the Tokyo metropolitan district. *Jpn J Med Biol.* 1990;43(1):7-14. http://doi.org/10.7883/yoken1952.43.7.

183. Lappin MR, Dowers K, Edsell D, Cheney J. Cryptosporidiosis and inflammatory bowel disease. *Fel Pract.* 1997;25(3):10-13.

184. Goodwin MA, Barsanti JA. Intractable diarrhea associated with intestinal cryptosporidiosis in a domestic cat also infected with feline leukemia virus. *J Am Anim Hosp Assoc*. 1990;26:365-368.

185. Brizee-Buxton BL, Crystal MA. Coincident enteric cryptosporidiosis (correspondence). *J Am Anim Hosp Assoc.* 1994;30:307.

186. Monticello TM, Levy MG, Bunch SE, et al. Cryptosporidiosis in a feline leukemia virus-positive cat. *J Am Vet Med Assoc.* 1987;191(6):705-706.

187. Uga S, Matsumura T, Ishibashi K, Yoda Y, Yatomi K, Kataoka N. Cryptosporidiosis in dogs and cats in Hyogo Prefecture, Japan. *Jap J Parasitol.* 1989;38:139-143.

188. Lent ST, Burkhardt JE, Bolka D. Coincident enteric cryptosporidiosis and lymphosarcoma in a cat with diarrhea. *J Am Anim Hosp Assoc.* 1993;29:492-496.

189. Bennet M, Baxby D, Blundell, Gaskell CJ, Hart CA, Kelly DF. Cryptosporidiosis in the domestic cat. *Vet Record.* 1985;116(3):73-74. doi:10.1136/vr.116.3.73.

190. Anderson BC, Donndelinger T, Wilkins RM, Smith J. Cryptosporidiosis in a veterinary student. *J Am Vet Med Assoc.* 1982;180(4):408-409.

191. Levine JF, Levy MG, Walker RL, Crittenden S. Cryptosporidiosis in veterinary students. *J Am Vet Med Assoc*. 1988;193(11):1413-1414.

192. Peng MM, Xiao LH, Freeman AR, et al. Genetic polymorphism among *Cryptosporidium parvum* isolates: Evidence of two human transmission cycles. *Emerg Infect Dis.* 1997;3(4):567-573. doi: 10.3201/eid0304.970423.

193. Mc Laughlin J, Amar C, Pedraza-Diaz S, Nichols GL. Molecular epidemiological analysis of *Cryptosporidium* spp. in the United Kingdom: Results of genotyping *Cryptosporidium* spp. in 1705 fecal samples from humans and 105 fecal samples from livestock animals. *J Clin Microbiol.* 2000;38(11):3984-3990.

194. Pedraza-Diaz S, Amar C, Iversen AM, Stanley PJ, McLauchlin J. Unusual *Cryptosporidium* species recovered from human feces: first description of *Cryptosporidium felis* and *Cryptosporidium* "dog type" from patients in England. *J Med Microbiol.* 2001;50(3):293-296. doi: 10.1099/0022-1317-50-3-293.

195. Xiao L, Bern C, Limor J, et al. Identification of 5 types of *Cryptosporidium* parasites in children in Lima, Peru. *J Infect Dis.* 2001;183(3):492-497. doi: 10.1086/318090.

196. Pienizaek NJ, Bornay Llinares FJ, Slemenda SB, et al. New *Cryptosporidium* genotypes in HIV-infected persons. *Emerg Infect Dis*. 1999;5(3):444-449. doi: 10.3201/eid0503.990318.

197. Morgan UM, Weber R, Xiao L, et al. Molecular characterization of *Cryptosporidium* isolates obtained from human immunodeficiency virus infected individuals living in Switzerland, Kenya and the United States. *J Clin Microbiol.* 2000;38:1180-1183.

198. Smith H. Diagnostics. In Fayer R, Xiao L, ed. *Cryptosporidium and Cryptosporidiosis*. Boca Raton, FL: CRC Press;2008;173-207.

199. Vastert D, Brinkman M, Wilke H, Mulder B. Diagnosis of *Cryptosporidium parvum* with microscopy, striptest, ELISA and realtime PCR [abstract P857]. Regional Public Health Laboratory, Enschede, the Netherlands.

200. Kuczynska E, Shelton DR. Method for detection and enumeration of *Cryptosporidium parvum* oocysts in feces, manures, and soils. *Appl Environ Microbiol*. 1999;65:2820–2826.

201. Arrowood MJ, Sterling CR. Isolation of *Cryptosporidium* oocysts and sporozoites using discontinuous sucrose and isopycnic Percoll gradient. *J Parasit.* 1987;73(2):314-319. doi: 10.2307/3282084.

202. Arrowood MJ, Donaldson K. Improved purification methods for calf-derived *Cryptosporidium parvum* oocysts using discontinuous sucrose and cesium chloride gradients. *J Eukaryot Microbiol.* 1996;43(5):89S. doi: 10.1111/j.1550-7408.1996.tb05015.x.

203. Casemore DP. Laboratory methods for diagnosing cryptosporidiosis. ACP Broadsheet 128. *J Clin Pathol.* 1991;128;445-451.

204. Petry F. Laboratory diagnosis of *Cryptosporidium parvum* infection. *Contrib Microbiol.* 2000;6:33-49. doi:10.1159/000060364.

205. Laboratory diagnosis of cryptosporidiosis: *Cryptosporidium* spp. Centers for Disease Control and Prevention Web site. Laboratory Identification of Parasites of Public Health Concern. http://www.cdc.gov/dpdx/resources/pdf/benchAids/Crypto_benchaid.pdf Accessed October 25, 2015.

206. Baxby D, Blundell N, Hart CA. The development and performance of a simple, sensitive method for the detection of *Cryptosporidium* oocysts in feces. *J Hyg Camb.* 1984;92:317-323.

207. Kehl KS, Cicirello H, Havens PL. Comparison of four different methods for detection of *Cryptosporidium* species. *J Clin Microbiol.* 1995;33(2):416–418.

208. Fluorescent antibodies: Fluorescent Antibody Technique. The Free Dictionary Web site. http://medical-dictionary.thefreedictionary.com/indirect+fluorescent+antibody+test Published 2003. October 27, 2015.

209. Cryptosporidiosis laboratory tests: Laboratory Findings. Wikidoc Web site. http://www.wikidoc.org/index.php/Cryptosporidiosis_laboratory_tests. Updated June 11, 2015. Accessed October 25, 2015.

210. Peeters JE, Villacorta I, Vanopdenbosch E. *Cryptosporidium parvum* in calves: kinetics and immunoblot analysis of specific serum and local antibody responses (Immunoglobulin A [IgA], Ig G, and IgM) after natural and experimental infections. *Infect Immun*. 1992;60(6):2309-2316.

211. Cirak VY, Bauer C. Comparison of conventional coproscopical methods and commercially copro-antigen ELISA kits for the detection of *Giardia* and *Cryptosporidium* infections in dogs and cats. *Berl Munch Tierarztl Wochenschr*. 2004;117(9-10):410-413.

212. Webster KA, Smith HV, Giles M, Dawson L, Robertson LJ. Detection of *Cryptosporidium parvum* oocysts in feces: comparison of conventional coproscopical methods and the polymerase chain reaction. *Vet Parasitol*. 1996;61(1-2):5-13. doi:10.1016/0304-4017(95)00811-X.

213. Alves M, Xiao L, Sulaiman IM, Lal AA, Matos O, Antunes F. Subgenotype analysis of *Cryptosporidium* isolates from humans, cattle, and zoo ruminants in Portugal. *J Clin Microbiol.* 2003;41(6):2744–2747. doi: 10.1128/JCM.41.6.2744-2747.2003.

214. Cacciò SM, Thompson RCA, McLaughlin J, Smith HV. Unravelling *Cryptosporidium* and *Giardia* epidemiology. *Trends Parasitol*. 2005;21(9):430-437. doi: 10.1016/j.pt.2005.06.013.

215. Aiello AE, Xiao LH, Limor JR, Liu C, Abrahamsen MS, Lal AA. Microsatellite analysis of the human and bovine genotypes of *Cryptosporidium parvum*. *J Eukariot Microbiol.* 1999;46(5):46S-47S.

216. Cacciò SM, Homan W, Camilli R, Traldi G, Kortbeek T, Pozio E. A microsatellite marker reveals population heterogeneity within human and animal genotypes of *Cryptosporidium parvum*. *Parasitol.* 2000;120(3):237-244.

217. Sulaiman IM, Morgan UM, Thompson RCA, Lal AA, Xiao L. Phylogenetic Relationships of Cryptosporidium Parasites Based on the Heat Shock Protein (HSP-70) Gene. *App Environm Microbiol*. 2000;66(6):2385-2391. doi: 10.1128/AEM.66.6.2385-2391.2000.

218. Peng MM, Matos O, Gatei W, et al. A comparison of *Cryptosporidium* subgenotypes from several geographic regions. *J Eukaryot Microbiol.* 2001;28S-31S. doi: 10.1111/j.1550-7408.2001.tb00442.x.

219. Sulaiman IM, Lal AA, Xiao L. A population genetic study of the *Cryptosporidium parvum* human genotype parasites. *J Eukaryot Microbiol.* 2001;Suppl:24S-27S.

220. Xiao LH, Limor J, Bern C, Lal AA. Tracking *Cryptosporidium parvum* by sequencing analysis of the small double-stranded RNA. *Emerg Infect Dis*. 2001;7(1):141-145.

221. Xiao L. Molecular epidemiology of cryptosporidiosis: An update. *Exp Parasitol.* 2010;124:80–89. doi:10.1016/j.exppara.2009.03.018.

222. Tangtrongsup S, Scorza V. Update on the diagnosis and management of *Giardia* spp. infections in dogs and cats. *Top Companion Anim Med.* 2010;25(3):155-62. doi:10.1053/j.tcam.2010.07.003.

223. Erickson MC, Ortega YR. Inactivation of protozoan parasites in food, water, and environmental systems. *J Food Prot.* 2006;69:2786–808.

224. Nichols G. Epidemiology. In: Fayer R, Xiao L, ed. *Cryptosporidium and Cryptosporidiosis*. New York, USA. CRC Press;2008:79-118.

225. Lappin MR. Diseases of the gastrointestinal tract. In: Washabau RJ, Day MJ, ed. *Canine and Feline Gastroenterology*. Missouri, USA. Elsevier Inc.; 2013:683-695.

226. Thompson RCA, Monis P. *Giardia* – from genome to proteome. *Adv Parasitol*. 2012;78:57-95. doi: 10.1016/B978-0-12-394303-3.00003-7.

227. Morgan-Ryan UM, Fall A, Ward LA, et al. *Cryptosporidium hominis* n. sp. (Apicomplexa: Cryptosporidiidae) from Homo sapiens. *J Eukaryot Microbiol.* 2002;49:433–440. doi: 10.1111/j.1550-7408.2002.tb00224.x.

228. Elwin K, Hadfield SJ, Robinson G, Crouch ND, Chalmers RM. *Cryptosporidium viatorum n. sp.* (Apicomplexa: Cryptosporidiidae) among travelers returning to Great Britain from the Indian subcontinent, 2007–2011. *Int J Parasitol.* 2012;42:675–682. doi: 10.1016/j.ijpara.2012.04.016.

229. Gibson-Kueh S, Yang R. Thuy NTN, Jones JB, Nicholls PK, Ryan U. The molecular characterization of an Eimeria and *Cryptosporidium* detected in Asian seabass *(Lates calcarifer*) cultured in Vietnam. *Vet Parasitol.* 2011;181:91–96. doi:10.1016/j.vetpar.2011.05.004.

230. Tyzzer EE. *Cryptosporidium parvum* (sp. nov.), a coccidium found in the small intestine of the common mouse. *Arch Protisenk.* 1912;26:394–412

231. Reid A, Lymbery A, Ng J, Tweedle S, Ryan U. Identification of novel and zoonotic *Cryptosporidium* species in marine fish. *Vet Parasitol.* 2010;168:190–195. doi:10.1016/j.vetpar.2009.11.015.

232. Fayer R, Santin M, Xiao L. *Cryptosporidium bovis* n. sp. (Apicomplexa: Cryptosporidiidae) in cattle (Bos taurus). *J Parasitol.* 2005;91:624–629. doi: http://dx.doi.org/10.1645/GE-3435.

233. Fayer R, Santin M, Trout JM. *Cryptosporidium ryanae* n. sp. (Apicomplexa: Cryptosporidiidae) in cattle (*Bos taurus*). *Vet Parasitol*. 2008;156:191–198. doi: 10.1016/j.vetpar.2008.05.024.

234. Lindsay DS, Upton SJ, Owens DS, Morgan UM, Mead JR, Blagburn BL. *Cryptosporidium andersoni* n. sp. (Apicomplexa: Cryptosporidiidae) from cattle, *Bos taurus*. *J Eukaryot Microbiol.* 2000;47:91–95.

235. Fayer R, Santin M. *Cryptosporidium xiaoi* n. sp. (Apicomplexa: Cryptosporidiidae) in sheep (*Ovis aries*). *Vet Parasitol.* 2009;164:192–200. doi: 10.1016/j.vetpar.2009.05.011.

236. Fayer R, Santin M, Macarisin D. *Cryptosporidium ubiquitum* n. sp. in animals and humans. *Vet Parasitol*. 2010;172:23–32. doi: 10.1016/j.vetpar.2010.04.028.

237. Santin M, Dixon BR, Fayer R. Genetic characterization of *Cryptosporidium* isolates from ringed seals (*Phoca hispida*) in Northern Quebec, Canada. *J Parasitol.* 2005;91:712–716. doi: http://dx.doi.org/10.1645/GE-3438RN.

238. Bass AL, Wallace CC, Yund PO, Ford TE. Detection of *Cryptosporidium* sp. in two new seal species, *Phoca vitulina* and *Cystophora cristata,* and a novel *Cryptosporidium* genotype in a third seal species, *Pagophilus groenlandicus*, from the Gulf of Maine. *J Parasitol.* 2012;98:316–322.

239. Ren X, Zhao J, Zhang L, et al. *Cryptosporidium tyzzeri* n. sp. (Apicomplexa: Cryptosporidiidae) in domestic mice (*Mus musculus*). *Exp Parasitol.* 2012;130:274–281. doi: 10.1016/j.exppara.2011.07.012.

240. Ryan UM, Monis P, Enemark HL, et al. *Cryptosporidium suis* n. sp. (Apicomplexa: Cryptosporidiidae) in pigs (*Sus scrofa*). *J Parasitol*. 2004;90:769–773. doi: 10.1645/GE-202R1.

241. Kváč M, Kestránová M, Pinková, et al. *Cryptosporidium scrofarum* n. sp. (Apicomplexa: Cryptosporidiidae) in domestic pigs (*Sus scrofa*). *Vet Parasitol.* 2013;191:218–227. doi: 10.1016/j.vetpar.2012.09.005.

242. Vetterling JM, Jervis HR Merrill TG, Sprinz H. *Cryptosporidium wrairi* sp. n. from the guinea pig *Cavia porcellus*, with an emendation of the genus. *J Protozool.* 1971;18:243–247. doi: 10.1111/j.1550-7408.1971.tb03315.x.

243. Robinson G, Wright S, Elwin K, et al. Re-description of *Cryptosporidium cuniculus* Inman and Takeuchi, 1979 (Apicomplexa: Cryptosporidiidae): morphology, biology and phylogeny. *Int J Parasitol*. 2010;40(13):1539–1548. doi: 10.1016/j.ijpara.2010.05.010.

244. Inman LR, Takeuchi A. Spontaneous cryptosporidiosis in an adult female rabbit. *Vet Pathol*. 1979;16:89–95.

245. Ryan UM, Power, Xiao L. *Cryptosporidium fayeri* n. sp. (Apicomplexa: Cryptosporidiidae) from the Red Kangaroo (*Macropus rufus*). *J Eukaryot Microbiol.* 2008;55:22–26. doi: 10.1111/j.1550-7408.2007.00299.x.

246. Power ML, Ryan UM. A new species of *Cryptosporidium* (Apicomplexa: Cryptosporidiidae) from eastern grey kangaroos (*Macropus giganteus*). *J Parasitol.* 94:1114–1117. doi: 10.1645/GE-1508.1.

247. Lindsay DS, Blagburn BL, Sandermann CA. Morphometric comparison of the oocysts of *Cryptosporidium meleagridis* and *Cryptosporidium baileyi* from birds. *Proc Helminthol Soc Wash*. 1989;56(1):91–92.

248. Morgan UM, Xiao L, Limor J, et al. *Cryptosporidium meleagridis* in an Indian ring-necked parrot (*Psittacu la krameri*). *Aust Vet J. 2000*;78:182–183. doi: 10.1111/j.1751-0813.2000.tb10589.x.

249. Morgan UM, Monis PT, Xiao L, et al. Molecular and phylogenetic characterization of *Cryptosporidium* from birds. *Int J Parasitol.* 2001;31:289–296. http://dx.doi.org/10.1016/S0020-7519(00)00164-8.

250. Glaberman S, Sulaiman IM, Bern C, et al. A multilocus genotypic analysis of *Cryptosporidium meleagridis*. *J Eukaryot Microbiol.* 2001;48:19S–22S. doi: 10.1111/j.1550-7408.2001.tb00439.x.

251. Abe N, Iseki M. Identification of *Cryptosporidium* isolates from cockatiels by direct sequencing of the PCR-amplified small subunit ribosomal RNA gene. *Parasitol Res.* 2004;92:523–526. doi: 10.1007/s00436-004-1082-z.

252. Page`s-Mante´ A, Page`s-Bosch M, Majó-Masferrer N, Gómez-Couso H, Ares-Mazás E. An outbreak of disease associated with Cryptosporidia on a red-legged partridge (*Alectoris rufa*) game farm. *Avian Pathol.* 2007;36:275–278. doi:10.1080/03079450701439389.

253. Abe N, Makino I. Multilocus genotypic analysis of *Cryptosporidium* isolates from cockatiels, Japan. *Parasitol Res*. 2010;106:1491–1497. doi: 10.1007/s00436-010-1810-5.

254. Wang R, Jian F, Sun Y, et al. Large-scale survey of *Cryptosporidium* spp. in chickens and Pekin ducks (*Anas platyrhynchos*) in Henan, China: prevalence and molecular characterization. *Avian Pathol*. 2010;39:447–451. doi: 10.1080/03079457.2010.518314.

255. Qi M, Wang R, Ning C, et al. *Cryptosporidium* spp. in pet birds: genetic diversity and potential public health significance. *Exp Parasitol.* 2011;128:336–340. doi: 10.1016/j.exppara.2011.04.003.

256. Berrilli F, D'Alfonso R, Giangaspero A, et al. *Giardia duodenalis* genotypes and *Cryptosporidium* species in humans and domestic animals in Côte d'Ivoire: occurrence and evidence for environmental contamination. *Trans R Soc Trop Med Hyg*. 2012;106:191–195. doi: 10.1016/j.trstmh.2011.12.005.

257. Wang R, Wang F, Zhao J, et al. *Cryptosporidium* spp. in quails (*Coturnix coturnix japonica*) in Henan, China: molecular characterization and public health significance. *Vet Parasitol*. 2012;187:534–537. doi: 10.1016/j.vetpar.2012.02.002.

258. Pathogen and environment: Causal Agent. Centers for Disease Control and Prevention Web site. http://www.cdc.gov/parasites/giardia/pathogen.html. Updated July 21, 2015. Accessed October 29, 2015.

259. Pathogen and environment: Causal Agent. Centers for Disease Control and Prevention Web site. http://www.cdc.gov/parasites/crypto/pathogen.html February 20, 2015. Accessed March 22, 2016.

CHAPTER 2: EVALUATION OF FACTORS ASSOCIATED WITH THE RISK OF TESTING POSITIVE TO *GIARDIA* SPP. AND *CRYPTOSPORIDIUM* SPP. IN PET DOGS AND CATS IN THE USA

Summary

Giardia spp. and *Cryptosporidium* spp. are protozoans that colonize and reproduce in the intestines of several domesticated animals, including dogs and cats and can include a range of manifestations from subclinical infection to severe diarrhea. *Cryptosporidium* spp. infection rates in dogs and cats are largely unknown as sensitive diagnostic procedures were not previously available. Polymerase chain reaction (PCR) assays are now available to amplify *Giardia* spp. and *Cryptosporidium* spp. DNA from feces.

The main purpose of this study was to estimate the risk of testing positive for *Giardia* or *Cryptosporidium* among pet dogs and cats in the USA. Additionally, whether the animals' age, sex, region of origin, and time of the year (season) were associated with the risk of testing positive for these pathogens were evaluated. Finally, coinfections due to *Giardia* spp. and *Cryptosporidium* spp. among the studied pets was quantified.

Data from fecal samples processed at ANTECH® Diagnostics during the period 2010-2015 were analyzed for this study. PCR assays were performed on fecal samples from 22,959 dogs and 16,273 cats. The assays amplified *Giardia* spp. and *Cryptosporidium* spp. in dogs and *Giardia* spp., *Cryptosporidium felis* and *Cryptosporidium* spp. in cats. Descriptive analysis, univariable, and multivariable logistic regression analyses were conducted to assess associations between age, sex, region, and season with the risk of testing positive to either *Giardia* spp. or *Cryptosporidium* spp. in pet dogs and cats.

The percentage of *Giardia* spp. positive test results among pet dogs was 7.7 (95% CI: 7.3, 8.0). For *Cryptosporidium* spp. in dogs the percentage of positive results was 5.4 (95% CI:

5.1, 5.7). Whereas in pet cats, the percentage of positives to *Giardia* spp. was 5.2 (95% CI: 4.9, 5.5), for *C. felis* was 5.1 (95% CI: 4.7, 5.4) and for *Cryptosporidium* spp. was 7.4 (95% CI: 7.0, 7.9).

The results of logistic regression models showed that age was a significant factor associated with both *Giardia* spp. and *Cryptosporidium* spp. in dogs and cats. Region was also significantly associated with the risk of *Cryptosporidium* spp. infections in dogs and cats. Season variable was significantly associated with the risk of *Giardia* spp. in pet dogs only.

Cryptosporidium spp. was detected in fecal samples of 336 (19.1%) of the 1,762 *Giardia* spp. positive dogs and *Giardia* spp. was detected in fecal samples of 336 (27.2%) of the 1,237 *Cryptosporidium* spp. positive dogs. For cats, *Cryptosporidium* spp. was detected in 132 (15.7%) of 843 *Giardia* spp. positive cats. Of the 843 *Giardia* spp. positive cats, 97 (11.8%) were positive for *C. felis*.

Our study results indicate that *Giardia* spp. and *Cryptosporidium* spp. are common pathogens in pet dogs and cats. In all multivariate models, the results showed that age was a significant predictor associated with *Giardia* spp. and *Cryptosporidium* spp. in dogs and cats. This finding calls suggests that infection by these protozoans should be suspected more highly in younger dogs and cats. Our multivariate analysis has also identified that the regional distribution is significantly associated with *Cryptosporidium* spp. infections in dogs and cats, whereas seasonal distribution is only associated with the risk of *Giardia* spp. in pet dogs. Thus, veterinarians in the Midwest region may be more likely to encounter *Cryptosporidium* infections in dogs and cats and veterinarians evaluating dogs with diarrhea in the summer season are more likely to encounter *Giardia* spp. infections. Coinfections with both pathogens can occur in pet dogs and cats.

As the histories from these dogs and cats are unknown, additional studies will be required to evaluate for associations of positive test results with clinical findings and to determine the likelihood dogs or cats are carrying zoonotic *Giardia* spp. or *Cryptosporidium* spp.

2.1 Introduction

Giardia spp. and *Cryptosporidium* spp. both are intestinal protozoan parasites that colonize and reproduce in the intestines of domesticated animals included dogs and cats and can be associated with diarrhea.[1] The clinical signs in dogs and cats depend greatly on the status of the host immune system, which is considered one determinant of whether disease will occur.[2] For instance, immune-competent cats may not develop diarrhea when they are infected with *Cryptosporidium felis* even though the cats are shedding the oocysts.[3] However, in immunocompromised cats, clinical signs associated with feline cryptosporidiosis can involve diarrhea, anorexia and weight loss.[4] Another determinant of whether disease will occur is the age of the host. For example, infections with *Cryptosporidium* spp. in dogs are more prevalent in young animals compared to older ones.[4] Furthermore, cryptosporidiosis can be acute if the animal had underlying conditions such as preexisting disease in the intestinal tract, or coinfection with other infectious or non-infectious agents.[4] For example, cryptosporidiosis in pups can result in severe diarrhea when associated with co-infections of parvovirus, distemper or parasitism.[5-7] Intestinal malabsorption was reported in an adult dog with cryptosporidiosis suggesting the infection can be chronic.[8]

Regarding feline giardiasis, it has been documented that kittens are more susceptible to the infection and diarrheal disease than adult cats.[9] Diarrhea in cats is usually mucoid, pale, soft, has a strong odor, and steatorrhea may also be seen.[9]

Canine giardiasis is common and *Giardia* associated diarrhea in dogs is usually self-limiting in immunocompetent animals. Immune-compromised animals may develop chronic malabsorption; therefore, weight loss may be detected.[10]

The diagnosis of these two protozoans still is challenging for a considerable number of veterinary practices. Even for animals presenting with diarrhea, direct diagnosis of the causal agent is not easy.[11] While conventional diagnosis techniques such as microscopy, immuno-fluorescence assay (IFA) or enzyme-linked immunosorbent assay (ELISA) are widely used in veterinary medicine to diagnose *Cryptosporidium* spp. and *Giardia* spp., these techniques have several limitations.

Cryptosporidium spp. diagnosis can be initially performed by microscopic examination as initial diagnostic workup. However, this technique is commonly falsely negative due to poor sensitivity and cannot be used to determine the species of *Cryptosporidium*-associated with the infection. In addition, infected dogs and cats shed *Cryptosporidium* spp. intermittently; thus, multiple samples from the same infected animal are required to confirm results.[12]

The diagnosis of giardiasis can be challenging for a number of reasons and can result in underdiagnosis, misdiagnosis or overdiagnosis.[13] *Giardia* cysts are shed intermittently which can cause false negative results; therefore, repeated fecal analysis may be required. Identification of *Giardia* cysts requires a trained microscopists as examination by less trained individual may result in a misdiagnosis.[12,13] Either pseudoparasites and yeasts can be easily mistaken as *Giardia* cysts giving false positive results. In addition, *Giardia* cysts can deteriorate in fecal flotation solutions giving false negative results.[13]

Several polymerase reaction chain (PCR) protocols have been used to amplify *Cryptosporidium* spp. and *Giardia* spp. DNA from fecal specimens of pet dogs and cats and now

are available in many commercial diagnostic laboratories. Genotyping can also be performed with some molecular techniques and has been used to study potentially zoonotic species of both protozoans and transmission probability to pet owners.[14-18]

Several studies[19-31] conducted throughout the USA that have used microscopy, ELISA and IFA have shown that intestinal parasites such as *Giardia* spp. and *Cryptosporidium* spp. are common in pet dogs and cats. Several of those studies were conducted during the period of 1999-2012. For instance, the reported prevalence of canine giardiasis ranged from 5.0 % - 7.0% in dogs with no clinical signs, 0.4 % - 16.0% in dogs with clinical signs, and 3.0% - 4.0% in dogs with unknown clinical signs.[19-25] For feline giardiasis, the reported prevalence estimates were 2.0% in cats with no clinical signs, and ranged from 8.0% - 14.0% in cats with clinical signs, and 2.0% in cats with unknown clinical signs.[22,26-29] The reported prevalence of canine cryptosporidiosis in dogs with clinical signs ranged from 2.3% - 5.6%, and 1.7% in dogs without clinical signs[20,25]. Whereas feline cryptosporidiosis estimates in cats with clinical signs ranged from 3.9% - 25.0% and from 1.9% - 10.0% in cats without clinical signs.[29,30,31]

Fewer studies have been conducted in different regions of the USA using PCR assays for genotyping or confirmation of a positive result for *Giardia* spp. and *Cryptosporidium* spp., during the period of 2000-2012.[20,28,32] In addition, fewer studies have evaluated the prevalence of *Giardia* spp. in dogs and cats by regional distribution in the United States.[23,24]

In this retrospective study, we analyzed results of PCR panels performed by a commercial service laboratory (ANTECH® Diagnostics) on feces from dogs and cats. The purpose of this cross-sectional study was to evaluate associations between the probability of testing positive to *Giardia* spp. and *Cryptosporidium* spp. and to determine potential risk factors

including animals' age, sex, region of origin, and time of the year in which fecal samples were collected (season).

2.2 Materials and Methods

2.2.1 Study Samples

PCR results of testing for *Giardia* spp. and *Cryptosporidium* spp. in canine and feline species were obtained from ANTECH® Diagnostic laboratories in January, 2015. The data contained information about fecal samples collected by veterinarians from fifty states in the United States from the period of 2010-2015. A total of 22,959 PCR canine test results for *Giardia* spp. and *Cryptosporidium* spp. and a total of 16,273 PCR feline test results for *Giardia* spp., *Cryptosporidium* spp. and *Cryptosporidium felis* were analyzed in this study.

2.2.2 Inclusion and Exclusion Criteria

The original data received from ANTECH contained test results of PCR panels for 13 pathogens. For the purpose of this study, only data regarding *Giardia* spp. and *Cryptosporidium* spp. in both dogs and cats was evaluated. Most of the samples were submitted by clinics located within the United States; however, the data also contained a few samples from commonwealth territories such as the Virgin Islands, Guam and Puerto Rico as well from Japan, Canada and South Korea. Test results from these regions were excluded from the dataset. The outcome of interest in our analysis was a "positive" or "negative" test results for *Giardia* spp. and *Cryptosporidium* spp. and only complete records containing test result information were included in our analysis.

2.2.3 Data Organization

The accession result identifier (i.e. animal identity) was used to track the same animal test results for the different pathogens. The fifty states of the United States were placed in the

Northeast (Connecticut, Delaware, Washington DC, Massachusetts, Maryland, Maine, New Hampshire, New Jersey, New York, Pennsylvania, Rhode Island, Vermont, Midwest (Iowa, Illinois, Indiana, Kansas, Michigan, Minnesota, Missouri, North Dakota, Nebraska, Ohio, South Dakota, Wisconsin), South (Alabama, Arkansas, Florida, Georgia, Kentucky, Louisiana, Mississippi, North Carolina, Oklahoma, South Carolina, Tennessee, Texas, Virginia, West Virginia), or West (Alaska, Arizona, California, Colorado, Hawaii, Idaho, Montana, New Mexico, Nevada, Oregon, Utah, Washington, Wyoming) according to the U.S. Census Bureau classification for regions as previously described and as displayed in Figure 2.1.[23]

The months in which the fecal sample was obtained were grouped and characterized based on the meteorological season classification of the northern hemisphere into four season categories: spring, summer, fall and winter. Each category contained three months.[33] Spring months were March, April and May, summer months were June, July, and August, autumn months were September, October, and November), and winter months were December, January, and February.[33] Animal age was categorized and analyzed as a categorical variable including five age categories were created: 1) <6 months, 2) 6 months – 1 year, 3) 1-2 years, 4) 3-7 years, and 5) >7 years similar to previously described.[23]

2.2.4 Causal Model

Causal model was created for the study variables as presented in Figure 2.2. The factors in the model are displayed per their causal order and relationship to the outcome variable on the far right (i.e. *Giardia* spp. and *Cryptosporidium* spp. infections in dogs and cats).

2.2.5 Regression Model Building Steps

Four predictor variables (age, sex, region, and season) were evaluated for inclusion in the models. The outcome variable in this study is dichotomous i.e. it represents the probability of

testing either positive or negative to either *Giardia* spp. or *Cryptosporidium* spp. or both. Thus, logistic regression analysis was used to analyze the data and identify associations between risk factors and the outcome of interest.

2.2.5.1 Descriptive Analysis

The outcome of interest and risk factors distributions were evaluated using standard descriptive statistics. The distribution (%'s) of test positive dogs and cats for *Giardia* spp. or *Cryptosporidium* spp. was calculated with the corresponding 95% CI, and similarly, the frequency distribution for age, sex, region and season, was calculated and presented as percentages with the corresponding 95% CI for each category.

2.2.5.2 Logistic Regression Analysis

As a first screening step, associations between individual risk factors and the outcome (testing positive or negative to *Giardia* spp., or *Cryptosporidium* spp.) were evaluated using univariable logistic regression analysis. Odds ratios and their corresponding 95% CIs were calculated. To account for the effect of multiple factors in the outcome and to control for potential confounding, associations between potential risk factors and the outcome were evaluated using a multivariable logistic regression. Factors showing a p-value <0.25 at the univariable analysis were used in the multivariable model. The likelihood ratio test (LRT) was used to determine the statistical significance of individual predictors in the multivariable model. The Hosmer – Lemeshow goodness of fit test was used to evaluate the overall fit of the final models generated. The presence of outliers and/or influential observations that could affect the model fit was evaluated. This evaluation was conducted by plotting the standardized residuals.[34]

2.3 Statistical Analysis

STATA® 13.0 (StataCorp. 2013. Stata Statistical Software: Release 13. College Station, TX: StataCorp LP.) was used for data manipulation, descriptive analysis, univariate and multivariate logistic regressions. These analyses were carried out to assess and compare of the risk of testing positive to *Giardia* spp. or *Cryptosporidium* spp., in dogs and cats. Associations between two predictor variables were evaluated using Chi-squared tests to determine collinearity. Logistic regression analysis was used to obtain the odds ratios with their corresponding 95% confidence interval and p-values when comparing the risk of testing positive to *Giardia* spp. or *Cryptosporidium* spp., in dogs and cats. Statistical significance was declared at $p<0.05$.

2.4 Results

2.4.1 Descriptive Analysis

The Outcome of Interest

The proportion of positive results for *Giardia* spp. and *Cryptosporidium* spp. in dogs and cats are shown in Tables 2.1-2.2. In total, 1,762 dogs were positive to *Giardia* spp., representing 7.7% (95% CI: 7.3, 8.0%) of the dog samples. In total, 1,237 dogs were positive to *Cryptosporidium* spp. representing 5.3% (95% CI: 5.1, 5.7%) of all dog samples. For cats, the percentage of positives for *Giardia* spp. was 5.2% (95% CI: 4.9, 5.5%), *C. felis* was 5.1% (95% CI: 4.7, 5.4%), and *Cryptosporidium* spp. was 7.4% (95% CI: 7.0, 7.9%) (Table 2.2).

Coinfections

Cryptosporidium spp. DNA was amplified from fecal samples of 336 (19.1%) of the 1,762 *Giardia* spp. positive dogs and *Giardia* spp. DNA was amplified from fecal samples of 336 (27.2%) of the 1,237 *Cryptosporidium* spp. positive dogs. For cats, *Cryptosporidium* spp.

DNA was amplified from 132 (15.7%) of 843 *Giardia* spp. PCR positive cats. Of the 843 *Giardia* spp. PCR positive cat samples, 97 (11.8%) were positive for *C. felis* DNA.

Risk Factors Distribution of Dogs and Cats

The distribution of dog and cat ages is shown in Table 2.3. The majority of dogs were at age between 3-7 years old (29.3%; 95% CI: 29, 30%) whereas the majority of cats were at age > 7 years old (29.4%; 95% CI: 29, 30%). The sex categories used in this study are described in Table 2.4. As shown in the Table, the majority of dogs were castrated males and spayed females (34%; 95% CI: 34, 35%), but the majority of cats were castrated males (42.1%; 95% CI: 41.3, 42.8%). The analysis of region variable is shown in Table 2.5 for dogs and cats. The majority of dogs (38.2%; 95% CI: 37.6, 38.9%) and cats (31.0%; 95% CI: 30.3, 31.7%) were located in the west region.

Four season categories for dogs and cats are shown in Table 2.6. For dogs, the majority of samples were submitted in autumn (26.4%; 95% CI: 25.9, 27.0%) as well as for cats (29.9%; 95% CI: 29.2, 30.6%).

Giardia spp. PCR Test Results Distribution by the Risk Factors of Dogs

The distribution of PCR test results (positive or negative) of *Giardia* spp. for dogs by age, sex, region and season are displayed in Table 2.7. For the age variable, the majority of test positive dogs were at age < 6 months (19.4%; 95% CI: 18.1, 20.8%). For the sex variable, the higher percentage positive were in intact female and male dogs (13.6%, 13.2%), respectively. The Midwest region had highest percentage positive compared to other region categories (9.9%; 95% CI: 8.7, 11.1%). For the season, spring represented highest percent positive dogs for *Giardia* PCR test (8.6%; 95% CI: 7.8, 9.3%).

Cryptosporidium spp. PCR Test Results Distribution by the Risk Factors of Dogs

The distribution of PCR test results of *Cryptosporidium* spp. for dogs by age, sex, region and season are shown in Table 2.8. For the age variable, the majority of test positive dogs were at age < 6 months (14.7%; 95% CI: 13.4, 15.9%). For the sex variable, the highest percentage positive was in intact female dogs (9.8%; 95% CI: 8.7, 11.0%). The west region had the highest percentage positive compared to other region categories (6.8%; 95% CI: 6.3, 7.3%). For the season, there was not much variability among the four categories.

Giardia spp. PCR Test Results Distribution by the Risk Factors of Cats

The distribution of PCR test results (positive or negative) of *Giardia* spp. for cats by age, sex, region and season are displayed in Table 2.9. For the age variable, the majority of test positive cats were at age between 6 months – one-year-old (8.8%; 95% CI: 7.4, 10.3%). For the sex variable, the highest percentage positive was in intact female cats (8.1%; 95% CI: 6.9, 9.4%). The Midwest region had highest percentage positive compared to other region categories (5.8%; 95% CI: 4.8, 6.9%). For the season, autumn represented highest percent positive cats for *Giardia* PCR test (5.6%; 95% CI: 5.0, 6.3%).

Cryptosporidium felis PCR Test Results Distribution by the Risk Factors of Cats

The distribution of test results (positive or negative) of *Cryptosporidium felis* for cats by age, sex, region and season is shown in Table 2.10. For the age variable, the majority of test positive cats were at age between 6 months – one-year-old (10.7%; 95% CI: 9.2, 12.4%). For the sex variable, the highest percentage positive was in intact female cats (6.8%; 95% CI: 5.7, 8.0%). The west region had highest percentage positive compared to other region categories (6.4%; 95% CI: 5.7, 7.1%). For the season, autumn represented highest percent positive cats for *C. felis* PCR test (5.5%; 95% CI: 4.9, 6.2%).

Cryptosporidium spp. PCR Test Results Distribution by the Risk Factors of Cats

The distribution of test results (positive or negative) of *Cryptosporidium* spp. for cats by age, sex, region and season is shown in Table 2.11. For the age variable, the majority of test positive cats were at age between 6 months – one-year-old (15.1%; 95% CI: 13.3, 17.1%). For the sex variable, the highest percentage positive was in intact male cats (9.8%; 95% CI: 8.5, 11.3%). The south region had highest percentage positive compared to other region categories (9.1%; 95% CI: 8.3, 10.0%). For the season, autumn represented highest percent positive cats for *Cryptosporidium* spp. PCR test (8.1%; 95% CI: 7.3, 8.9%).

2.4.2 Univariate Logistic Regression Analysis

2.4.2.1 Univariate Analysis for Dogs

***Giardia* spp.**

The results of univariate logistic regression for individual risk factors for *Giardia* spp. are shown in Table 2.12. For age, all categories were compared to the reference category (>7 years old) with 21,622 total dogs with complete data for this factor. There was no difference in the odds of testing positive to *Giardia* spp. when comparing dogs aged 3 -7 years old to dogs older than 7 years old ($p = 0.62$). The odds of testing positive for *Giardia* spp. in dogs aged 1-2 years old were 3.0 times higher than the odds of testing positive for *Giardia* spp.in dogs older than 7 years old, and this difference was statistically significant ($p < 0.0001$). The odds of testing positive for *Giardia* spp. in dogs aged 6 months to one-year-old were 6.6 times higher than the odds of testing positive for *Giardia* spp. in dogs older than 7 years old, and this difference was statistically significant ($p < 0.0001$). The odds of testing positive for *Giardia* spp. in dogs aged less than six months old were 7.8 times higher than the odds of testing positive for *Giardia* spp. in dogs older than 7 years old, and this difference was also statistically significant ($p < 0.0001$).

The overall p-value for this variable was p <0.00001; therefore, age category met the p < 0.25 entry criteria and it was subsequently included in the multivariate logistic regression analysis.

Regarding sex, all categories were compared to the reference category castrated male with 22,629 total dogs with complete data regarding sex. The odds of testing positive to *Giardia* spp. in intact females was 2.6 times higher than in castrated males (p < 0.0001). The odds of testing positive for *Giardia* spp. in intact male dogs were 2.5 times higher than the odds of testing positive for *Giardia* spp. in castrated male dogs (p < 0.0001). The odds of testing positive for *Giardia* spp. decrease as female cats are spayed (OR= 0.83, p < 0.01). There was no difference in the odds of testing positive to *Giardia* spp. when comparing dogs with unknown sex to castrated male dogs (OR = 1.3, p 0.16). The overall p-value for sex was p < 0.00001; therefore, sex met the p < 0.25 entry criteria and it was subsequently included in the multivariate logistic regression analysis.

The Northeast region was used as the reference category when comparing the risk of testing positive to *Giardia* spp. among regions. Complete data on region was available for 22,948 dogs. The odds of testing positive to *Giardia* spp. in dogs from the Midwest were 1.3 times higher than those located in the Northeast, and this difference was statistically significant (p < 0.0001). However, there was no difference in the odds of testing positive to *Giardia* spp. in dogs from the Southern (OR = 0.90, p = 0.14) or Western (OR = 1.0, p = 0.85) regions in the USA compared to Northeast region. The overall p-value for region was p < 0.0001; therefore, region met the p < 0.25 entry criteria and it was subsequently included in the multivariate logistic regression analysis.

Winter was used as the reference category in the season variable with complete information being available from 22,959 total dogs. There was no difference in the odds for dog

samples submitted in spring compared to samples submitted in winter ($p = 0.65$). However, the odds of testing positive for *Giardia* spp. in samples submitted in summer were 0.79 times less than the odds of testing positive for *Giardia* spp. in winter time, and this difference was statistically significant ($p < 0.001$). Also, the odds of testing positive for *Giardia* spp. in samples submitted in autumn were 0.87 times the odds of testing positive for *Giardia* spp. in winter. This difference was statistically significant ($p = 0.04$). The overall p-value for season was $p < 0.0003$; therefore, season met the $p < 0.25$ entry criteria and it was subsequently included in the multivariate logistic regression analysis.

***Cryptosporidium* spp.**

The results of univariate logistic regression for individual risk factors for *Cryptosporidium* spp. are shown in Table 2.13. For age, all categories were compared to the reference category (> 7 years old) with 21,622 total dogs with complete data for this factor. The odds of testing positive for *Cryptosporidium* spp. in dogs aged 3-7 years old were 0.63 times less than the odds of testing positive for *Cryptosporidium* spp. in dogs older than 7 years old, and this difference was statistically significant ($p < 0.0001$). There was no difference in the odds of testing positive to *Cryptosporidium* spp. when comparing dogs aged 1-2 years old to dogs older than 7 years old ($p = 0.25$). The odds of testing positive for *Cryptosporidium* spp. in dogs aged 6 months to one-year-old were 2.1 times higher than the odds of testing positive for *Cryptosporidium* spp. in dogs older than 7 years old, and this difference was statistically significant ($p < 0.0001$). The odds of testing positive for *Cryptosporidium* spp. in dogs aged less than six months old were 4.4 times higher than the odds of testing positive for *Cryptosporidium* spp. in dogs older than 7 years old, and this difference was also statistically significant ($p < 0.0001$). The overall p-value for this variable was $p < 0.00001$; therefore, age category met the p

< 0.25 entry criteria and it was subsequently included in the multivariate logistic regression analysis.

Regarding dogs' sex, all categories were compared to the reference category castrated male with 22,630 total dogs with complete data regarding sex. The odds of testing positive to *Cryptosporidium* spp. in intact females was 2.5 times higher than in castrated males ($p < 0.0001$). The odds of testing positive for *Cryptosporidium* spp. in intact male dogs were 2.3 times higher than the odds of testing positive for *Cryptosporidium* spp. in castrated male dogs ($p < 0.0001$). The odds of testing positive for *Cryptosporidium* spp. decrease as female cats are spayed (OR= 0.82, $p < 0.02$). The odds of testing positive for *Cryptosporidium* spp. were 1.9 in dogs with unknown sex compared to the reference category. This difference was statistically significant ($p < 0.001$). The overall p-value for sex was $p < 0.00001$; therefore, sex met the $p < 0.25$ entry criteria and it was subsequently included in the multivariate logistic regression analysis.

The Northeast region was used as the reference category when comparing the risk of testing positive to *Cryptosporidium* spp. among regions. Complete data on region was available for 22,948 dogs. The odds of testing positive to *Cryptosporidium* spp. in dogs from the Midwest were 1.8 times higher than those located in the Northeast, and this difference was statistically significant ($p < 0.0001$). However, there was no difference in the odds of testing positive to *Cryptosporidium* spp. in dogs from the Southern (OR = 1.2, $p = 0.12$) region. The odds of testing positive to *Cryptosporidium* spp. in dogs from Western region were 1.8 times higher than those in the Northeast region and this difference was statistically significant ($p < 0.0001$). The overall p-value for region was $p < 0.00001$; therefore, region met the $p <0.25$ entry criteria and it was subsequently included in the multivariate logistic regression analysis.

Winter was used as the reference category in the season variable with 22,959 total number of dogs for season variable. The univariate analysis for season revealed this variable insignificant at 0.05 level of significance. The overall p-value for season was $p < 0.60$; therefore, season did not meet the $p < 0.25$ entry criteria and it was excluded from the multivariate logistic regression analysis.

2.4.2.2 Univariate Analysis for Cats

Giardia spp.

The results of univariate logistic regression for individual risk factors for *Giardia* spp. are shown in Table 2.14. For age, all categories were compared to the reference category (> 7 years old) with 15,003 total cats with complete data for this factor. The odds of testing positive for *Giardia* spp. in cats aged 3-7 years old were 2.3 times higher than the odds of testing positive for *Giardia* spp. in cats older than 7 years old, and this difference was statistically significant ($p < 0.0001$). The odds of testing positive for *Giardia* spp. in cats aged 1-2 years old were 4.6 times higher than the odds of testing positive for *Giardia* spp. in cats older than 7 years old, and this difference was statistically significant ($p < 0.0001$). The odds of testing positive for *Giardia* spp. in cats aged 6 months to one-year-old were 5.4 times higher than the odds of testing positive for *Giardia* spp. in dogs older than 7 years old, and this difference was statistically significant ($p < 0.0001$). The odds of testing positive for *Giardia* spp. in cats aged less than six months old were 4.3 times higher than the odds of testing positive for *Giardia* spp. in cats older than 7 years old, and this difference was also statistically significant ($p < 0.0001$). The overall p-value for this variable was ($p < 0.00001$); therefore, age category met the ($p < 0.25$) entry criteria and it was subsequently included in the multivariate logistic regression analysis.

Regarding cats' sex, all categories were compared to the reference category castrated male with 15,946 total cats with complete data regarding sex. The odds of testing positive to *Giardia* spp. in intact females was 1.9 times higher than in castrated males ($p < 0.0001$). The odds of testing positive for *Giardia* spp. in intact male dogs were 1.7 times higher than the odds of testing positive for *Giardia* spp. in castrated male dogs ($p < 0.0001$). There was no difference in the odds of testing positive to *Cryptosporidium* spp. in spayed female cats ($p = 0.34$) and cats with unknown sex ($p = 0.42$) compared to castrated males. The overall p-value for sex was ($p < 0.00001$); therefore, sex met the ($p < 0.25$) entry criteria and it was subsequently included in the multivariate logistic regression analysis.

The Northeast region was used as the reference category when comparing the risk of testing positive to *Giardia* spp. among regions. Complete data on region was available for 16, 269 cats. The univariate analysis for region showed that none of this variables categories was significant at ($p = 0.05$). The overall p-value for region was ($p = 0.43$); therefore, region did not meet the ($p < 0.25$) entry criteria and it was excluded from the multivariate logistic regression analysis.

Winter was used as the reference category with 16,273 total number of cats for season variable. There was no difference in the odds for cat samples submitted in spring compared to samples submitted in winter ($p = 0.07$). In addition, there was no significant difference in the odds of testing positive to *Giardia* for cat samples submitted in summer ($p = 0.12$) and autumn ($p = 0.88$). The overall p-value for season was ($p = 0.08$); therefore, season met the ($p < 0.25$) entry criteria and it was subsequently included in the multivariate logistic regression analysis.

Cryptosporidium felis

The results of univariate logistic regression for individual risk factors for *Cryptosporidium felis* are shown in Table 2.15. For age, all categories were compared to the reference category (> 7 years old) with 15,003 total cats with complete data for this factor. There was no difference in the odds of testing positive to *C. felis* when comparing cats aged 3-7 years old to cats older than 7 years old ($p = 0.23$). The odds of testing positive for *C. felis* in dogs aged 1-2 years old were 3.2 times higher than the odds of testing positive for *C. felis* in cats older than 7 years old, and this difference was statistically significant ($p < 0.0001$). The odds of testing positive for *C. felis* in cats aged 6 months to one-year-old were 6.6 times higher than the odds of testing positive for *C. felis* in cats older than 7 years old, and this difference was statistically significant ($p < 0.0001$). The odds of testing positive for *C. felis* in cats aged less than six months old were 5.6 times higher than the odds of testing positive for *C. felis* in cats older than 7 years old, and this difference was also statistically significant ($p < 0.0001$). The overall p-value for this variable was ($p < 0.00001$); therefore, age category met the ($p < 0.25$) entry criteria and it was subsequently included in the multivariate logistic regression analysis.

Regarding cat's sex, all categories were compared to the reference category castrated male with 15,946 total cats with complete data regarding sex. The odds of testing positive to *C. felis* in intact females was 1.4 times higher than in castrated males ($p < 0.001$). The odds of testing positive for *C. felis* in intact male cats were also 1.4 times higher than the odds of testing positive for *C. felis* in castrated male cats ($p < 0.004$). The odds of testing positive for *C. felis* decrease as female cats are spayed (OR = 0.81, $p < 0.02$). There was no significant difference in the odds of testing positive to *C. felis* in cats with unknown sex compared to the reference category ($p = 0.34$). The overall p-value for sex was ($p < 0.00001$); therefore, sex met the ($p <$

0.25) entry criteria and it was subsequently included in the multivariate logistic regression analysis.

The Northeast region was used as the reference category when comparing the risk of testing positive to *C. felis* among regions. Complete data on region was available for 16,269 cats. The odds of testing positive to *C. felis* in cats from the Midwest were 2.2 times higher than those located in the Northeast, and this difference was statistically significant ($p < 0.0001$). The odds of testing positive to *C. felis* in cats from Southern region were 2.4 times higher than those in the Northeast region and this difference was statistically significant ($p < 0.0001$). The odds of testing positive to *C. felis* in cats from Western region were 2.6 times higher than those in the Northeast region and this difference was statistically significant ($p < 0.0001$). The overall p-value for region was ($p < 0.00001$); therefore, region met the ($p < 0.25$) entry criteria and it was subsequently included in the multivariate logistic regression analysis.

Winter was used as the reference category in the season variable with 16,273 total number of cats. There was no significant difference in the odds of testing positive to *C. felis* compared to the reference category ($p = 0.69$). The odds of testing positive to *C. felis* from cat samples collected in summer were 0.71 times less than the reference category and this difference was statistically significant ($p < 0.001$). The overall p-value for season was $p < 0.002$; therefore, season met the ($p < 0.25$) entry criteria and it was subsequently included in the multivariate logistic regression analysis.

***Cryptosporidium* spp.**

The results of univariate logistic regression for individual risk factors for *Cryptosporidium* spp. are shown in Table 2.16. For age, all categories were compared to the reference category (> 7 years old) with 15,003 total cats with complete data for this factor. There

was no difference in the odds of testing positive to *Cryptosporidium* spp. when comparing cats aged 3-7 years old to cats older than 7 years old ($p = 0.33$). The odds of testing positive for *Cryptosporidium* spp. in dogs aged 1-2 years old were 2.8 times higher than the odds of testing positive for *Cryptosporidium* spp. in cats older than 7 years old, and this difference was statistically significant ($p < 0.0001$). The odds of testing positive for *Cryptosporidium* spp. in cats aged 6 months to one-year-old were 5.7 times higher than the odds of testing positive for *Cryptosporidium* spp. in cats older than 7 years old, and this difference was statistically significant ($p < 0.0001$). The odds of testing positive for *Cryptosporidium* spp. in cats aged less than six months old were 4.9 times higher than the odds of testing positive for *Cryptosporidium* spp. in cats older than 7 years old, and this difference was also statistically significant ($p < 0.0001$). The overall p-value for this variable was ($p < 0.00001$); therefore, age category met the ($p < 0.25$) entry criteria and it was subsequently included in the multivariate logistic regression analysis.

Regarding cat's sex, all categories were compared to the reference category castrated male with 15,946 total cats with complete data regarding sex. The odds of testing positive to *Cryptosporidium* spp. in intact females was 1.4 times higher than in castrated males ($p < 0.0001$). The odds of testing positive for *Cryptosporidium* spp. in intact male cats were 1.5 times higher than the odds of testing positive for *Cryptosporidium* spp. in castrated male cats ($p < 0.0001$). There was no significant difference in the odds of testing positive to *Cryptosporidium* spp. in spayed female cats ($p = 0.10$) and cats with unknown sex ($p = 0.80$) compared to the reference category. The overall p-value for sex was ($p < 0.00001$); therefore, sex met the ($p < 0.25$) entry criteria and it was subsequently included in the multivariate logistic regression analysis.

The Northeast region was used as the reference category when comparing the risk of testing positive to *Cryptosporidium* spp. among regions. Complete data on region was available for 16,269 cats. The odds of testing positive to *Cryptosporidium* spp. in cats from the Midwest were 1.8 times higher than those located in the Northeast, and this difference was statistically significant ($p < 0.0001$). The odds of testing positive to *Cryptosporidium* spp. in cats from Southern region were 2.1 times higher than those in the Northeast region and this difference was statistically significant ($p < 0.0001$). The odds of testing positive to *Cryptosporidium* spp. in cats from Western region were 2.0 times higher than those in the Northeast region and this difference was statistically significant ($p < 0.0001$). The overall p-value for region was ($p < 0.00001$); therefore, region met the ($p < 0.25$) entry criteria and it was subsequently included in the multivariate logistic regression analysis.

Winter was used as the reference category in the season variable with 16,273 total number of cats. There was no significant difference in the odds of testing positive to *Cryptosporidium* spp. in cat samples collected in spring ($p = 0.62$) and samples collected in autumn ($p = 0.60$) compared to the reference category. The odds of testing positive to *Cryptosporidium* spp. from cat samples collected in summer were 0.81 times less than the reference category and this difference was statistically significant ($p < 0.02$). The overall p-value for season was ($p < 0.02$); therefore, season met the ($p < 0.25$) entry criteria and it was subsequently included in the multivariate logistic regression analysis.

2.4.3 Multivariate Logistic Regression Analysis

2.4.3.1 Multivariate Analysis for Dogs

***Giardia* spp.**

The results of multivariate logistic regression are shown in Table 2.17. After adjusting for the effect of other factors in the model, age, region and season were included in the final main effects model for dogs with *Giardia* spp..

As shown in Table 2.17, there was no difference in the odds of testing positive to *Giardia* spp. when comparing 3-7 years old to > 7 years old dogs (OR=0.95, 95% CI: 0.77, 1.2, p = 0.63). The odds of testing positive for *Giardia* spp. in dogs aged 1-2 years old were 3.0 times higher than the odds of testing positive for *Giardia* spp. in dogs older than 7 years old. This difference was statistically significant (p < 0.0001) and the 95% CI (2.5, 3.6). The odds of testing positive for *Giardia* spp. in dogs aged 6 months – one-year-old were 6.6 times higher than the odds of testing positive for *Giardia* spp.in dogs older than 7 years old. This difference was statistically significant (p < 0.0001) and the 95% CI (5.4, 8.0). The odds of testing positive for *Giardia* spp.in dogs aged <6 months old were 7.8 times higher than the odds of testing positive for *Giardia* spp. in dogs older than 7 years old. This difference was statistically significant (p < 0.0001) and the 95% CI (6.6, 9.2).

For region, the odds of testing positive for *Giardia* spp. in dogs located in the Midwest region are 1.3 times higher than dogs located in the Northeast, and this difference was statistically significant (p < 0.001) and the 95% CI (1.1, 1.6). The odds of testing positive for *Giardia* spp. in dogs located in the South are 0.84 less than dogs located in the Northeast. This difference was statistically significant (p = 0.03) and the 95% CI (0.72, 0.98). However, there

was no difference between dogs located in the West than dogs located in the Northeast (OR=1.0, p = 0.52) and the 95% CI (0.73, 1.0).

For the season variable, there was no significant difference in samples submitted in spring than those submitted in winter time (OR=1.0, 95% CI: 0.90, 1.2, p = 0.60). However, the odds of testing positive to *Giardia* spp. in samples submitted in the summer time were 0.80 less than those submitted in winter time, and this difference was statistically significant (p = 0.002) and the 95% CI (0.69, 0.92). Also, the odds of testing positive to *Giardia* spp. in samples submitted in autumn were 0.84 less than those submitted in winter time. This difference was statistically significant (p = 0.02) and the 95% CI (0.73, 1.0). The total number of dogs of this final model were 21,612.

***Cryptosporidium* spp.**

Table 2.18 contained a summary of multivariable analysis for the predictor variables age, sex and region with testing positive to *Cryptosporidium* spp. in dogs as the binary outcome. After adjusting for the effect of other factors in the model, age, sex and region were the final predictors for dogs with *Cryptosporidium* spp.

As shown in Table 2.18, the odds of testing positive to *Cryptosporidium* spp. in dogs significantly decrease as age increase by one year (OR=0.62, 95% CI: 0.50, 0.77, p < 0.0001). There was no significant difference of testing positive to *Cryptosporidium* spp. between dogs that are 1-2 years old and those that are more than 7 years old (OR=1.1, 95% CI: 0.90, 1.3, p = 0.37). However, the odds of testing positive to *Cryptosporidium* spp. in dogs aged 6 months – 1 year were 2.0 times higher than those that are more than 7 years old. This difference is statistically significant (p < 0.0001) and the 95% CI (1.6, 2.6). In addition, the odds of testing

positive to *Cryptosporidium* spp. in dogs aged < 6 months were 4.0 times higher than those that are more than 7 years old and the 95% CI (3.2, 4.8).

Regarding the sex, there was no significant difference in the probability of testing positive to *Cryptosporidium* spp. in intact females compared to castrated males (OR = 1.2, 95% CI: 0.94, 1.4, p = 0.20). Likewise, there was no significant difference in the probability of testing positive to *Cryptosporidium* spp. in intact males compared to castrated males (OR = 1.2, 95% CI: 0.95, 1.4, p = 0.10). However, odds of testing positive to *Cryptosporidium* spp. in spayed females decrease by 0.84 compared to castrated males. This difference was statistically significant (p = 0.05) and the 95% CI (0.71, 1.0). There was no significant difference in the probability of testing positive to *Cryptosporidium* spp. in unknown sex dogs compared to castrated males (OR = 1.4, 95% CI: 0.81, 2.4, p = 0.23).

For the region variable, the odds of testing positive to *Cryptosporidium* spp. in dogs located in the Midwest were 1.7 times higher than those located in the Northeast. This difference was statistically significant (p < 0.0001) and the 95% CI (1.4, 2.1). In contrast, there was no difference in the odds of testing positive to *Cryptosporidium* spp. in dogs located in the South and those located in the Northeast (OR=1.1, 95% CI: 0.90, 1.3, p = 0.35). However, the odds of testing positive to *Cryptosporidium* spp. in dogs located in the West were 2.0 times higher than those located in the Northeastern region, as shown in Table 2.18 and the 95% CI (1.7, 2.3). The total number of dogs of this final model were 21,448.

2.4.3.2 Multivariate Analysis for Cats

***Giardia* spp.**

Regarding multivariate logistic regression models in cats, Table 2.19 represented a summary of multivariable analysis for the predictor variables age and sex with testing positive to

Giardia spp. as the binary outcome. After adjusting for the effect of other factors in the model, age, and sex were identified as significant predictors for *Giardia* spp. in cats.

As shown in Table 2.19, the odds of testing *Giardia* spp. in cats aged 3-7 years old were 2.4 times higher than those aged more than 7 years. This difference was statistically significant $p < 0.0001$ and the 95% CI (1.8, 3.2). Also, the odds of testing *Giardia* spp. in cats aged 1-2 years old were 4.5 times higher than those aged more than 7 years. This difference was statistically significant $p < 0.0001$ and the 95% CI (3.4, 5.9). Furthermore, the odds of testing *Giardia* spp. in cats aged 6 months to one-year-old were 5.2 times higher than those aged more than 7 years. This difference was statistically significant $p < 0.0001$ and the 95% CI (4.0, 6.9). Finally, the odds of testing *Giardia* spp. in cats aged less than 6 months were 3.7 times higher than those aged more than 7 years. This difference was statistically significant $p < 0.0001$ and the 95% CI (2.8, 4.9).

The odds of testing positive to *Giardia* spp. in intact female cats were 1.3 times higher than in castrated male cats. The difference was statistically significant ($p < 0.0001$) and the 95% CI (1.1, 1.7). However, the odds of testing positive to *Giardia* spp. in intact male cats were not statistically significant than in castrated male cats (OR = 1.2, $p = 0.07$) and the 95% CI (0.98, 1.6). Likewise, the odds of testing positive to *Giardia* spp. in spayed female cats were not statistically significant than in castrated male cats (OR = 0.91, 95% CI: 0.76, 1.1, $p = 0.33$). Also, the odds of testing positive to *Giardia* spp. in unknown sex cats were not statistically significant than in castrated male cats (OR = 0.92, 95% CI: 0.40, 2.1, $p = 0.85$). The total number of cats in this final model were 14,891.

Cryptosporidium felis

Table 2.20 display a summary of multivariable analysis for the predictor variables age, region, and season with testing positive to C*ryptosporidium felis* as the binary outcome. After adjusting for the effect of other factors in the model, age, region, and season were identified as significant predictors for *C. felis* in cats.

As shown in Table 2.20, there was no significant difference for testing positive to *C. felis* in cats that are 3-7 years old compared to cats that are more than 7 years old (OR = 1.2, 95% CI: 0.88, 1.7, p = 0.21). However, the difference was statistically significant (p < 0.0001) in cats aged 1-2 years old compared to the reference category. The odds of testing positive to *C. felis* in cats aged from 1-2 years are 3.3 times higher than in cats that are older than 7 years and the 95% CI (2.5, 4.3). The odds of testing positive to *C. felis* in cats aged from 6 months to one-year-old are 6.4 times higher than in cats that are older than 7 years. The difference was statistically significant (p < 0.0001) and the 95% CI (4.9, 8.5). Additionally, the odds of testing positive to *C. felis* in cats aged less than 6 months are 5.6 times higher than in cats that are older than 7 years. The difference was statistically significant (p < 0.0001) and the 95% CI (4.5, 7.2).

Regarding region variable, the odds of testing positive to *C. felis* in cats located in the Midwest are 2.1 times higher than in cats that were located in the Northeast. The difference was statistically significant (p < 0.0001) and the 95% CI (1.6, 2.8). Also, the odds of testing positive to *C. felis* in cats located in the South were 2.2 times higher than in cats that are located in the Northeast. The difference was statistically significant (p < 0.0001) and the 95% CI (1.7, 2.8). Furthermore, the odds of testing positive to *C. felis* in cats located in the West were 2.5 times higher than in cats that are located in the Northeast. The difference was statistically significant (p < 0.0001) and the 95% CI (2.0, 3.2).

Regarding season, there was no significant difference in the odds of testing positive to *C. felis* in cats between samples submitted in spring to samples submitted in winter time (OR=1.0, p = 0.77) and the 95% CI (0.83, 1.3). Whereas the odds of testing positive to *C. felis* in samples submitted in summer time were 0.7 times less than in samples submitted in winter time. The difference was statistically significant (p = 0.002) and the 95% CI (0.60, 0.88). Lastly, there was no significant difference in the odds of testing positive to *C. felis* in cats between samples submitted in autumn to samples submitted in winter time (OR = 1.0, p = 0.94) and the 95% CI (0.82, 1.2). The total number of cats for this final model were 14,999.

***Cryptosporidium* spp.**

Table 2.21 represented a summary of multivariable analysis for the predictor variables age and region with testing positive to *Cryptosporidium* spp. as the binary outcome. After adjusting for the effect of other factors in the model, age, and region were identified as significant predictors for *Cryptosporidium* spp. in cats.

As shown in Table 2.21, there was no significant difference for testing positive to *Cryptosporidium* spp. in cats that are 3-7 years old compared to cats that are more than 7 years old (OR = 1.1, p = 0.30) and the 95% CI (0.88, 1.5). However, the difference was statistically significant (p < 0.0001) in cats aged 1-2 years old compared to the reference category. The odds of testing positive to *Cryptosporidium* spp. in cats aged from 1-2 years are 2.8 times higher than in cats that are older than 7 years and the 95% CI (2.3, 3.6). Also, the odds of testing positive to *Cryptosporidium* spp. in cats aged from 6 months to one-year-old are 5.7 times higher than in cats that are older than 7 years. The difference was statistically significant (p < 0.0001) and the 95% CI (4.6, 7.2). Additionally, the odds of testing positive to *Cryptosporidium* spp. in cats aged

less than 6 months are 4.9 times higher than in cats that were older than 7 years. The difference was statistically significant ($p < 0.0001$) and the 95% CI (4.0, 5.9).

Regarding region variable, the odds of testing positive to *Cryptosporidium* spp. in cats located in the Midwest are 1.8 times higher than in cats that are located in the Northeast. The difference was statistically significant ($p < 0.0001$). Also, the odds of testing positive to *Cryptosporidium* spp. in cats located in the South are 2.0 times higher than in cats that are located in the Northeast. The difference was statistically significant ($p < 0.0001$) and the 95% CI (1.7, 2.4). Furthermore, the odds of testing positive to *Cryptosporidium* spp. in cats located in the West are 2.1 times higher than in cats that are located in the Northeast. The difference was statistically significant ($p < 0.0001$) and the 95% CI (1.7, 2.5). The total number of cats for this final model were also 14,999 cats.

2.4.4 Model Evaluation

Hosmer – Lemeshow (HL) goodness – of – fit test for *Giardia* spp. model in dogs indicated no significant lack of fit ($p = 0.07$). For *Cryptosporidium* spp. model in dogs, the HL goodness – of – fit test indicated no significant lack of fit ($p = 0.37$). The HL goodness – of – fit test for *Giardia* spp. model in cats indicated no significant lack of fit ($p = 0.16$). For *C. felis* model in cats, the HL goodness – of – fit test indicated no significant lack of fit ($p = 0.12$) and for *Cryptosporidium* spp. model indicated no significant lack of fit ($p = 0.36$). The evaluation of outliers and/or influential observations showed no effect of these observations on the model fit (Figures 2.3-2.4).

2.5 Discussion

Intestinal parasites such as *Giardia* spp. and *Cryptosporidium* spp. are common in pet dogs and cats in the United States. According to the USA source book in 2012, there are about

70 million pet dogs in the United States.[34] Several studies have identified the prevalence of different intestinal parasites in pets in the USA using diagnostics such as microscopy, ELISA, IFA or PCR.[20-23,25,26,27,29,30,35] In these studies, sample sizes ranged from 129-16,114 dogs or cats or both. Our study is considered novel in that a large data set was evaluated (22,959 pet dogs and 16,273 pet cats), information was available from all states in the USA, and a standardized and sensitive test modality was used (PCR assays). In addition, our study evaluated the prevalence of *Giardia* spp. and *Cryptosporidium* spp. in pet dogs and cats using the real-time PCR assay and evaluated risk factors such as age, sex, region and season. Most veterinarians order PCR tests only for dogs and cats with clinical signs (i.e. diarrhea) and not for those without clinical signs. Therefore, we suspect the samples collected from dogs and cats in this study were from those with diarrhea.

The results of this study showed that the prevalence of positive tests for *Giardia* spp. in dog samples was 7.7%. This result falls into the range of prevalence estimates (0.4% – 16.0%) in pet dogs in the USA with clinical signs of diarrhea diagnosed by centrifugal fecal flotation, ELISA and IFA.[19-25] Regarding *Cryptosporidium* spp. prevalence in pet dogs, the proportion was 5.4%, and also this result falls within the range of estimates of 2.3% and 5.6% for pet dogs with diarrhea diagnosed by microscopy and ELISA.[20,25]

Regarding cat percentage of positive estimates, the prevalence of *Giardia* spp. in diarrheic cats was 5.2% as shown in Table 2.2. This estimate was less than prevalence estimates (8.0% - 14.0%) of cat giardiasis determined by previous studies.[22,26-29] This disagreement in the estimates could merely be due to increased false negative rates measured by the PCR assays of our study. Fecal PCR inhibitors are known to lead to false negative results in *Giardia* PCR assays.[36] Regarding the prevalence of *Cryptosporidium* spp. DNA in feline feces, the average of

proportion of positives due to *C. felis* and *Cryptosporidium* spp. was approximately 6.3% (Table 2.2). This estimate is within the range of prevalence estimates (3.9% -25.0%) in pet cats with diarrhea.[29,30,31]

Co-infections in dogs and cats can occur. Our study showed that nearly 30% of dogs with *Cryptosporidium* spp. were also positive to *Giardia* spp. Also, a smaller proportion of cats positive to *Cryptosporidium* were positive to *Giardia* spp.. One study[37] found that dual or triple infections are significantly associated with the clinical signs, specifically, diarrhea. In that study, the prevalence for *Giardia* spp. and *Cryptosporidium* spp. with bacterial agents was 6.2% and triple infection with viral and bacterial agents was approximately 21.9%.[37] Also, some studies reported that dogs that are naturally infected with C*ryptosporidium* spp. are likely to be infected with *Giardia* spp.[10,38] In a study, cats infected with *Giardia* spp. were more likely to shed *Cryptosporidium* spp. oocysts.[28] Triple or quadruple infections with pathogens other than *Giardia* spp. and *Cryptosporidium* spp. were not investigated in this study. Coinfections with *Giardia* spp. and *Cryptosporidium* spp. are not surprising as both have similar risk factors, are immediately infectious when passed in feces, and are both transmitted primarily by fecal-oral contact.

The distribution of PCR test results with the risk factor, age, showed also that the majority of animals tested positive to *Giardia* spp. and *Cryptosporidium* spp. in dogs and cats were in puppies and kittens less than 6 months old. This indicates that the likelihood of disease or infection due to both protozoans is more prevalent in young animals which is with accordance with previous study.[19] One study conducted nationally found the prevalence of *Giardia* spp. using microscopy examination in dogs that were less than 6 months old was 13.1% compared to <1.0% in dogs that were greater than 3 years old.[23] In another study, *Giardia* antigen was

detected in 6.1% pet cats that were less than 1 year of age.[29] The increased risk of infection due to both pathogens in younger animals can be attributed to their immature immune systems.[39]

The descriptive analysis of this showed that the majority were from dogs (69.5%) and (73.5%) from cats that were spayed or neutered (Table 2.4). This is attributed to legislations of some states that require pets to be spayed or neutered. However, this procedure is not mandatory, but the American Society for the Prevention of Cruelty to Animals (ASPCA) supports spay/neuter programs at low to no cost in some states.[40] In our study, the risk of testing positive to *Giardia* spp. in dogs was higher in intact males and females (13.4%) compared to castrated males and spayed females (5.3%) (Table 2.7). Similarly, the risk of testing positive to *Cryptosporidium* spp. was higher in intact male and female dogs (9.3%) compared to castrated males and spayed females (3.8%) (Table 2.8). This result is consistent with different studies conducted elsewhere and the reason why the percentage positive is higher in intact males and females compared to neutered or spayed is merely related to differences in the exposure to both parasites of these two animal groups.[41,42] However, one study indicated no significant difference of testing positive to *Giardia* spp. in dogs between intact males or females vs spayed/neutered males or females (OR= 1.31 vs 1.0), respectively.[19]

Regarding region, statistics showed that the majority (38.2%) of the dogs were located in the Western region (Table 2.5). However, the risk of contracting *Giardia* spp. was higher in the Midwest (9.9%) (Table 2.7). Per the National Oceanic and Atmospheric Administration (NOAA), the Midwest region characterized by a wide range of temperature and precipitation extremes due to cold air masses from the far north, and warm, humid air masses from the Gulf of Mexico.[43] Since *Giardia* cysts are affected by humidity, temperature, and

freeze–thaw cycle, climatic conditions of the Midwest region are favorable to those cysts which might remain infective for longer periods of time.[44,45] A study found that the prevalence of *Giardia* spp. by state was highest in Maine (4.0%) and Colorado (2.6%) and lowest (0.10%) in 11 different states (WA, ID, ND, IA, OK, AR, LA, MS, AL, GA, and FL). Also, the highest regional prevalence of *Giardia* in pet dogs was reported in the Mountain region (1.4%) followed by the New England region (0.80%).[19] The actual regional differences in *Giardia* transmission cycles or giardiasis reporting capacity across states might be related to the geographic differences in the prevalence estimates of the disease.[46] The risk of contracting *Cryptosporidium* spp. in dogs was not different among all four regions, but it was the lowest in the Northeastern region (3.8%) (Table 2.8). For cats, most samples also were obtained from the Western region (31.0%). Cross-tabulation with the outcome, the risk of contracting *Cryptosporidium* spp. was highest in cats located in the South (9.1%) (Table 2.11) and there was no difference in the risk of contracting both *Giardia* spp. and *C. felis* (Tables 2.9, and 2.10).

The descriptive analysis of the variable, season, showed the highest percentages of positives noticed in spring (8.6%) and autumn (7.3%) (Table 2.7). This finding is consistent with seasonal patterns observed in a different study where the increased rates of the disease can be related to the increased outdoor activities (i.e. camping, hiking, swimming, etc.).[46] In contrast, a study evaluated the prevalence by season or dogs tested positive to *Giardia* spp. and found slight seasonal increases during the winter and summer.[19] For dogs with cryptosporidiosis, there was no difference in the percentage of positives due to seasonal distributions (Table 2.8). For cats with *Giardia* spp., there was no difference in the percentage of positives due to seasonal distributions (Table 2.9). The percentage of positives in cats infected with *C. felis* was lowest in the summer time (3.9%) compared to other seasons (5.4%) (Table 2.10). For cats with

Cryptosporidium spp., the percentage of positives was highest in autumn (8.1%) (Table 2.11). This finding of variations in percentages positives from season to the other may be coincides with the effect of climate on the parasite or host physiology.[47,48]

2.6 Conclusion

Giardia spp. and *Cryptosporidium* spp. are common protozoan pathogens that can be associated with diarrhea in pet dogs and cats. Age was identified as significant predictor that is associated with the probability of testing positive to *Giardia* spp. and *Cryptosporidium* spp. in both dogs and cats. The probability of testing positive to either pathogens was likely in the very young animals which calls for more attention should be paid in this population. Additionally, region and season were identified as significant predictors of both pathogens in pet dogs and cats by the logistic regression models. One strength of this study was the use of a highly sensitive and standardized PCR assay to estimate prevalence rates of the infections. However, since the clinical histories are not known, we cannot use the results to determine associations with diarrhea. Additionally, this study successfully identified risk factors associated with infection in a large sample size. This study provided a base of future studies to be conducted for pet dogs and cats using PCR test results. Future research may involve an evaluation for associations of positive test results with clinical findings in pets. In addition, research should be performed to determine the proportion of dogs or cats that are carrying zoonotic species of *Giardia* spp. and or *Cryptosporidium* spp..

2.7 Tables

Table 2.1 Percentage of positive PCR test results of *Giardia* spp., *Cryptosporidium* spp. in dogs

Pathogen	**Total**	**Positives**	**Percentage**	**95% CI**
Giardia spp.	22,959	1,762	7.7	(7.3, 8.0)
Cryptosporidium spp.	22,959	1,237	5.4	(5.1, 5.7)

Table 2.2 Percentage of positive PCR test results of *Giardia* spp., *Cryptosporidium* spp. in cats

Pathogen	**Total**	**Positives**	**Percentage**	**95% CI**
Giardia spp.	16,273	843	5.2	(4.9, 5.5)
Cryptosporidium felis	16,273	824	5.1	(4.7, 5.4)
Cryptosporidium spp.	16,273	1,211	7.4	(7.0, 7.9)

Table 2.3 Dogs and cats age distribution from submitted fecal samples

Variable (Age)	**Frequency**	**Percent**	**95% CI**
Species:			
Dogs			
>7 yrs.	6,112	28.1	(28.0, 29.0)
3-7 yrs.	6,358	29.3	(29.0, 30.0)
1-2 yrs.	4,254	19.6	(19.0, 20.0)
6 mo. -1 yr.	1,728	8.0	(7.0, 8.0)
<6 mo.	3,245	15.0	(14.0, 15.0)
Total	**21,697**	**100**	
Cats			
>7 yrs.	4,428	29.4	(29.0, 30.0)
3-7 yrs.	3,173	21.1	(20.0, 22.0)
1-2 yrs.	2,425	16.1	(16.0, 17.0)
6 mo. -1 yr.	1,483	9.9	(9.0, 10.0)
<6 mo.	3,538	23.5	(23.0, 24.0)
Total	**15,047**	**100**	

Table 2.4 Distribution of fecal samples by sex among dogs and cats

Variable (SEX)	Frequency	Percent	95% CI
Species:			
Dogs			
Castrated Male (CM)	7,927	34.9	(34.3, 35.0)
Intact Female (F)	2,651	11.7	(11.3, 12.0)
Intact Male (M)	3,818	16.8	(16.3, 17.3)
Spayed Female (SF)	7,866	34.6	(34.0, 35.3)
Unknown Sex (U)	447	2.0	(1.8, 2.2)
Total	**22,709**	**100**	
Cats			
Castrated Male (CM)	6,731	42.1	(41.3, 42.8)
Intact Female (F)	1,941	12.1	(11.6, 12.6)
Intact Male (M)	1,905	11.9	(11.4, 12.4)
Spayed Female (SF)	5,023	31.4	(30.8, 32.1)
Unknown Sex (U)	398	2.5	(2.3, 2.7)
Total	**15,998**	**100**	

Table 2.5 Distribution of fecal samples by region among dogs and cats

Variable (REGION)	Frequency	Percent	95% CI
Species:			
Dogs			
Northeast	6,410	27.8	(27.3, 28.4)
Midwest	2,612	11.3	(10.9, 11.8)
South	5,201	22.6	(22.0, 23.1)
West	8,807	38.2	(37.6, 38.9)
Total	**23,030**	**100**	
Cats			
Northeast	4,693	28.8	(28.0, 29.5)
Midwest	2,004	12.3	(11.8, 12.8)
South	4,571	28.0	(27.3, 28.7)
West	5,053	31.0	(30.3, 31.7)
Total	**16,321**	**100**	

Table 2.6 Descriptive statistics of season

Variable (SEASON)	Frequency	Percent	95% CI
Species:			
Dogs			
Winter (12,1,2)	5,419	23.5	(23.0, 24.1)
Spring (3,4,5)	5,560	24.1	(23.6, 24.7)
Summer (6,7,8)	5,972	25.9	(25.4, 26.5)
Autumn (9,10,11)	6,090	26.4	(25.9, 27.0)
Total	**23,041**	**100**	
Cats			
Winter (12,1,2)	4,244	26.0	(25.3, 26.7)
Spring (3,4,5)	3,288	20.1	(19.5, 20.8)
Summer (6,7,8)	3,909	23.9	(23.3, 24.6)
Autumn (9,10,11)	4,884	29.9	(29.2, 30.6)
Total	**16,325**	**100**	

Table 2.7 Distribution of PCR test results for *Giardia* spp. by age, sex, region and season for dogs

Variable	Level	Negative	Positive	Total	95% CI
Age	>7 yrs.	5,909 (97.0%)	183 (3.0%)	6,092	(2.6, 3.5)
	3-7 yrs.	6,156 (97.1%)	181 (2.9%)	6,338	(2.5, 3.3)
	1-2 yrs.	3,881 (91.5%)	359 (8.5%)	4,240	(7.6, 9.3)
	6 mo. – 1 yr.	1,430 (83.1%)	291 (16.9%)	1,721	(15.2, 18.8)
	< 6 mo.	2,604 (80.6%)	628 (19.4%)	3,232	(18.1, 20.8)
	Total	19,980 (92.4%)	1,642 (7.6%)	**21,622**	
Sex	CM	7,445 (94.2%)	456 (5.8%)	7,901	(5.3, 6.3)
	F	2,283 (86.4%)	358 (13.6%)	2,641	(12.3, 14.9)
	M	3,302 (86.8%)	502 (13.2%)	3,804	(12.1, 14.3)
	SF	7,457 (95.2)	379 (4.8%)	7,837	(4.4, 5.3)
	U	414 (92.6%)	33 (7.4%)	447	(5.1, 10.2)
	Total	20,901 (92.4%)	1,728 (7.6%)	**22,629**	
Region	Northeast	5,891 (92.4%)	485 (7.6%)	6,377	(7.0, 8.3)
	Midwest	2,349 (90.1%)	257 (9.9%)	2,606	(8.7, 11.1)
	South	4,814 (93.1%)	356 (6.9%)	5,170	(6.2, 7.6)
	West	8,134 (92.5%)	662 (7.5%)	8,796	(7.0, 8.1)
	Total	21,188 (92.3%)	1,760 (7.7%)	**22,948**	
Season	Winter	4,961 (91.7%)	449 (8.3%)	5,410	(7.6, 9.1)
	Spring	5,064 (91.4%)	474 (8.6%)	5,538	(7.8, 9.3)
	Summer	5,549 (93.3%)	397 (6.7%)	5,946	(6.1, 7.3)
	Autumn	5,623 (92.7%)	442 (7.3%)	6,066	(6.6, 8.0)
	Total	21,197 (92.3%)	1,762 (7.7%)	**22,959**	

Table 2.8 Distribution of PCR test results for *Cryptosporidium* spp. by age, sex, region and season for dogs

Variable	Level	Negative	Positive	Total	95% CI
Age	>7 yrs.	5,868 (96.3%)	227 (3.7%)	6,091	(3.3, 4.2)
	3-7 yrs.	6,188 (97.6%)	152 (2.4%)	6,339	(2.03, 2.8)
	1-2 yrs.	4,064 (95.8%)	177 (4.2%)	4,240	(3.6, 4.8)
	6 mo. – 1 yr.	1,591 (92.4%)	131 (7.6%)	1,721	(6.4, 9.0)
	<6 mo.	2,759 (85.3%)	474 (14.7%)	3,231	(13.4, 15.9)
	Total	20,461(94.6%)	1,161 (5.4%)	**21,622**	
Sex	CM	7,581 (95.9%)	323 (4.1%)	7,904	(3.7, 4.5)
	F	2,382 (90.2%)	258 (9.8%)	2,640	(8.7, 11.0)
	M	3,470 (91.3%)	333 (8.8%)	3,803	(7.9, 9.7)
	SF	7,571 (96.6%)	265 (3.4%)	7,836	(3.0, 3.8)
	U	414 (92.6%)	33 (7.4%)	447	(5.1, 10.2)
	Total	21,418 (94.7%)	1,212 (5.4%)	**22,630**	
Region	Northeast	6,134 (96.2%)	242 (3.8%)	6,376	(3.3, 4.3)
	Midwest	2,433 (93.5%)	170 (6.5%)	2,603	(5.6, 7.5)
	South	4,949 (95.6%)	226 (4.4%)	5,175	(3.8, 5.0)
	West	8,197 (93.2%)	597 (6.8%)	8,794	(6.3, 7.3)
	Total	21,713 (94.6%)	1,235 (5.4%)	**22,948**	
Season	Winter	5,115 (94.5%)	296 (5.5%)	5,411	(4.9, 6.1)
	Spring	5,235 (94.6%)	298 (5.4%)	5,533	(4.8, 6.0)
	Summer	5,647 (94.9%)	302 (5.1%)	5,949	(4.5, 5.7)
	Autumn	5,725 (94.4%)	341 (5.6%)	6,066	(5.1, 6.2)
	Total	21,722 (94.6%)	1,237 (5.4%)	**22,959**	

Table 2.9 Distribution of PCR test results for *Giardia* spp. by age, sex, region and season for cats

Variable	Level	Negative	Positive	Total	95% CI
Age	>7 yrs.	4,335 (98.2%)	78 (1.8%)	4,413	(1.4, 2.2)
	3-7 yrs.	3,037 (96.0%)	128 (4.1%)	3,165	(3.4, 4.8)
	1-2 yrs.	2,234 (92.4%)	184 (7.6%)	2,418	(6.6, 8.7)
	6 mo. – 1 yr.	1,349 (91.2%)	130 (8.8%)	1,479	(7.4, 10.3)
	<6 mo.	3,276 (92.9%)	252 (7.1%)	3,528	(6.3, 8.0)
	Total	14,232 (94.9%)	772 (5.2%)	**15,003**	
Sex	CM	6,412 (95.5%)	299 (4.5%)	6,711	(4.0, 5.0)
	F	1,777 (91.9%)	157 (8.1%)	1,934	(6.9, 9.4)
	M	1,764 (92.9%)	136 (7.2%)	1,900	(6.0, 8.4)
	SF	4,802 (95.9%)	205 (4.1%)	5,007	(3.6, 4.7)
	U	373 (94.7%)	21 (5.3%)	394	(3.3, 8.0)
	Total	15,129 (94.9%)	818 (5.1%)	**15,946**	
Region	Northeast	4,441 (95.1%)	227 (4.9%)	4,668	(4.3, 5.5)
	Midwest	1,884 (94.2%)	116 (5.8%)	2,000	(4.8, 6.9)
	South	4,324 (94.9%)	232 (5.1%)	4,556	(4.5, 5.8)
	West	4,777 (94.7%)	268 (5.3%)	5,045	(4.7, 6.0)
	Total	15,426 (94.8%)	843 (5.2%)	**16,269**	
Season	Winter	3,996 (94.5%)	234 (5.5%)	4,230	(4.9, 6.3)
	Spring	3,124 (95.4%)	150 (4.6%)	3,274	(3.9, 5.4)
	Summer	3,713 (95.2%)	186 (4.8%)	3,899	(4.1, 5.5)
	Autumn	4,597 (94.4%)	273 (5.6%)	4,870	(5.0, 6.3)
	Total	15,430 (94.8%)	843 (5.2%)	**16,273**	

Table 2.10 Distribution of PCR test results for *Cryptosporidium felis* by age, sex, region and season for cats

Variable	Level	Negative	Positive	Total	95% CI
Age	>7 yrs.	4,336 (98.2%)	79 (1.8%)	4,415	(1.4, 2.2)
	3-7 yrs.	3,094 (97.8%)	69 (2.2%)	3,163	(1.7, 2.8)
	1-2 yrs.	2,282 (94.5%)	134 (5.5%)	2,416	(4.7, 6.5)
	6 mo. – 1 yr.	1,322 (89.3%)	159 (10.7%)	1,481	(9.2, 12.4)
	< 6 mo.	3,202 (90.8%)	326 (9.2%)	3,528	(8.3, 10.2)
	Total	14,236 (94.9%)	767 (5.1%)	**15,003**	
Sex	CM	6,384 (95.1%)	327 (4.9%)	6,715	(4.4, 5.4)
	F	1,802 (93.2%)	131 (6.8%)	1,934	(5.7, 8.0)
	M	1,777 (93.5%)	124 (6.5%)	1,901	(5.5, 7.7)
	SF	4,807 (96.01%)	200 (4.0%)	5,008	(3.5, 4.6)
	U	379 (96.2%)	15 (3.8%)	394	(2.1, 6.2)
	Total	15,149 (95.0%)	797 (5.0%)	**15,946**	
Region	Northeast	4,547 (97.4%)	120 (2.6%)	4,667	(2.1, 3.1)
	Midwest	1,892 (94.6%)	108 (5.4%)	2,000	(4.5, 6.5)
	South	4,283 (94.0%)	273 (6.0%)	4,556	(5.3, 6.7)
	West	4,723 (93.6%)	323 (6.4%)	5,046	(5.7, 7.1)
	Total	15,445 (94.9%)	824 (5.1%)	**16,269**	
Season	Winter	3,999 (94.6%)	230 (5.4%)	4,229	(4.8, 6.2)
	Spring	3,099 (94.8%)	171 (5.2%)	3,270	(4.5, 6.0)
	Summer	3,749 (96.1%)	153 (3.9%)	3,902	(3.3, 4.6)
	Autumn	4,602 (94.5%)	270 (5.5%)	4,872	(4.9, 6.2)
	Total	15,449 (94.9%)	824 (5.1%)	**16,273**	

Table 2.11 Distribution of PCR test results for *Cryptosporidium* spp. by age, sex, region and season for cats

Variable	Level	Negative	Positive	Total	95% CI
Age	>7 yrs.	4,282 (97.0%)	133 (3.0%)	4,415	(2.5, 3.6)
	3-7 yrs.	3,055 (96.6%)	108 (3.4%)	3,163	(2.8, 4.1)
	1-2 yrs.	2,222 (92.0%)	194 (8.0%)	2,416	(7.0, 9.2)
	6 mo. – 1 yr.	1,257 (84.9%)	224 (15.1%)	1,481	(13.3, 17.1)
	< 6 mo.	3,063 (86.8%)	465 (13.2%)	3,528	(12.1, 14.3)
	Total	13,879 (92.5%)	1,124 (7.5%)	**15,003**	
Sex	CM	6,244 (93.1%)	467 (7.0%)	6,711	(6.4, 7.6)
	F	1,750 (90.5%)	183 (9.5%)	1,933	(8.2, 10.9)
	M	1,714 (90.2%)	187 (9.8%)	1,901	(8.5, 11.3)
	SF	4,697 (93.8%)	310 (6.2%)	5,007	(5.5, 6.9)
	U	365 (92.6%)	29 (7.4%)	394	(5.0, 10.4)
	Total	14,770 (92.6%)	1,176 (7.4%)	**15,946**	
Region	Northeast	4,458 (95.5%)	209 (4.5%)	4,667	(3.9, 5.1)
	Midwest	1,848 (92.4%)	152 (7.6%)	2,000	(6.5, 8.8)
	South	4,142 (90.9%)	414 (9.1%)	4,556	(8.3, 10.0)
	West	4,610 (91.4%)	436 (8.6%)	5,046	(7.9, 9.4)
	Total	15,058 (92.6%)	1,211 (7.4%)	**16,269**	
Season	Winter	3,902 (92.3%)	327 (7.7%)	4,229	(6.9, 8.6)
	Spring	3,027 (92.6%)	243 (7.4%)	3,270	(6.5, 8.4)
	Summer	3,654 (93.6%)	248 (6.4%)	3,902	(5.6, 7.2)
	Autumn	4,479 (91.9%)	393 (8.1%)	4,872	(7.3, 8.9)
	Total	15,062 (92.6%)	1,211 (7.4%)	**16,273**	

Table 2.12 Univariate logistic regression analysis to evaluate associations between individual risk factors and the probability of testing positive to *Giardia* spp. for dogs

Variable	Level	OR	95% CI		P Value	Overall P Value
Age	>7 yrs.	Ref.				
	3-7 yrs.	0.95	0.77	1.2	0.62	
	1-2 yrs.	3.0	2.5	3.6	<0.0001	
	6 mo. – 1 yr.	6.6	5.4	8.0	<0.0001	<0.00001
	<6 mo.	7.8	6.6	9.2	<0.0001	
Sex	CM	Ref.				
	F	2.6	2.2	3.0	<0.0001	
	M	2.5	2.2	2.8	<0.0001	<0.00001
	SP	0.83	0.72	0.96	0.01	
	U	1.3	0.90	1.9	0.16	
Region	Northeast	Ref.				
	Midwest	1.3	1.1	1.6	<0.0001	
	South	0.90	0.78	1.0	0.14	<0.0001
	West	1.0	0.06	1.1	0.85	
Season	Winter	Ref.				
	Spring	1.0	0.90	1.18	0.65	
	Summer	0.79	0.69	0.91	0.001	<0.0003
	Autumn	0.87	0.76	1.0	0.04	

Table 2.13 Univariate logistic regression analysis to evaluate associations between individual risk factors and the probability of testing positive to *Cryptosporidium* spp. for dogs

Variable	Level	OR	95% CI		P Value	Overall P Value
Age	>7 yrs.	Ref.				
	3-7 yrs.	0.63	0.52	0.78	<0.0001	
	1-2 yrs.	1.1	0.92	1.4	0.25	<0.00001
	6 mo. – 1 yr.	2.1	1.7	2.7	<0.0001	
	<6 mo.	4.4	3.8	5.2	<0.0001	
Sex	CM	Ref.				
	F	2.5	2.1	3.0	<0.0001	
	M	2.3	1.9	2.6	<0.0001	<0.00001
	SP	0.82	0.70	1.0	0.02	
	U	1.9	1.3	2.7	<0.001	
Region	Northeast	Ref.				
	Midwest	1.8	1.4	2.2	<0.0001	
	South	1.2	0.96	1.4	0.12	<0.00001
	West	1.8	1.6	2.2	<0.0001	
Season	Winter	Ref.				
	Spring	0.98	0.83	1.2	0.84	
	Summer	0.92	0.78	1.1	0.35	0.60
	Autumn	1.0	0.88	1.2	0.72	

Table 2.14 Univariate logistic regression analysis to evaluate associations between individual risk factors and the probability of testing positive to *Giardia* spp. for cats

Variable	Level	OR	95% CI		P Value	Overall P Value
Age	>7 yrs.	Ref.				
	3-7 yrs.	2.3	1.8	3.1	<0.0001	
	1-2 yrs.	4.6	3.5	6.0	<0.0001	<0.00001
	6 mo. – 1 yr.	5.4	4.0	7.1	<0.0001	
	<6 mo.	4.3	3.3	5.5	<0.0001	
Sex	CM	Ref.				
	F	1.9	1.5	2.3	<0.0001	
	M	1.7	1.3	2.0	<0.0001	<0.00001
	SP	0.92	0.76	1.1	0.34	
	U	1.2	0.77	1.9	0.42	
Region	Northeast	Ref.				
	Midwest	1.2	0.96	1.5	0.11	
	South	1.0	0.87	1.3	0.61	0.43
	West	1.1	0.92	1.3	0.32	
Season	Winter	Ref.				
	Spring	0.82	0.66	1.0	0.07	
	Summer	0.85	0.70	1.0	0.12	0.08
	Autumn	1.0	0.85	1.2	0.88	

Table 2.15 Univariate logistic regression analysis to evaluate associations between individual risk factors and the probability of testing positive to *Cryptosporidium felis* for cats

Variable	Level	OR	95% CI		P Value	Overall P Value
Age	>7 yrs.	Ref.				
	3-7 yrs.	1.2	0.88	1.7	0.23	
	1-2 yrs.	3.2	2.4	4.3	<0.0001	
	6 mo. – 1 yr.	6.6	5.0	8.7	<0.0001	<0.00001
	<6 mo.	5.6	4.4	7.2	<0.0001	
Sex	CM	Ref.				
	F	1.4	1.2	1.7	<0.001	
	M	1.4	1.1	1.7	0.004	<0.00001
	SP	0.81	0.68	0.97	0.02	
	U	0.77	0.46	1.3	0.34	
Region	Northeast	Ref.				
	Midwest	2.2	1.7	2.8	<0.0001	
	South	2.4	1.9	3.0	<0.0001	<0.00001
	West	2.6	2.1	3.2	<0.0001	
Season	Winter	Ref.				
	Spring	0.96	0.78	1.2	0.69	
	Summer	0.71	0.58	0.87	<0.001	0.002
	Autumn	1.0	0.85	1.2	0.83	

Table 2.16 Univariate logistic regression analysis to evaluate associations between individual risk factors and the probability of testing positive to *Cryptosporidium* spp. for cats

Variable	Level	OR	95% CI		P Value	Overall P Value
Age	>7 yrs.	Ref.				
	3-7 yrs.	1.1	0.88	1.5	0.33	
	1-2 yrs.	2.8	2.2	3.5	<0.0001	
	6 mo. – 1 yr.	5.7	4.6	7.2	<0.0001	<0.00001
	<6 mo.	4.9	4.0	6.0	<0.0001	
Sex	CM	Ref.				
	F	1.4	1.2	1.7	<0.0001	
	M	1.5	1.2	1.7	<0.0001	<0.00001
	SP	0.88	0.76	1.0	0.10	
	U	1.1	0.72	1.6	0.80	
Region	Northeast	Ref.				
	Midwest	1.8	1.4	2.2	<0.0001	
	South	2.1	1.8	2.5	<0.0001	<0.00001
	West	2.0	1.7	2.4	<0.0001	
Season	Winter	Ref.				
	Spring	0.96	0.81	1.1	0.62	
	Summer	0.81	0.68	0.96	0.02	0.02
	Autumn	1.0	0.90	1.2	0.60	

Table 2.17 Multivariate logistic regression analysis to evaluate associations between multiple risk factors and the probability of testing positive to *Giardia* spp. for dogs

Variable	Level	Odds Ratio	95% CI		P Value	Total No. of Dogs
Age	>7 yrs.	Ref.				
	3-7 yrs.	0.95	0.77	1.2	0.63	21,612
	1-2 yrs.	3.0	2.5	3.6	<0.0001	
	6 mo. – 1 yr.	6.6	5.4	8.0	<0.0001	
	<6 mo.	7.8	6.6	9.2	<0.0001	
Region	Northeast	Ref.				
	Midwest	1.3	1.1	1.6	0.001	
	South	0.84	0.72	0.98	0.03	
	West	1.0	0.92	1.2	0.52	
Season	Winter	Ref.				
	Spring	1.0	0.90	1.2	0.60	
	Summer	0.80	0.69	0.92	0.002	
	Autumn	0.84	0.73	1.0	0.02	

Table 2.18 Multivariate logistic regression analysis to evaluate associations between multiple risk factors and the probability of testing positive to *Cryptosporidium* spp. for dogs

Variable	Level	Odds Ratio	95% CI		P Value	Total No. of Dogs
Age	>7 yrs.	Ref.				
	3-7 yrs.	0.62	0.50	0.77	<0.0001	21,448
	1-2 yrs.	1.1	0.90	1.3	0.38	
	6 mo. – 1 yr.	2.0	1.6	2.6	<0.0001	
	<6 mo.	4.0	3.2	4.8	<0.0001	
Sex	CM	Ref.				
	F	1.2	0.94	1.4	0.20	
	M	1.2	0.95	1.4	0.10	
	SP	0.84	0.71	1.0	0.05	
	U	1.4	0.81	2.4	0.23	
Region	Northeast	Ref.				
	Midwest	1.7	1.4	2.1	<0.0001	
	South	1.1	0.90	1.3	0.35	
	West	2.0	1.7	2.3	<0.0001	

Table 2.19 Multivariate logistic regression analysis to evaluate associations between multiple risk factors and the probability of testing positive to *Giardia* spp. for cats

Variable	Level	Odds Ratio	95% CI		P Value	Total No. of Cats
Age	>7 yrs.	Ref.				
	3-7 yrs.	2.4	1.8	3.2	<0.0001	14,891
	1-2 yrs.	4.5	3.4	5.9	<0.0001	
	6 mo. – 1 yr.	5.2	4.0	6.9	<0.0001	
	<6 mo.	3.7	2.8	4.9	<0.0001	
Sex	CM	Ref.				
	F	1.3	1.1	1.7	0.01	
	M	1.2	0.98	1.6	0.07	
	SP	0.91	0.76	1.1	0.33	
	U	0.92	0.40	2.1	0.85	

Table 2.20 Multivariate logistic regression analysis to evaluate associations between multiple risk factors and the probability of testing positive to *Cryptosporidium felis* for cats

Variable	Level	Odds Ratio	95% CI		P Value	Total No. of Cats
Age	>7 yrs.	Ref.				
	3-7 yrs.	1.2	0.88	1.7	0.21	14,999
	1-2 yrs.	3.3	2.5	4.3	<0.0001	
	6 mo. – 1 yr.	6.4	4.9	8.5	<0.0001	
	<6 mo.	5.6	4.5	7.2	<0.0001	
Region	Northeast	Ref.				
	Midwest	2.1	1.6	2.8	<0.0001	
	South	2.2	1.7	2.8	<0.0001	
	West	2.5	2.0	3.2	<0.0001	
Season	Winter	Ref.				
	Spring	1.0	0.83	1.3	0.77	
	Summer	0.7	0.60	0.88	0.002	
	Autumn	1.0	0.82	1.2	0.94	

Table 2.21 Multivariate logistic regression analysis to evaluate associations between multiple risk factors and the probability of testing positive to *Cryptosporidium* spp. for cats

Variable	Level	Odds Ratio	95% CI		P Value	Total No. of Cats
Age	>7 yrs.	Ref.				
	3-7 yrs.	1.1	0.88	1.5	0.30	14,999
	1-2 yrs.	2.8	2.3	3.6	<0.0001	
	6 mo. – 1 yr.	5.7	4.6	7.2	<0.0001	
	<6 mo.	4.9	4.0	5.9	<0.0001	
Region	Northeast	Ref.				
	Midwest	1.8	1.4	2.2	<0.0001	
	South	2.0	1.7	2.4	<0.0001	
	West	2.1	1.7	2.5	<0.0001	

2.8 Figures

Figure 2.1 USA Census Bureau classification for regions[a]
[a]Adapted from http://www.spinward.com/us_map/us_map.htm

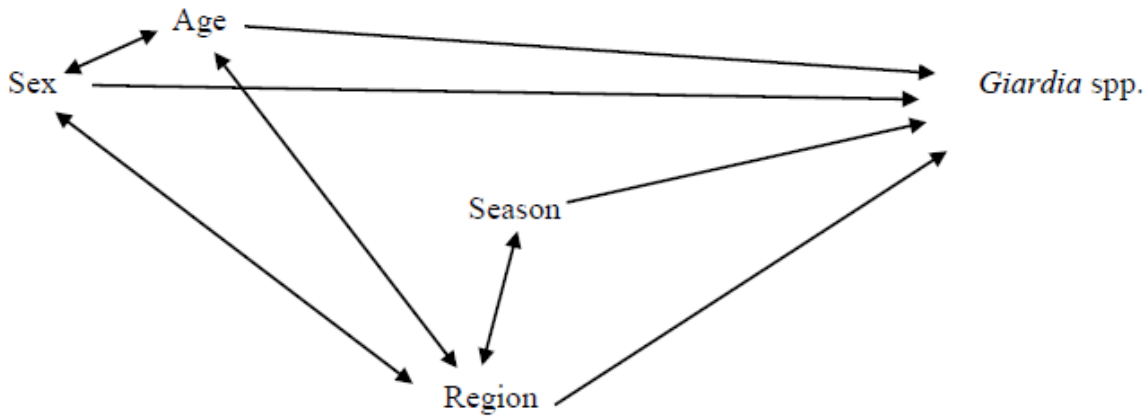


Figure 2.2 A causal model for *Giardia* spp. in pet dogs in the United States

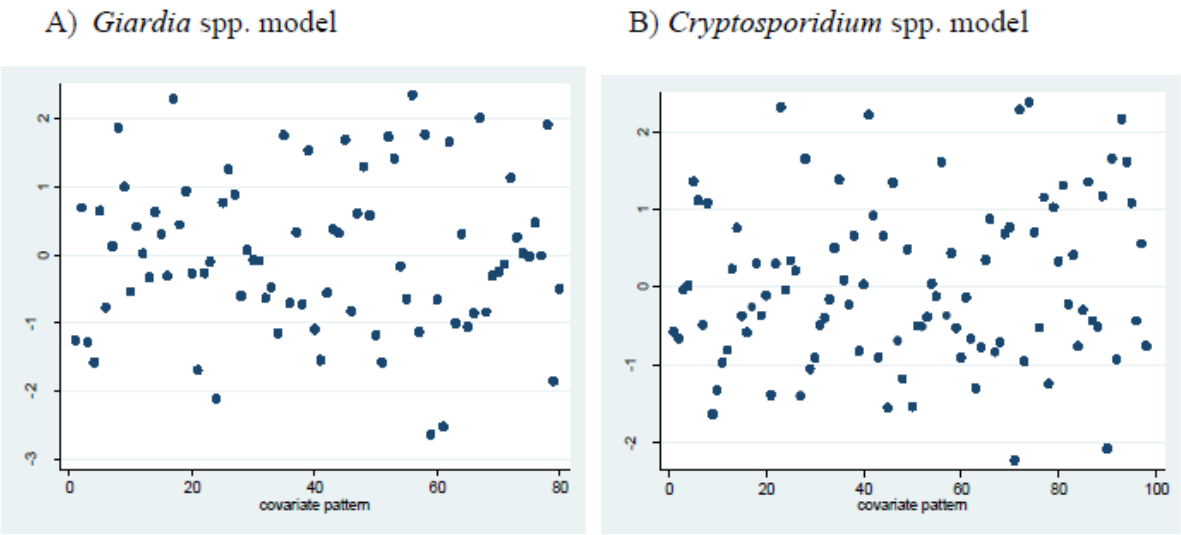


Figure 2.3 Plot of standardized residuals of *Giardia* spp. and *Cryptosporidium* spp. model for dogs

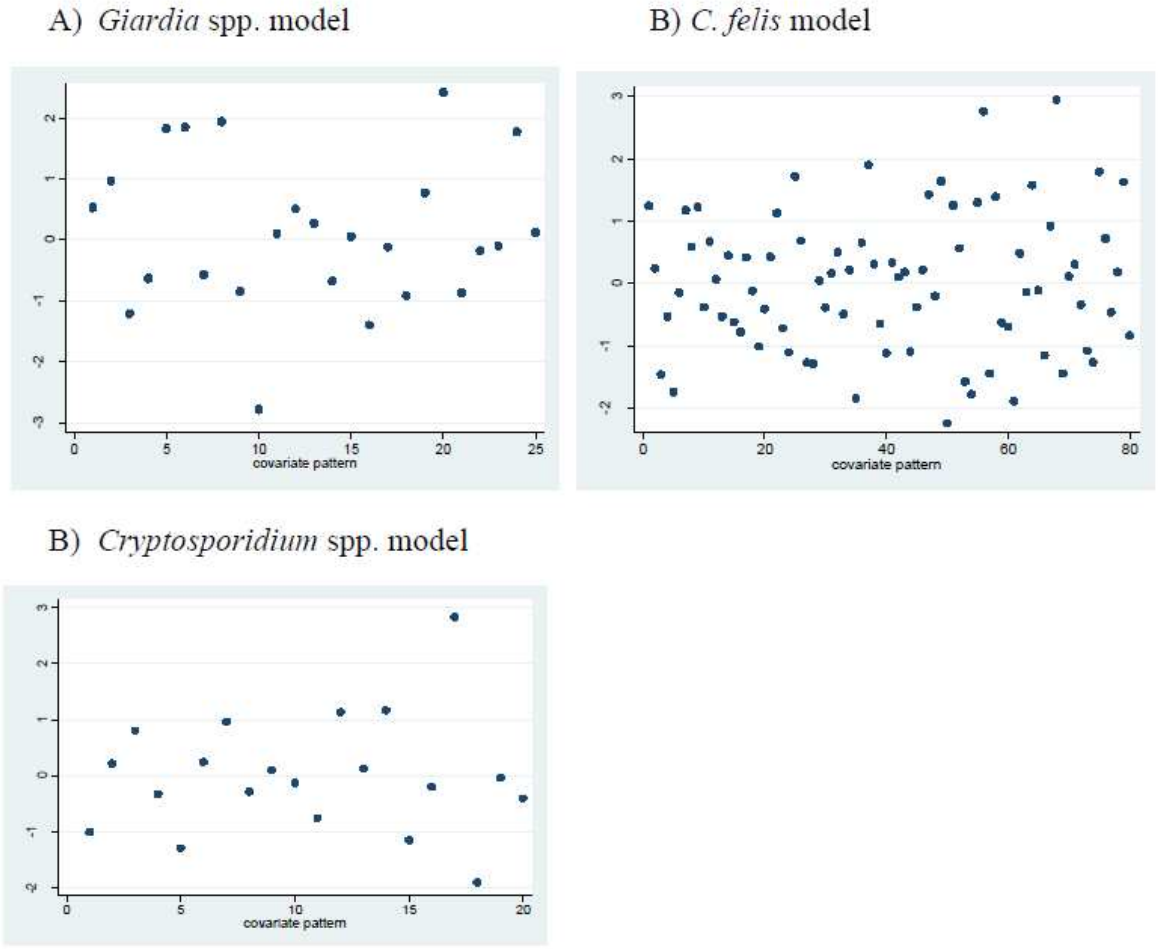


Figure 2.4 Plot of standardized residuals of *Giardia* spp., *C. felis* and *Cryptosporidium* spp. model for cats

REFERENCES

1. Sotiriadou I, Pantchev N, Gassmann D, Karanis P. Molecular identification of *Giardia* and *Cryptosporidium* from dogs and cats. *Parasite.* 2013;20(8):1-7. doi: 10.1051/parasite/2013008.

2. Faubert GM. The immune response to *Giardia*. *Parasitol Today.* 1996;12:140–145.

3. Asahi H, Koyama T, Arai H, et al. Biological nature of *Cryptosporidium* spp. isolated from a cat. *Parasitol Res*. 1991;77:237-240. doi: 10.1007/BF00930864.

4. Lappin MR, Dowers K, Edsell D, Cheney J. Cryptosporidiosis and inflammatory bowel disease. *Fel Pract.* 1997;25(3):10-13.

5. Willard MD, Bouly D. Cryptosporidiosis, coccidiosis and total colonic mucosal collapse in an immunosuppressed puppy. *J Am Anim Hosp Assoc*. 1999;35(5):405-409. doi: 10.5326/15473317-35-5-405.

6. Denholm KM, Haitjema H, Gwynne BJ, Morgan UM, Irwin PJ. Concurrent *Cryptosporidium* and parvovirus infections in a puppy. *Aust Vet J* . 2001;79:98-101. doi: 10.1111/j.1751-0813.2001.tb10708.x.

7. Aydin Y, Güvenç T, Beyaz L, Sancak A. Intestinal cryptosporidiosis associated with distemper in a dog. *Ankara Univ Vet Fak Derg* 2004;51(3):233-235 (Abstract).

8. Greene CE, Jacobs GJ, Pricket, D. Intestinal malabsorption and cryptosporidiosis in an adult dog. *J Vet Med Assoc*. 1990;197(3):365-367.

9. Kirkpatrick CE. Feline giardiasis: A review. *J Small Anim Pract.* 1986;27:69-80. doi: 10.1111/j.1748-5827.1986.tb02124.x.

10. Tangtrongsup S, Scorza V. Update on the diagnosis and management of *Giardia* spp. infections in dogs and cats. *Top Companion Anim Med*. 2010;155-162. doi:10.1053/j.tcam.2010.07.003.

11. Epe C, Rehkterb G, Schniedera T, Lorentzen L, Kreienbrock L. *Giardia* in symptomatic dogs and cats in Europe – results of a European study. *Vet Parasitol.* 2010;173:32–38. doi:10.1016/j.vetpar.2010.06.015.

12. Fall A, Thompson RCA, Hobbs RP, Morgan-Ryan U. Morphology is not a reliable tool for delineating species within *Cryptosporidium*. *J Parasitol.* 2003;89(2):399-402. doi: 10.1645/0022-3395(2003)089[0399:MINART]2.0.CO;2.

13. Dryden MW, Payne PA, Smith V. Accurate diagnosis of *Giardia* spp. and proper fecal examination procedures. *Vet Ther.* 2006;7(1):4-14.

14. Scorza AV, Lappin MR. Giardiosis. In: Beugnet F, Halos L, ed. *Parasitoses and Vector Borne Diseases of Cats*. Lyon, France: Merial S.A.S.;2015:67-73.

15. Bouzid M, Halai K, Jeffreys D. The prevalence of *Giardia* infection in dogs and cats, asystematic review and meta-analysis of prevalence studies from stool samples. *Vet Parasitol.* 2015;207:181–202. http://dx.doi.org/10.1016/j.vetpar.2014.12.011

16. Pallant L, Barutzki D, Schaper R., Thompson RC. The epidemiology of infections with *Giardia* species and genotypes in well cared for dogs and cats in Germany. *Parasit Vectors.* 2015;8(2):1-14. doi: 10.1186/s13071-014-0615-2.

17. Scorza AV, Ballweber LR, Tangtrongsup S, Panuska C, Lappin MR. Comparisons of mammalian *Giardia duodenalis* assemblages based on the β-giardin, glutamate dehydrogenase and triose phosphate. *Vet Parasitol.* 2012;189:182-188. doi: 10.1016/j.vetpar.2012.04.032.

18. Li W, Li Y, Song M, et al. Prevalence and genetic characteristics of *Cryptosporidium, Enterocytozoon bieneusi* and *Giardia duodenalis* in cats and dogs in Heilongjiang province, China. *Vet Parasitol.* 2015;208:125-134. http://dx.doi.org/10.1016/j.vetpar.2015.01.014

19. Mohamed AS, Glickman LT, Camp Jr. JW, Lund E, Moore GE. Prevalence and risk factors for *Giardia* spp. infection in a large national sample of pet dogs visiting veterinary hospitals in the United States (2003–2009). *Vet Parasitol.* 2013;195:35–41. doi:10.1016/j.vetpar.2012.12.049.

20. Wang A, Ruch-Gallie R, Scorza V, Lin P, Lappin MR. Prevalence of *Giardia* and *Cryptosporidium* species in dog park attending dogs compared to non-dog park attending dogs in one region of Colorado. *Vet Parasitol.* 2012; 184: 335–340. doi:10.1016/j.vetpar.2011.08.019.

21. Covacin C, Aucoin DP, Elliot A, Thompson RC. Genotypic characterisation of *Giardia* from domestic dogs in the USA. *Vet Parasitol.* 2011;177:28–32. doi:10.1016/j.vetpar.2010.11.029.

22. Gates MC, Nolan TJ. Endoparasite prevalence and recurrence across different age groups of dogs and cats. *Vet Parasitol.* 2009;166:153–158.doi:10.1016/j.vetpar.2009.07.041

23. Little SE, Johnson EM, Lewis D, et al. Prevalence of intestinal parasites in pet dogs in the United States. *Vet Parasitol.* 2009; 166:144–152. doi:10.1016/j.vetpar.2009.07.044.

24. Carlin EP, Bowman DD, Scarlett JM, Garrett J, Lorentzen L. 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):199–206.

25. Hackett T, Lappin MR. Prevalence of enteric pathogens in dogs of north-central Colorado. *J Am Anim Hosp Assoc.* 2003;39:52–56. doi: 10.5326/0390052.

26. Queen EV, Marks SL, Farver TB. Prevalence of selected bacterial and parasitic agents in feces from diarrheic and healthy control cats from Northern California. *J Vet Intern Med.* 2012;26:54–60. doi: 10.1111/j.1939-1676.2011.00843.x.

27. De Santis-Kerr AC, Raghavan M, Glickman NW, et al. Prevalence and risk factors for *Giardia* and coccidia species of pet cats in 2003–2004. *J Feline Med Surg.* 2006;8:292–301. doi: 10.1016/j.jfms.2006.02.005.

28. Ballweber LR, Panuska C, Huston CL, Vasilopulos R, Pharr GT, Mackin A. Prevalence of and risk factors associated with shedding of *Cryptosporidium felis* in domestic cats of Mississippi and Alabama. *Vet Parasitol.* 2009;160:306–310. http://dx.doi.org/10.1016/j.vetpar.2008.11.018.

29. Spain CV, Scarlett JM, Wade SE, McDonough P. Prevalence of enteric zoonotic agents in cats less than 1 year old in Central New York State. *J Vet Intern Med.* 2001;15:33–38. doi: 10.1111/j.1939-1676.2001.tb02294.x.

30. McReynolds C, Lappin MR, McReynolds LM, et al. Regional seroprevalence of *Cryptosporidium parvum* IgG specific antibodies of cats in the United States. *Vet Parasitol.* 1999;80(3):187-195. doi:10.1016/S0304-4017(98)00219-2.

31. Hill S, Cheney J, Tatton-Allen GF, Reif JS, Bruns C, Lappin MR. Prevalence of enteric zoonotic organisms in cats. *J Am Vet Med Assoc.* 2000;216:687-692. doi: 10.2460/javma.2000.216.687.

32. Ballweber LR, Xiao L, Bowman DD, Khan G, Cama VA. Giardiasis in dogs and cats: update on epidemiology and public health significance. *Trends Parasitol.* 2010;26(4):180-189. doi:10.1016/j.pt.2010.02.005.

33. Meteorological Reckoning: Wikipedia Web site: https://en.wikipedia.org/wiki/Spring_(season). Updated October 20, 2015. Accessed October 21, 2015.

34. U.S. Pet Ownership and Demographics Sourcebook (USPODS) 2012. AVMA Web site https://www.avma.org/KB/Resources/Statistics/Pages/Market-research-statistics-US-Pet-Ownership-Demographics-Sourcebook.aspx. Published 2012. Updated 2015. Accessed August 5, 2015.

35. Vasilopulos RJ, Rickard LG, Mackin AJ, Pharr GT, Huston CL. Genotypic analysis of *Giardia duodenalis* in domestic cats. *J Vet Intern Med.* 2007;21:352–355.

36. Hawash Y, Ghonaim MM, Al-Shehri SS. An improved PCR-RFLP assay for detection and genotyping of asymptomatic *Giardia lamblia* infection in a resource-poor setting. *Korean J Parasitol.* 2016;54(1):1-8. doi: 10.3347/kjp.2016.54.1.1.

37. Gizzi AB, Oliveira ST, Leutenegger CM, et al. Presence of infectious agents and co-infections in diarrheic dogs determined with a real-time polymerase chain reaction-based panel. *BMC Vet Res*. 2014;10(23):1-8. doi: 10.1186/1746-6148-10-23.

38. Scorza AV, Lappin MR. 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 States. Fort Collins, CO, Clinical Sciences, Colorado State University, 2007.

39. Palmer CS, Traub RJ, Robertson ID, Devlin G, Rees R, Thompson RC. Determining the zoonotic significance of *Giardia* and *Cryptosporidium* in Australian dogs and cats. *Vet Parasitol*. 2008;154:142–147. doi:10.1016/j.vetpar.2008.02.031.

40. State Summary Report. Mandatory spay/neuter laws. AVMA Web site https://www.avma.org/Advocacy/StateAndLocal/Pages/sr-spay-neuter-laws.aspx Updated 2013. Accessed 6, 2015.

41. Hoskins JD, Malone, JB, Smith PH. Prevalence of parasitism diagnosed by fecal examination in Louisiana dogs. *Am J Vet Res.* 1982;431106–1109.

42. Lightner L, Christensen BM, Beran GW. Epidemiologic findings on canine and feline intestinal nematode infections from records of the Iowa State University Veterinary Clinic. *J Am Vet Med Assoc*. 1978;172:564–567.

43. National Oceanic and Atmospheric Administration (NOAA) 2013. Regional Climate Trends and Scenarios for the US National Climate Assessment. Part 3. Climate of the Midwest. NOAA Technical Report NESDIS 142-143. United States Department of Commerce. EPA Web site https://www.epa.gov/climate-impacts/climate-impacts-midwest. Accessed October 20, 2016.

44. Robertson LJ, Gjerde BK. Fate of *Cryptosporidium* oocysts and *Giardia* cysts in the Norwegian aquatic environment over winter. *Microb Ecol.* 2006;52:597–602. doi: 10.1007/s00248-006-9005-4

45. Robertson LJ, Lim YAL. Waterborne and Environmentally-Borne Giardiasis. In: Luján HD, Svärd S, ed. *Giardia*. Wien, New York: Springer; 2011:29–69.

46. Painter JE, Hlavsa MC, Collier SA, Yoder JS. Cryptosporidiosis surveillance — United States, 2011–2012. Giardiasis surveillance — United States, 2011–2012. *Morb Mortal Wkly Rep*. 2015;64(3):1-28.

47. Becker SV, Selby LA, Hutcheson DP, Hacker DV. The association of selected climatic factors with natural alimentary parasites of the dog. *Environ Res.* 1977;14:141-151. doi:10.1016/0013-9351(77)90075-5.

48. Kirkpatrick CE. Epizootiology of endoparasitic infections in pet dogs and cats presented to a veterinary teaching hospital. *Vet Parasitol.* 1988; 30,113-124.doi: 10.1016/0304-

4017(88)90158-6.

CHAPTER 3: VALIDATION OF A POLYMERASE CHAIN REACTION ASSAY FOR THE SUBTYPING OF *CRYPTOSPORIDIUM* SPP. ISOLATES OF HUMAN ORIGIN

Summary

Cryptosporidium spp. have been associated with diarrhea in a wide range of hosts including humans. The objective of this study was to present the optimization and validation of a polymerase chain reaction (PCR) assay to subtype *Cryptosporidium parvum* and *Cryptosporidium hominis* from positive human isolates for application in a study of veterinary students. A published 60 kDa glycoprotein (gp60) gene-based PCR assay was previously optimized elsewhere for subtyping of *C. parvum* and *C. hominis*. In this study, the gp60 PCR assay was validated in negative fecal samples experimentally inoculated with *C. parvum* oocysts and *C. hominis* DNA. After optimization experiments, the optimal primer concentration (0.2 µM), DNA volume (2.0 µl) and annealing temperature (55°C) were selected to achieve the highest sensitivity. The gp60 assay amplified DNA from *C. parvum* and *C. hominis* as expected.

To evaluate the analytic sensitivity of the gp60 assay, five replicates of serial dilutions for *C. parvum* were prepared in three different methods: Method A involved preparing five sets of serial dilutions of the *C. parvum* oocysts. Method B involved making five sets of dilutions by adding 0.25 g of the fecal matter to the purified oocysts. Method C involved making five sets of dilutions by adding 2.5 g of fecal matter to the oocysts. All *C. parvum* oocysts were at concentrations from 10^6 to 10^1/mL in PBS-EDTA in each set of dilutions. The lowest limit of detection for the gp60 PCR assay was $1x10^3$ oocysts/mL for *C. parvum* oocysts and 0.001 ng/mL for *C. hominis* DNA, respectively.

To determine the analytic specificity of the gp60 PCR assay, DNA was extracted from *C. parvum*, *C. canis*, *C. felis*, as well as the *C. hominis* DNA. In addition, the DNA was extracted from non-*Cryptosporidium* spp. such as *Cyclospora cayetanensis Toxoplasma gondii*, and

Giardia duodenalis. While the gp60 PCR assay amplified DNA of *C. hominis* and *C. parvum*, no other non – targeted DNA was amplified.

In this study, we have presented the optimization and evaluation processes for the gp60 assay. In our study, method C has been identified to be superior for *C. parvum* oocysts and it is recommended for use in the future research. The gp60 assay optimized is suitable to genotype and subgenotype isolate of human origin and this is considered a novel approach for our laboratory.

3.1 Introduction

Cryptosporidium spp. are protozoans that can cause gastrointestinal disease in several species including cats, dogs and humans. Serologic studies report that 25% or more of the USA population has been exposed to *Cryptosporidium* spp..[1]

Human cryptosporidiosis is mainly caused by *Cryptosporidium hominis* (previously known as the *Cryptosporidium parvum* anthroponotic genotype or genotype 1) and *Cryptosporidium parvum.*[2,3] *Cryptosporidium hominis* is harbored almost exclusively by humans, but *C. parvum* is found in domestic livestock, wild animals, and humans.[3] Since both *Cryptosporidium* spp. are detected in humans, two cycles of infection: the anthroponotic and the zoonotic cycles can cause human cryptosporidiosis.[3]

Several molecular techniques have been developed for the differentiation of *Cryptosporidium* at the genotype or species level.[4] However, to understand the transmission dynamics of human cryptosporidiosis, subtyping within a species is critical. Subtyping analysis is more informative than genotyping alone as it can clarify genetic variation on a finer scale. Thus, to determine the proportion of *C. parvum* infections in humans attributable to zoonotic transmission, subtyping of this species is crucial.[5]

One of the popular subtyping tools is the PCR amplification and DNA sequence analysis of the 60 kDa glycoprotein (gp60).[4] An important feature of this gene is its high degree of sequence polymorphism, particularly among *C. hominis* and *C. parvum* isolates.[2,3] The gp60 gene has tandem repeats of the serine-coding trinucleotide at the end of the gene and also has extensive sequence differences in the non-repeat regions, which categorize *C. parvum* and *C. hominis* each to several subtype families.[4] The gp60 PCR has shown at least 10 subtype allele families, IIa–IIj, among *C. parvum* isolates from humans and animals.[6-8] Among these subtype families, only IIa and IId have been detected in cattle.[8]

The Center for Companion Animal Studies has extensive experience of performing *Cryptosporidium* spp. genotyping from canine and feline isolates. However, the Center has not performed the gp60 PCR assay on isolates from humans. The hypothesis of this study is that the gp60 PCR assay followed by DNA sequencing analysis will allow for the *C. parvum* human and bovine subtype allele families' differentiation from human isolates as previously reported. The specific aim was for this technique to be optimized for use in a subsequent experiment using veterinary student feces (Chapter IV).

3.2 Materials and Methods

3.2.1 Gp60 Assay Verification

A published gp60 PCR assay was selected from published articles.[3,9,10] The assay was verified in a feline fecal specimen (that was negative to *Cryptosporidium* spp. using the IFA and PCR assays) and experimentally inoculated with *C. parvum* oocysts and in *C. hominis* DNA. The sample was then evaluated in serial dilutions. The DNA of *C. parvum* oocysts was extracted using a FastDNA® Kit (MP Biomedicals, LLC. Solon, OH) as previously described.[11] The *C. parvum* and *C. hominis* isolates were then used for the analytic sensitivity testing.

3.2.2 Gp60 Optimization Process

The optimization process involved comparing different concentrations of each PCR component to determine which combination performs better under our laboratory conditions. AmpliTaq Gold™ DNA Polymerase (Life Technologies, Grand Island, NY) and two commercial premade, ready to use master mixes, these are: HotStar Taq Master Mix (QIAgen, Valencia, CA), and the Fast Cycling PCR Kit (QIAgen, Valencia, CA) were compared against each other. Annealing gradient ranged from (50°C - 65°C) were chosen to check which temperature would perform best. The primer concentrations (0.1, 0.2, and 0.5 µm) and different DNA volumes (1.0, 2.0, and 5.0 µl) were tested in the gp60 PCR assay.

3.2.3 Assay Validation

Validation provides the essential evidence to justify the continued use of the assay in further research projects.[12]

3.2.3.1 Analytic Sensitivity Testing

The *C. parvum* oocysts isolate (99-13) was obtained from a positive cow. It was stored in (0.9 x10^8 /mL) phosphate buffer saline and antibiotics (1,000 U Penicillin 1,000 µg Streptomycin) at 4°C prior until required. Pure DNA of *C. hominis* from a positive human patient was donated by the global bioresource center (NR – 2520) of the American Type Culture Collection (ATCC/ Manassas, VA) as a commercial source of oocysts was not available.

A 1:9 dilution was made with *C. parvum* oocysts as (10.0 µl of the oocyst solution in 90.0 µl of PCR water). Then, 10.0 µl of this solution were placed in a hemacytometer in order to count the oocysts. This procedure was repeated three times.

An average of three counts resulted in enumerating approximately 980 oocysts. The hemacytometer calculation was as follows: 980 (oocysts) x 10 (dilution factor) x 2500=24,500,000 oocysts/mL. The oocysts were diluted to a concentration of 2,450,000

oocysts/mL. This stock was serially diluted to create oocyst concentrations from 1 x 10^6 to 1 x 10^1 oocysts/mL. Five replicates of the serial dilutions for the *C. parvum* oocysts were prepared to be used for sensitivity testing.

The gp60 PCR assay detection limit was compared in three different methods of dilutions. Additionally, to determine the overall performance of the assay, it was compared with the IFA as well as two different PCR assays that include the Heat shock protein 70 (hsp70) and 18SrRNA performed as described previously.[13,14]

Method A

Cryptosporidium parvum oocysts with a concentration from 10^6 to 10^1 oocysts/mL were replicated five times in PBS-EDTA dilution. No fecal matter was added to these concentrations using this method.

Method B

Cryptosporidium parvum oocysts with a concentration from 10^6 to10^1 oocysts/mL were replicated five times in PBS-EDTA dilution. Then, an amount of 0.2-0.25 grams of a feline fecal specimen (that was negative to *Cryptosporidium* spp. using the IFA and PCR assays) was added to these concentrations.

Method C

Cryptosporidium parvum oocysts with a concentration from 10^6 to 10^1 oocysts/mL were replicated five times in 5.0 mL of PBS-EDTA dilution. Then, an amount of 2.5-3.0 grams of a feline fecal specimen (that was negative to *Cryptosporidium* spp. using the IFA and PCR assays) was added to these concentrations. The samples then were concentrated using Sheather's sugar centrifugation technique. The DNA for each method of dilutions was extracted using the FastDNA® extraction kit.[11]

***Cryptosporidium hominis* Dilutions:**

Due to the unavailability of *C. hominis* oocysts, the three methods of dilutions (A, B and C) were not prepared. Instead, DNA of *C. hominis* with concentration of 10 ng/mL was used to make five sets of serial dilutions starting with 1.0 ng/mL to 0.0001 ng/mL.

3.2.3.2 Analytic Specificity Testing

The main purpose of conducting the analytic specificity testing is to confirm whether the primers that have been chosen for this assay are binding only to the desired sequences or to additional irrelevant sequences. The analytic specificity of the gp60 PCR assay was assessed by testing the DNA from a range of *Cryptosporidium* spp.: *C. parvum* (obtained from a calf isolate), *C. hominis* (DNA), *C. canis, C. felis,* and non-*Cryptosporidium* spp.: *C. cayetanensis* (obtained from a human isolate), *T. gondii, and G. duodenalis* (obtained from a dog and a cow isolate).

3.3 Results

3.3.1 Gp60 Verification and Optimization Process

In terms of the assay optimization process, comparing the HotStarTaq and AmpliTaq Gold™ DNA Polymerase master mixes showed that the HotStar Taq master mix was more efficient because it produced brighter bands and less PCR byproducts compared to the AmpliTaq Gold. The Fast Cycling PCR master mix did not produce any bands. Therefore, the HotStar Taq master mix was selected for performing all PCR reactions. The non-specific amplification has not occurred in any of the selected annealing temperature, however the highest yield was observed in annealing gradient of 55°C. The results of this optimization process were displayed in Figure 3.1. In terms of primer and DNA concentrations, optimal primer concentration that has worked best was 0.2 µM and 2.0 µl best volume for the DNA template.

3.3.2 Gp60 Assay Validation

3.3.2.1 Analytic Sensitivity

To determine the overall performance of the gp60 PCR, this assay was compared to 18SrRNA PCR assay, hsp70 *Cryptosporidium* PCR assay, and the IFA. The maximal number of replicates detected by the gp60 assay varied by method (Tables 3.1, 3.2, and 3.3). All 5 replicates were positive in method A at the concentration of 10^6 oocysts/mL, method B at the concentration of 10^6 oocysts/mL, and method C at the concentration of 10^4 oocysts/mL. The lowest concentration of oocysts to be detected in the gp60 assay were 1 of 5 replicates positive at the concentration of 10^4 oocysts/mL in method A, 3 of 5 replicates at the concentration of 10^3 oocysts/mL in method B, and 2 of 5 replicates at the concentration of 10^3 oocysts/mL in method C. The gp60 assay was positive for C. *hominis* DNA in all 5 replicates at the concentration 0.001 ng/mL and 2 of 5 replicates at the concentration of 0.0001 ng/mL (Table 3.4).

The results were similar for the 18sRNA PCR, the hsp70 PCR assay and the IFA (with some slight differences in the concentrations for which the most replicates were positive (Tables 3.1, 3.2, and 3.3).

Cryptosporidium hominis DNA (Table 3.4) was amplified from more replicates at the 0.0001 ng/mL for the gp60 (2 replicates) and hsp70 assay (4 replicates) than the 18sRNA PCR (0 replicates). Since *C. hominis* oocysts were not available, the IFA technique was not performed for this method of dilutions.

3.3.2.2 Analytic Specificity

The results of blasting the primers selected in the *C. parvum* and *C. hominis* PCR reaction showed that those primers as specific to amplify the DNA of these two pathogens only and did not amplify the DNA of other pathogens included in the PCR reaction. As shown in Figure 3.2, the gp60 PCR assay revealed no cross-reactions with other genera and detected all the *C. hominis* and two out of six *C. parvum* samples correctly.

3.4 Discussion

Cryptosporidium species are indistinguishable morphologically and species can only be identified using molecular techniques. The gp60 PCR assay and DNA sequence analysis have been widely used in molecular epidemiology because it allows for both genotyping and subtyping of *Cryptosporidium* spp.[4] Due to its high sequence polymorphism resolution, particularly among *C. hominis* and *C. parvum* isolates, and because these two species are commonly isolated from human samples, a gp60 PCR assay have been optimized to be applied for human samples.

In the sensitivity evaluation of the assay, dilutions prepared with purified *C. parvum* oocysts were used to spike feline feces. The use of feline feces instead of the human feces was merely because this protocol has been previously used in similar experiments in the laboratory. We do not believe that the use of cat feces affected the sensitivity of the assay even though human and feline feces were not compared. Our laboratory spiked different fecal matrices (bobcats, foxes, and other carnivores) with *C. parvum* oocysts and similar detection limits were observed. One study showed that spiking cat feces with a known number of *C. parvum* oocysts did not affect the much improved sensitivity of the PCR assay in detecting *C. parvum* DNA compared to the IFA method.[17]

In these experiments, method A was expected to give the highest analytical sensitivity as feces was not present which should increase sensitivity because of the lack of potential PCR inhibitors. One study that stated the presence of PCR inhibitors in the sample matrix can lead to a reduced efficiency of the PCR assay itself.[19] However, all 3 PCR assays had fewer positive replicates at the concentration of 10^4 oocysts than the IFA, a technique that is predicted to be less sensitive than PCR (as was documented in Table 3.3). These results can only be explained by a laboratory error. The diluted oocysts used in method A were stored at 4°C for a much longer period of time for method A (two months) compared to the other two methods (two months). This storage condition might have negatively affected the amplification yield of the DNA. The DNA in low DNA concentration samples might degrade and become unavailable for the PCR amplification under such storage situations. One study documented that there was significant difference in the amplification yield between samples stored frozen in any buffer and those that are stored in 4°C.[18] Additionally, in method C, concentration technique before DNA extraction was performed. This technique is reported to enhance the recovery of *Cryptosporidium* spp. oocysts.[15,16] Overall, all replicates were positive consistently only at the concentration of 10^4 oocysts/mL regardless of PCR assay. Due to other published research (as of our knowledge) did not report the sensitivity of the gp60, 18SrRNA and the hsp70 PCR assays, we could not compare this level of sensitivity (10^4 oocysts/mL) to that previously reported with these three PCR assays.[3,13,14] The results of these experiments show that analysis of a single fecal sample in a clinically ill person or animal could have false negative results and so the combination of assays or evaluating more than one sample might be indicated.

Table 3.5 shows the average DNA concentrations for all five replicates in the three dilution methods for *C. parvum*. In methods B and C, the spiked samples contained a large

amount of DNA that can be attributed to the fecal matter. For method C, the hsp70 PCR assay performed best compared to other assays, detecting a few more positive replicates at 10^2 and 10^3 oocyst concentrations than the other assays. A possible explanation of this result is that the hsp70 PCR protocol is nested which increases the sensitivity of this assay.[13]

Since oocysts of *C. hominis* were not available, the analytic sensitivity of gp60 PCR assay could not be completely determined. In addition, the IFA method could not be assessed at all as it requires intact oocysts. Regardless, the 18SrRNA PCR assay appeared to be less sensitive than the other two assays. This finding may relate to the fact that both the gp60 and hsp70 are nested PCR reactions which can be considered more sensitive. As the *C. hominis* DNA was not diluted in feces, it is unknown the effect PCR inhibitors have on this assay.

Evaluation of analytical specificity of the gp60 PCR assay revealed no cross-reactions with other genera and detected all the *C. parvum* and *C. hominis* isolates correctly. That indicates that this PCR assay was 100% specific for *C. parvum* and *C. hominis*. Furthermore, another study conducted to evaluate the analytical specificity of primers designed to detect *C. cuniculus* using both nested, and real time gp60 PCR assay in a panel of 97 fecal samples contained *Cryptosporidium* spp. and non-*Cryptosporidium* spp. DNA. The primers amplified all *Cryptosporidium* spp. DNA and did not amplify other non-target DNA.[20] The analytic sensitivity and specificity of gp60 assay in this study was in accordance with studies conducted previously.[2,3,9,10]

3.5 Conclusion

In the present study, the gp60 PCR assay consistently detected DNA of *C. parvum* if oocysts were present at 10^4/mL if the spiked fecal sample was concentrated before assay (method C). This method of dilutions is recommended to be used for future fecal samples processing in

this research. The analytic sensitivity of *C. hominis* in this study was detected in the lowest DNA concentration studied, but was not studied in feces and so the effect of fecal inhibitors is unknown. The strength of the optimized Gp60 assay is that it is suitable to genotype and sub-genotype isolates of human origin. This assay will be utilized in Chapter 4 of this dissertation.

3.6 Tables

Table 3.1 Analytic sensitivity of the IFA and PCR assays using purified *C. parvum* oocysts diluted in PBS-EDTA solution

Method A	**Serial Dilution**					
	10^1 oocysts/mL	**10^2 oocysts/mL**	**10^3 oocysts/mL**	**10^4 oocysts/mL**	**10^5 oocysts/mL**	**10^6 oocysts/mL**
Gp60	0/5	0/5	0/5	1/5	3/5	5/5
18SrRNA	1/5	1/5	2/5	2/5	4/5	4/5
Hsp70	0/5	1/5	0/5	1/5	3/5	5/5
IFA	1/5	2/5	0/5	4/5	5/5	5/5

Table 3.2 Analytic sensitivity of the IFA and PCR assays using *C. parvum* oocysts spiked into 0.20-0.25 gram of feces

Method B	**Serial Dilutions**					
	10^1 oocysts/mL	**10^2 oocysts/mL**	**10^3 oocysts/mL**	**10^4 oocysts/mL**	**10^5 oocysts/mL**	**10^6 oocysts/mL**
Gp60	0/5	0/5	3/5	4/5	4/5	5/5
18SrRNA	0/5	0/5	2/5	4/5	5/5	5/5
Hsp70	0/5	0/5	0/5	2/5	3/5	5/5
IFA	0/5	0/5	0/5	3/5	5/5	5/5

Table 3.3 Analytic sensitivity of the IFA and PCR assays using *C. parvum* oocysts spiked into 2.0-3.0 grams of feces

Method C	**Serial Dilution**					
	10^1 oocysts/mL	**10^2 oocysts/mL**	**10^3 oocysts/mL**	**10^4 oocysts/mL**	**10^5 oocysts/mL**	**10^6 oocysts/mL**
Gp60	0/5	0/5	2/5	5/5	5/5	5/5
18SrRNA	0/5	0/5	1/5	5/5	5/5	5/5
Hsp70	0/5	2/5	3/5	5/5	5/5	5/5
IFA	0/5	0/5	0/5	2/5	3/5	5/5

Table 3.4 Analytic sensitivity of the three PCR assays using dilutions of *C. hominis* DNA dilutions

C. hominis	**Serial Dilution**				
	1.0 ng/mL	**0.1 ng/mL**	**0.01 ng/mL**	**0.001 ng/mL**	**0.0001 ng/mL**
Gp60	5/5	5/5	5/5	5/5	2/5
18SrRNA	5/5	5/5	5/5	2/5	0/5
Hsp70	5/5	5/5	5/5	5/5	4/5

Table 3.5 DNA concentration per each method of *C. parvum* dilutions

Serial Dilutions Oocysts/mL	**DNA Concentration ng/µl**
Method A:	
10^1	37.10
10^2	30.99
10^3	37.48
10^4	42.63
10^5	40.70
10^6	42.07
Method B:	
10^1	135.12
10^2	159.43
10^3	142.08
10^4	176.59
10^5	191.86
10^6	161.38
Method C:	
10^1	41.27
10^2	50.44
10^3	40.91
10^4	40.87
10^5	75.84
10^6	46.92

3.7 Figures

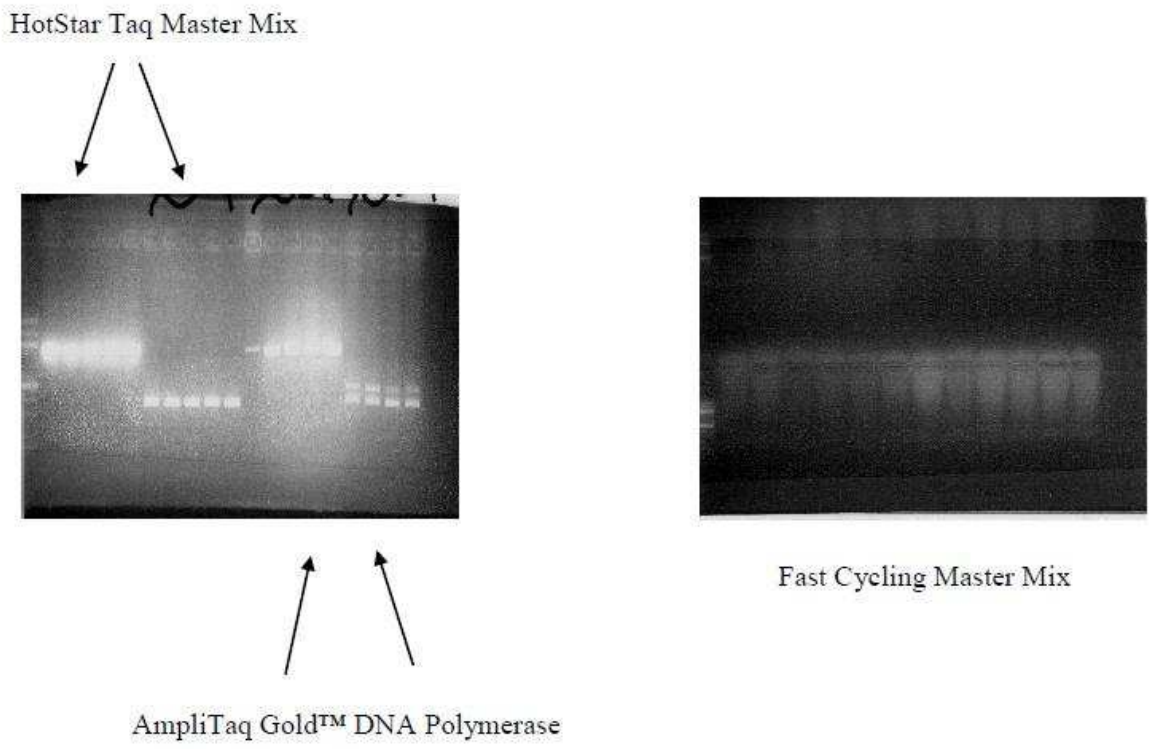


Figure 3.1 Gp60 Optimization Process

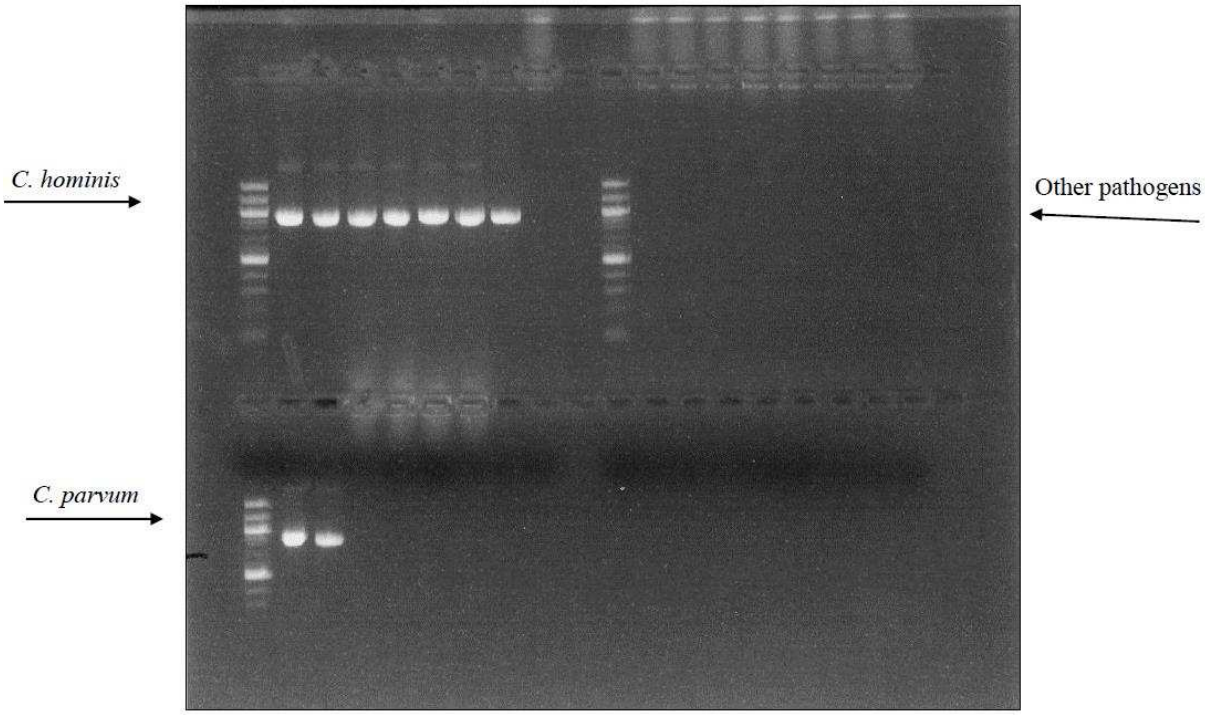


Figure 3.2 Analytic specificity of gp60 primers on 1.5% Agarose gel electrophoresis

REFERENCES

1. Chappell CL, Okhuysen PC, Langer-Curry R, et al. *Cryptosporidium hominis*: experimental challenge of healthy adults. *Am J Trop Med Hyg*. 2006:75(5):851-857.

2. Peng MM, Matos W, Gatei P, et al. A comparison of *Cryptosporidium* subgenotypes from several geographic regions. *J Eukaryot Microbiol.* 2001;(Suppl.):28S–31S. doi: 10.1111/j.1550-7408.2001.tb00442.x.

3. Alves M, Xiao L, Sulaiman IM, Lal AA, Matos O, Antunes F. Subgenotype analysis of *Cryptosporidium* isolates from humans, cattle, and zoo ruminants in Portugal. *J Clin Microbiol.* 2003;41(6):2744–2747. doi: 10.1128/JCM.41.6.2744-2747.2003.

4. Xiao L. Molecular epidemiology of cryptosporidiosis: An update. *Experimen Parasitol.* 2010;124:80–89. doi: 10.1016/j.exppara.2009.03.018.

5. Xiao L, Feng Y. Zoonotic cryptosporidiosis. *FEMS Immunol Med Microbiol.* 2008;52:309–323. doi: 10.1111/j.1574-695X.2008.00377.x.

6. Sulaiman IM, Lal AA, Xiao L. A population genetic study of the *Cryptosporidium parvum* human genotype parasites. *J Eukaryot Microbiol.* 2001;48(Suppl):S24–S27. doi: 10.1111/j.1550-7408.2001.tb00441.x.

7. Sulaiman IM, Hira PR, Zhou L, et al. Unique endemicity of cryptosporidiosis in children in Kuwait. *Clin Microbiol.* 2005;43:2805–2809. doi: 10.1128/JCM.43.6.2805-2809.2005.

8. Budu-Amoako E, Greenwood SJ, Dixon BR, et al. Molecular epidemiology of *Cryptosporidium* and *Giardia* in humans on Prince Edward Island, Canada: Evidence of zoonotic transmission from cattle. *Zoonoses Public Health.* 2012;59(6):424–433. doi: 10.1111/j.1863-2378.2012.01474.x.

9. Chalmers RM, Ferguson C, Cacciò S, et al. Direct comparison of selected methods for genetic categorization of *Cryptosporidium parvum* and *Cryptosporidium hominis* species. *Int J Parasitol.* 2005;35:397–410. doi:10.1016/j.ijpara.2005.01.001.

10. Chalmers RM, Hadfield SJ, Jackson CJ, Elwin K, Xiao L, Hunter P. Geographic linkage and variation in *Cryptosporidium hominis*. *Emerg Infect Dis.* 2008;14(3):496-498. doi: 10.3201/eid1403.071320.

11. Da Silva AJ, Bornay-Llinares FJ, Moura NS, Slemenda SB, Tuttle JL, Pieniazek NJ. Fast and reliable extraction of protozoan parasite DNA from fecal specimens. *Mol Diag.*1999;4(1):57-64. doi: 10.1016/S1084-8592(99)80050-2.

12. Bustin SA, Benes V, Garson JA, et al. The MIQE guidelines: minimum information for publication of quantitative real-time PCR experiments. *Clin Chem*. 2009;55:611-622. doi: 10.1373/dinchem.2008.112797.

13. Morgan UM, Monis PT, Xiao L, et al. Molecular and phylogenetic characterisation of *Cryptosporidium* from birds. *Int J Parasitol.* 2001;31(3):289-296. http://dx.doi.org/10.1016/S0020-7519(00)00164-8.

14. Rochelle PA, De Leon R, Stewart MH, Wolfe RL. Comparison of primers and optimization of PCR conditions for detection of *Cryptosporidium parvum* and *Giardia lamblia* in water. *Appl Environ Microbiol.* 1997;63:106–114.

15. O'Handley RM, Olson ME, Fraser D, Adams P, Thompson RCA. Prevalence and genotypic characterisation of *Giardia* in dairy calves from Western Australia and Western Canada. *Vet Parasitol.* 2000. 90;193-200. http://dx.doi.org/10.1016/S0304-4017(00)00235-1

16. Scorza AV. Diagnosis And Treatment Of Cryptosporidiosis And Giardiasis In Cats And Dogs In The United States [doctoral dissertation]. United States: Colorado, Colorado State University;2007.

17. Scorza AV, Brewer MM, Lappin MR. Polymerase chain reaction for the detection of *Cryptosporidium* spp. in cat feces. *J Parasitol.* 2003;89:423-426. doi: http://dx.doi.org/10.1645/0022-3395(2003)089[0423:PCRFTD]2.0.CO;2

18. Smith S, Morin PH. Optimal storage conditions for highly dilute DNA samples: A role for Trehalose as a preserving agent. *J Forensic Sci.* 2005;50(5):1-8.

19. Yang R, Murphy C, Song Y, et al. Specific and quantitative detection and identification of *Cryptosporidium hominis* and *C. parvum* in clinical and environmental samples. *Exp Parasitol.* 2013;135:142-147. http://dx.doi.org/10.1016/j.exppara.2013.06.014.

20. Hadfield SJ, Chalmers RM. Detection and characterization of *Cryptosporidium cuniculus* by real-time PCR. *Parasitol Res.* 2012;111:1385–1390. doi 10.1007/s00436-012-2874-1.

CHAPTER 4: RISK ASSESSMENT OF FACTORS AND PREVALENCE OF *GIARDIA DUODENALIS* AND *CRYPTOSPORIDIUM* SPP. INFECTIONS IN VETERINARY STUDENTS AND THEIR DOGS AND CATS

Summary

Giardia duodenalis and *Cryptosporidium* spp. are considered zoonotic agents, however, few data are available assessing the genotypes of these organisms in humans and their companion animals. The primary objectives of this study were to determine potential risk factors associated with *G. duodenalis* and *Cryptosporidium* spp. infections in senior veterinary students and their dogs and cats. The secondary objective was to identify and to molecularly characterize *G. duodenalis* and *Cryptosporidium* spp. isolates obtained from feces of senior veterinary students, as well from their dogs and cats.

All senior veterinary students (N=137) were contacted by a hard copy letter as well via email and were invited to participate in an anonymous, voluntary study. The students were requested to complete a survey designed to obtain information on potential risk factors associated with exposure or infection due to *G. duodenalis* and *Cryptosporidium* spp.. Additionally, students were requested to supply a personal fecal sample as well as a fecal sample from one dog and one cat if present in the household. Respondents' demographics, their pet demographics, and their clinical rotations were descriptively analyzed.

Feces were analyzed using microscopic examination for parasite eggs, cysts and oocysts after using Sheather's sugar centrifugation. For detection of *Cryptosporidium* spp. oocysts and *Giardia* spp. cysts a commercially available IFA (MERIFLUOR® *Cryptosporidium/Giardia*, Meridian Biosciences) was used. DNA was extracted from each fecal sample and was assayed for *G. duodenalis* using PCR assays for the glutamate dehydrogenase, triosephosphate isomerase,

and ß-giardin genes. DNA extracted from each human fecal sample was assayed for *Cryptosporidium* spp. using PCR assays for the 18SrRNA and gp60 genes whereas dog and cat DNA extracts were evaluated by PCR assays for the 18SrRNA and heat shock protein 70 genes.

Fifty-one (n=51) students participated in the online survey, corresponding to a response rate of 37.2%. The majority of respondents (72.6%) completed the online survey and supplied human and pet fecal samples. Potential factors evaluated for the risk of *G. duodenalis* and *Cryptosporidium* spp. in senior veterinary students included: veterinary track preference, gender, pet ownership, and farm exposure. Veterinary students supplied 42 personal fecal samples; 31 dog fecal samples, and 17 cat fecal samples. *Giardia duodenalis*, *Cryptosporidium* spp. and hookworm were the only parasites detected in the human, dog, and cat samples tested.

Out of 42 student fecal samples, five students were positive to either *Giardia* or *Cryptosporidium*. DNA sequencing was conducted in five dogs, one cat and one human fecal sample. Two dogs were positive for *G. duodenalis* Assemblage D using tpi and gdh genes, one dog was positive for *G. duodenalis* Assemblage C using β-giardin and gdh genes. One dog that was positive for *G. duodenalis* was also positive for *Cryptosporidium* spp. using the 18SrRNA gene. The two other dogs were positive for *Cryptosporidium* spp. and *C. felis* using the hsp70 gene. One cat DNA sequencing result showed that this cat was infected with *Cryptosporidium* spp.. The one human positive for *Cryptosporidium* DNA was infected with *C. parvum* subtype family (allele) IIa.

The stratification of risk factors including track preference, gender, pet ownership and farm exposure by laboratory results using IFA and PCR assays showed that none of these factors was statistically associated with the risk of infection for either pathogen.

The results of this study show that both protozoans are isolated from humans, dogs, and cats in the region. The *Giardia* positive dogs that were successfully sequenced harbored a host-adapted assemblage and the owners were negative which suggests that zoonotic transmission did not occur in these households. The strength of this study involved providing valuable information about senior veterinary students via the survey and identify and characterize isolates using highly sensitive assay. The main limitation of this study is the low participation and a single fecal sample collected. For future evaluation of the risk for zoonotic transmission of *G. duodenalis* and *Cryptosporidium* spp., a larger sample size is required.

4.1 Introduction

Based on several studies conducted in developed countries such as the United States of America (USA), the United Kingdom (UK) and Australia, the main risk factors associated with human cryptosporidiosis are direct contact with *Cryptosporidium* spp. infected individuals or those with diarrhea (< 2 -11 years old), international travel and contact with cattle/calf as well as swallowing freshwater that is not filtered.[1,2] However, contact with companion animals such as pets was not associated with the risk of infection or disease.[1,2] Other risk factors can also include caregiving to children or toddlers who are < 5 years old and consuming contaminated raw fruits and vegetables.[3, 4] According to human cryptosporidiosis surveillance conducted in the USA for the period of 2011-2012, the risk of infection increased in females rather than males aged more than 15 years old. In addition, the peak onset of symptoms increased in late summer months about 4.4-fold compared to other seasons.[5]

Regarding human giardiasis, the same risk factors associated with cryptosporidiosis seem to apply for giardiasis. Based on several case-control studies in industrialized countries, human giardiasis can be associated with consumption of contaminated surface water, swallowing

recreational water, foreign travel, contact with farm animals, contact with pets, presence of children or toddler in the house and nursing mothers.[6-10] Additionally, cases of human giardiasis were more frequently reported in children aged 1-4 years and adults aged 45-49 years old and symptoms increased in early summer through fall.[5]

Serologic studies report that 25% or more of the U.S. population has been exposed to *Cryptosporidium* spp.[11] and giardiasis is the most frequently reported intestinal parasitic infection in the USA where it is estimated that 1.2 million cases occurs annually.[12]

A very low number (n=10) of *Giardia* cysts or *Cryptosporidium* oocysts is enough to cause infection in people.[11,12] Infected cats and dogs can shed up to 10^3 *Cryptosporidium* oocysts/gram of feces and up to 10^5 *Giardia* cysts/gram of feces.[13,14] Infected humans can shed up to 10^8–10^9 *Giardia* cysts or *Cryptosporidium* oocysts in their stool per day and both pets and humans can excrete cysts or oocysts for months.[11,12] Prevalence estimates can be over 20% for both organisms in animals presented with diarrhea.[15]

Epidemiological investigations support the theory that zoonotic infections due to *G. duodenalis* and *Cryptosporidium* spp. amongst humans, dogs and cats exists.[16-21] Dogs and cats harbor host-adapted strains of *Giardia* (assemblages C and D for dogs and F for cats) and *Cryptosporidium* (*C. canis* and *C. felis*) and also zoonotic strains (*Giardia* assemblages A and B and *C. parvum*).[12-22] Most *Cryptosporidium* infections in humans are caused by *C. hominis* and *C. parvum* and less frequently by *C. canis* and *C. felis*.[22]

While human *Giardia* genotypes (assemblage A and B) are occasionally detected within feces of dogs, and *C. felis* and *C. canis* are occasionally detected within feces of people, there have been almost no studies that directly compare the results of sensitive assays (immune-

fluorescent assay (IFA) and polymerase chain reaction assay (PCR) using samples from people and their personal dogs or cats.[23-24]

Commercial diagnostic laboratories in some countries (including the USA) currently offer PCR assays to amplify the DNA of *Giardia* spp. and *Cryptosporidium* spp. from feces of dogs and cats. However, genotyping assays are not routinely provided and so whether dogs or cats are carrying zoonotic or host-adapted genotypes is unknown. An inadvertent sequela from increased recognition of *G. duodenalis* and *Cryptosporidium* spp. infections in dogs and cats is an increase in pet relinquishment due to fear of zoonotic transmission to human family members. A study conducted by the National Council on Pet Population and Study Policy showed pet illness is one of the top ten reasons why pets are relinquished, which has negative impact on companion animal welfare.[25] There is a critical need to study and evaluate the role of pets in transmitting *Giardia* and *Cryptosporidium* related infections to their owners and vice versa.

To date, there has been no research directly comparing *Giardia* spp. and *Cryptosporidium* spp. isolates from owners and their pets using highly sensitive assays capable of genotyping. Additionally, fewer studies have evaluated factors associated with infections in humans and their pets. We selected senior veterinary students and their dogs and cats as our study source populations since veterinary students are usually pet owners and occupationally exposed to small and large animals. Thus, the objectives of this study were to determine factors associated with *G. duodenalis* and or *Cryptosporidium* spp. infections in senior veterinary students and to identify and characterize *G. duodenalis* and or *Cryptosporidium* spp. isolates of human, dog and cat origin.

4.2 Materials and Methods

4.2.1 Recruitment

All senior veterinary students (N=137) were provided information concerning this anonymous, elective study by hard copy and email. This contact was established after an approval from the Institutional Review Board (IRB) on June 26, 2014. There were two phases of recruitment. The first phase of student recruitment took place in the first six months of the curriculum (between May – October). In this phase, students were asked to complete an online survey (Appendix 1). Students were also asked to supply a personal fecal sample as well as a fecal sample from one dog and one cat if present in the household.

The IRB approved a compensation of $75.00 for students that submit a personal fecal sample, a pet fecal sample and complete the survey, and a stipend of $50.00 for those who take the survey and submit a personal fecal sample without pet samples.

The second phase of the recruitment took place in the last six months of the curriculum (November – May). In this phase, due to the low participation, students were requested to submit pet samples without the requirement to submit human samples. They were also requested to take the survey only without submitting any fecal samples. During this phase, a renewal to the IRB protocol was needed and an amendment to the protocol was needed. The amendment involved an increase to the student compensation in order to enhance students' participation in the study. The compensation involved a $100.00 for students who take the survey and submit their own fecal samples. In addition, those who completed the survey and submit their pet samples, received $50.00. Lastly, those who completed the online survey without submitting fecal samples received $25.00. From the beginning of the study until the end, five to six reminders for participation in the study were sent via email to the students. The protocol amendment and IRB

approval of this protocol was received on April 17, 2015. The study actively started on July 2014 and ended in May, 2015. The two official IRB letters of approval are provided in Appendices 2 and 3.

4.2.2 Questionnaire Development and Delivery

Structured questionnaire was developed using SurveyMonkey® Inc. software. The survey entitled "Evaluation of Zoonotic *G. duodenalis* and *Cryptosporidium* spp. Infection amongst Veterinary Students and their Dogs and Cats". The objective of the survey was to collect information regarding factors that can potentially be risk factors for acquiring *G. duodenalis* and *Cryptosporidium* spp. infections in human, dog and cat populations. A total of 59 questions were developed and the survey was pilot tested at the veterinary teaching hospital. The survey was divided into seven main sections. The first section involved veterinary students' demographics. The second section involved an assessment of student health of seven required questions. Third section consisted of one required pet ownership question. Fourth section involved seven questions of dog demographics, health assessment and husbandry practices. The fifth section involved seven questions cat demographics, health assessment and husbandry practices. The sixth section involved 11 questions of dog and cat demographics, health assessment and husbandry practices. The last section involved 14 questions on students' clinical rotations. Closed-ended questions with multiple choice type of questions were provided in the survey. Response choices were limited to specified response categories, a (yes or no) question, or time frequency response categories that include: always, most of the time, sometimes, rarely, and never.

Fecal Sample Collection

Students were asked to collect the study fecal sample kits that have their unique sample identifier that they should also use in the survey. This approach was used to in linking between samples and surveys. Students were provided with sample kits. Each kit included: 1) labeled stool container for human sample, 2) labeled stool container for dog sample, 3) labeled stool container for cat sample, 4) unlabeled brown bag, 5) pair of latex gloves, and 6) three tongue depressors. Pet fecal sample containers were labeled with the same sample identifier as the human fecal sample container identifier. Fecal samples submission was anonymous i.e., students were directed to submit their samples to the laboratory refrigerator.

In the recruitment, students were requested to collect their samples in the second week of a clinical rotation that involves intensive handling of small or large animals. Fecal character (i.e. texture and consistency) for dog and cat samples was reported subjectively by the researcher using the Nestle Purina Fecal Scoring System for dogs and cats (Nestle-Purina Pet Food Co, St Louis, MO). Fecal scores of 1-3 were considered as normal, whereas scores with 4-7 were classified as diarrheic. All fecal samples were stored in the laboratory refrigerator at 4˚C until processed.

4.2.3 Diagnostic Tests and Procedures

The diagnostic test for *G. duodenalis* and *Cryptosporidium* spp. included microscopic examination of the cysts and oocysts and molecular techniques to identify both pathogens genomic DNA. *G. duodenalis* case definition was based on the CDC definition in 2011 of cases which involved:

Laboratory-confirmed giardiasis should meet the criteria as the detection of *Giardia* organisms/cyst using IFA, or amplification of DNA from stool by PCR (molecular characterization (e.g., assemblage designation) should be reported.[5] *Cryptosporidium* spp. case definition was based on the CDC definition in 2012 of cases which involved:

A confirmed case of cryptosporidiosis is defined as having evidence of *Cryptosporidium* oocysts by IFA or amplification of DNA from stool by PCR tests.[5]

4.2.3.1 Fecal Flotation

All fecal samples were subjected to fecal flotation procedure as previously described.[30] The protocol involved weighing 2-3 grams of feces. A volume of 3-5 ml of distilled water (depending on the stool consistency), were used to wash the fecal matter instead of PBS-EDTA which was a little adjustment of the previous protocol. The distilled water was then filtered through gauze. The filtrate was placed in a clean 15 ml tube and was centrifuged at 500 X g for 3 minutes. The filtrate then was mixed with Sheather's sugar flotation solution (Jorgensen Labs. Loveland, CO) with specific gravity of 1.27. The tube was centrifuged at 1,500 X g for 10 minutes. A coverslip was placed on top of the solution to give a positive meniscus. After 10 minutes, the coverslip was transferred on a microscope slide to be later examined under the light microscope for *Giardia* cysts and other potential parasites. The slide was initially screened at magnification power with 10x, then if potential parasites were noted, the examination was continued at 20x or 40x to confirm the findings.

4.2.3.2 Concentration Technique and Immuno-Fluorescent Assay (IFA)

All fecal samples were subjected to fecal concentration technique as described in previous study.[26,27] This procedure enhances the recovery of *Giardia* spp. cysts and *Cryptosporidium* spp. oocysts.[28]

4.2.3.3 PCR DNA Extraction

All fecal specimens were subjected to DNA extraction. The genomic DNA of *G. duodenalis* and *Cryptosporidium* spp. in the concentrated fecal samples was extracted using FastDNA® Kit (MP Biomedicals, LLC. Solon, OH) following published protocols.[28,29,30] The extracted DNA was stored at 4°C until used for molecular analysis.

4.2.3.4 Molecular Analysis

***G. duodenalis* PCR**

The isolates of *G. duodenalis* were genetically characterized using gdh[26,31], β-giardin[32] and tpi[26,33,34] loci.

***Cryptosporidium* spp. PCR**

The isolates of *Cryptosporidium* spp. were genetically characterized using 18SrRNA[35,36], and gp60[37] loci for human isolates and 18SrRNA[35,36] and hsp70 for animal isolates[38].

4.2.3.5 Stratification of Laboratory Results by Risk Factors

Fisher's exact test was used to assess associations between positive and negative laboratory results for IFA and PCR assays of human fecal samples by the study selected risk factors. The factors involved in this stratification included: student track preference, gender, pet ownership, and farm exposure. This analysis was conducted merely for descriptive purposes.

4.2.3.6 DNA Sequencing

All PCR-positive samples were subjected to sequencing as previously published.[26] All PCR products (10.0 µl) were separated by electrophoresis in 1.5% agarose gels stained with 2.0 µl EZ-Vision® One DNA dye (Biochemicals and Life Science Research Products, Solon, OH) for 30-35 minutes. After the positive band has been identified using the BIO-RAD (Gel DocTM EZ Imager), the remaining PCR reaction (40.0 µl) was then re-separated by the electrophoresis

in 1.5% agarose gel stained with 5.0 µl EZ-Vision®. The gel was visualized by the Spectroline® UV Transilluminator (Slimline™ Series) and the positive band DNA was sliced. The gel was extracted using QIAquick Gel Extraction kit (QIAgen, cat. No. 28704, Germany) per the manufacturer's protocol. PCR products were sequenced in both forward and reverse directions. The DNA sequence data from *G. duodenalis* and *Cryptosporidium* spp. isolates was compared by BLAST analysis with sequences from the nucleotide database from the GenBank.

4.3 Data Analysis

STATA® 13.0 (StataCorp. 2013. Stata Statistical Software: Release 13. College Station, TX: StataCorp LP.) was used for data formatting and descriptive analysis. The survey data analysis included descriptive analysis of the characteristics related to students and pet demographics and student clinical rotations as previously described and did not contain any open-ended questions. Statistical significance was declared at $p < 0.05$.

4.4 Results

4.4.1 Results of the Survey

Survey Participation

Survey responses were received from 51 (37.2%) of senior veterinary students (Table 4.1).

Section I – Veterinary Student Demographics

The majority of respondents (n = 37, 72.6%) took the online survey and also submitted human and pet samples. Most samples (n = 12, 23.5%) were submitted in May, 2015 (Table 4.1).

The majority of respondents (n = 44), (86.3%; 95% CI: 73, 94%) were females. The mean age of all respondents was 28 years old with 95% corresponding confidence interval between (27, 28.9%). The majority of respondents were in the small animal track (n = 23),

(45.1%; 95% CI: 31, 59%). In terms of the type of residence for the students, the majority (n = 44), (86.3%; 95% CI: 73, 94%) of students lived in single family residence where no cattle were housed. Forty-five percent (n = 23), (45.1%; 95% CI: 31, 59%) of respondents have lived with one adult other than the respondent. The majority of students (n = 48), (94.12%; 95% CI: 83, 98%) had no children under five years old in the household, and only three students (5.9%) had children under five years old in their household. The majority of respondents (n = 32, 62.8%; 95% CI: 48, 75.0%) had worked in an operation that was their own or owned by others where cattle of any age were housed or fed in the last 10 years and the majority of respondents (n = 21, 67.7%; 95% CI: 27, 55%) have worked on these farms within last year. In terms of activities performed on these farms, the majority of respondents were involved in adult cattle and calf treatments (n = 25 (86.2%; 95% CI: 68, 96%), n=21 (72.4%; 95% CI: 52, 87%), respectively (Table 4.1).

Section II – Veterinary Students Health Assessment

Forty-nine (n = 49, 96.1%; 95% CI: 86, 99%) respondents had no diabetes, cancer, HIV, or leukemia. Of the 44 respondents who were females, only three were pregnant (6.8%). The majority of respondents (n = 49, 96.1%; 95% CI: 86, 99%) had not taken medications that suppress their immune system. The majority (n = 46, 90.2%; 95% CI: 78, 96%) of students responded not-applicable (N/A) to the question asking about use of filter when drinking freshwater from water surface that is not chlorinated (Table 4.2). Water sources and number of times water swallowed accidentally or intentionally by respondents were displayed in Tables 4.3-4.4. The majority of respondents reported that they have not swallowed water from the mentioned water sources neither accidentally or intentionally. In terms of the frequency of

diarrhea, the majority of students had never had diarrhea in the last 3, 6 or 12 months (71.4%, 75.5%, or 60.8%), respectively (Table 4.5).

Section III – Pet Ownership

In terms of pet ownership, 20 students (39.2%; 95% CI: 25, 53%) of the students had dogs only and same percentage of students had both dogs and cats. Whereas 13.7% of respondents (n = 7) had cats only and 7.8% (n=4) had neither dogs nor cats (Table 4.6).

Section IV – Dog Demographics, Health Assessment, Husbandry

For those students who had only dogs (n=20; 95% CI: 19, 63%), eight students had at least one dog that their age range was from 1-5 years old. The majority (n = 13, 65.0%; 95% CI: 40, 84%) of students adopted their dogs from the shelter or rescue group. Regarding medication use such as dewormers and heartworm preventatives, the majority of respondents (n = 13, 65.0%; 95% CI: 40, 84%) had used Heartgard® for their dogs. Fifty percent (95% CI: 27, 72%) of respondents (n = 10) indicated that their dogs did not have abnormal health events. Furthermore, 80.0% of respondents (n = 16; 95% CI: 56, 94%) indicated that their dogs have never been used for herding or hunting. Additionally, 35.0% of respondents (n = 7; 95% CI: 15, 59%) had never taken their dogs to a dog park. However, 30.0% of respondents (n = 6) had taken their dogs to a dog park at least less than once a week. For the frequency of diarrhea in the last three months, 80.0% of students (n=16; 95% CI: 56, 94%) reported that their dogs have never had diarrhea, but 15.0% of students (n = 3) reported that their dogs have had diarrhea once in the last three months (Table 4.7).

Section V – Cat Demographics, Health Assessment, Husbandry

For those responders who had only cats (n = 7; 95% CI: 9, 81%), three students had at least two cats that their age range was from 6-12 years old. Five responders (71.4%; 95% CI: 29, 96%) reported that their cats were adopted from the shelter or rescue group. Regarding medication use such as dewormers and heartworm preventatives, the majority of respondents (n = 6) (85.7%; 95% CI: 42, 99%) had not used any cat dewormers or heart worm preventatives. Four respondents (57.1%) indicated that their cats did not have health events. Furthermore, three responders (42.9% 95% CI: 9, 81%) indicated that they clean the litterbox daily. Additionally, six respondents (85.7%; 95% CI: 42, 99%) have always used to wash their hands after handling the litterbox. For the frequency of diarrhea in the last three months, six students (85.7%; 95% CI: 42, 99%) reported that their cats have never had diarrhea, but one student (14.3%) reported that their cat have had diarrhea once per three months (Table 4.8).

Section VI – Dog and Cat Demographics, Health Assessment, Husbandry

For those 20 students that had both dogs and cats, nine students (60.0%; 95% CI: 23, 68%) had at least one dog with an age ranging from 1-5 years old and 10 students (50.0%; 95% CI: 27, 72%) had at least one cat aged from 1-5 years old. All owners (100.0%; 95% CI: 83, 100%) adopted their dogs and cats from the shelter or rescue group. Regarding medication use such as dewormers and heartworm preventatives, the majority of responders (n = 11, 55.0%; 95% CI: 31, 76%) had used Heartgard® for their dogs and (50.0%) of responders (n=10; 95% CI: 31, 76%) have not used any cat dewormers or heartworm preventatives. However, seven respondents (35.0%) have used Advantage Multi for their cats. Seven responders (35.0%; CI: 15, 59%) indicated that their dogs or cats did not have abnormal health events. However, seven respondents (35.0%; 95% CI: 15, 59%) also indicated that their dogs or cats have had acute

health conditions. Furthermore, 18 responders (90.0%; 95% CI: 68, 98%) indicated that they have never used their dogs for herding or hunting. Also, 10 responders (35.0%; 95% CI: 31, 76%) have taken their dogs to the dog park at least once per week. Additionally, six responders (30.0%) have cleaned the litterbox daily and 11 responders (55.0%; 95% CI: 31, 76%) have always used to wash their hands after handling the litterbox. For the frequency of diarrhea in the last three months, 15 students (75.0%; 95% CI: 50, 91%) reported that their dogs or cats have never had diarrhea, but three students (15.0%) reported that their dogs have had diarrhea at least once per three months (Table 4.9).

Section VII – Clinical Rotations

A list of procedures performed for adult cattle or calves on senior practicum whether core or elective rotations is displayed in Table 4.10. Those procedures involve intensive exposure to large animals such as cattle and calves' treatments. The majority of respondents responded yes to some of these procedures. Table 4.11 displays the frequency of handwashing procedure after handling cattle or calves. As shown in the table, the majority of students (n=31, 60.8%; 95% CI: 46, 74%) have always washed their hands after performing activities listed in Table 4.10. Also, forty-nine percent of students (n = 25; 95% CI: 34, 63%) reported that they sometimes would eat or drink within an hour after performing any of the activities listed in Table 4.10. In terms of washing coveralls or rubber boots, 22 students (43.1%; 95% CI: 29, 57%) indicated that they wash their coveralls or boots daily, whereas (41.2%) of students (n = 21; 95% CI: 27, 55%) answered not-applicable (Table 4.11). The frequency of use of personal protective equipment (PPE) when handling diarrheic and non-diarrheic cattle or claves is displayed in Table 4.12. The majority of students were used the PPE whether cattle or calves were diarrheic or non-diarrheic. Additionally, a list of procedures performed for dogs and cats on senior practicum whether core

or elective rotations is displayed in Table 4.13. Those procedures involved intensive exposure to dogs and cats such as physical exams and diagnostic tests. The majority of students responded yes to performing these activities.

As shown in Table 4.14, 26 students (51.0%; 95% CI: 36, 65%) washed their hands most of the time after performing any activities listed in the previous table (Table 4.13). Additionally, 55% (95% CI: 40, 68%) of students (n = 28) indicated that they have washed their hands between patients most of the time. A total of 55% (95% CI: 40.0, 68.0%) of students (n = 28) reported that they would eat and drink sometimes within an hour after handling dogs and cats. Furthermore, 21 students (41.2%; 95% CI: 27.0, 55.0%) washed their clinic smocks or cloth surgical gowns at least once per week (Table 4.14). The frequency of use of personal protective equipment when handling diarrheic and non-diarrheic dogs or cats is displayed in Table 4.15. In addition, the frequency of rotations that have completed from the beginning of the senior practicum until the time of survey was listed in Table 4.16.

4.4.2 Fecal Samples Diagnostic Tests Results

A total of 42 human, 31 dog, and 17 cat fecal samples were submitted to the Center of Companion Animal Studies at the Department of Clinical Sciences at Colorado State University laboratories by the senior veterinary students.

G. duodenalis Diagnostic Tests

Results from fecal flotation, IFA, and molecular analysis are shown in Table 4.17. All human fecal samples tested negative to the fecal flotation test, one dog fecal sample tested positive to *G. duodenalis* (3.23%; 95% CI: 0.1,16.7%) and one hookworm egg was identified in one *Giardia* negative dog. None of the cat samples tested positive in the fecal flotation test. In the IFA, *G. duodenalis* cysts were identified in one out of 42 human fecal samples (2.38%; 95%

CI: 0.1, 12.6%), and three fecal samples from dogs (9.68%; 95% CI: 2.0, 25.8%). None of the cat samples tested positive to *Giardia* using the IFA test. Regarding the PCR results in humans (see also Table 4.18), three out of 42 samples (7.14%; 95% CI: 1.5, 19.5%) tested positive for *G. duodenalis*, all the positive human isolates were typed by the tpi gene (7.14%). Three out of 31 dog samples (9.68%; 95% CI: 2.0, 25.8%) tested positive to *G. duodenalis*, two of the isolates were typed by the gdh gene (6.45%), three were typed by the tpi gene (9.67%) and one isolate were typed by the β-giardin gene (3.22%). Also, one out of 17 samples (5.88%; 95% CI: 0.1, 28.7) tested positive *G. duodenalis* in cats. This one isolate was typed by the β-giardin gene.

Cryptosporidium spp. Diagnostic Tests

Results of the fecal flotation, IFA, and molecular analysis are shown in Table 4.19. Regarding the fecal flotation test, none of human, dog or cat samples tested positive to *Cryptosporidium* spp.. In the IFA, *Cryptosporidium* spp. oocysts were identified in one of 42 human samples (2.38%; 95% CI: 0.1, 12.6%) and one of 31 dogs (3.23%; 95% CI: 0.1, 16.7%) tested positive. None of the cat samples tested positive to *Cryptosporidium* spp. using the IFA test. Regarding the PCR results in humans (see also Table 4.20), one out of 42 samples (2.38%; 95% CI: 0.1, 12.6%) tested positive for *Cryptosporidium* spp.. This human positive isolate was typed by gp60 gene (2.38%). Four out of 31 dog samples (12.9%; 95% CI: 3.6, 29.8%) tested positive to *Cryptosporidium* spp.. The four isolates were typed by hsp70 gene (12.9%) and two of these isolates were also typed by 18SrRNA gene (6.45%). In cats, one out of 17 samples (5.88%; 95% CI: 0.1, 28.7%) tested positive to *Cryptosporidium* spp. that were typed by hsp70 gene (5.88%).

Stratification of Laboratory Results by Risk Factors

Tables 4.21-4.23 describe the probability of testing positive to *G. duodenalis* or *Cryptosporidium* spp. in senior veterinary students by track preference, gender, pet ownership and farm exposure. As shown in the Table 4.21, one student of 20 students (5.0%; 95% CI: 0.12, 25%) in the small animal track was positive to *G. duodenalis* and *Cryptosporidium* spp. using the IFA test. This student was female (2.8%; 95% CI: 0.1, 14.5%) who owned pets (2.6%; 95% CI: 0.1, 13.5%) and had worked on a farm that had cattle (3.8%; 95% CI: 0.1, 19.6%). Table 4.22 shows *G. duodenalis* PCR test results of veterinary students stratified by track preference, gender, pet ownership and farm exposure. Two of 20 students (10.0%; 95% CI: 1.2, 32%) positive to *G. duodenalis* were in the small animal track and one student of 14 (7.1%; 95% CI: 0.2, 33.9%) was in the general track. Each of these students were females (8.3%; 95% CI: 1.7, 22.5%), all owned pets (7.7%; 1.6, 20.9%), and all had farm exposure (11.5%; 95% CI: 2.4, 30.2%). As noted in Table 4.23, one student positive to *Cryptosporidium* spp. using the PCR test was in the large animal track (12.5%; 95% CI: 0.3, 52.7%), male (16.7%; 95% CI: 0.4, 64.1%), owned pets (2.6%; 0.1, 13.5%) and had farm exposure (3.8%; 95% CI: 0.1, 19.6%). However, none of these factors was significantly associated with using Fisher's exact test.

Results of DNA Sequencing

The DNA sequencing was completed in one human, five dogs and one cat samples. As shown in Table 4.24, dogs who were diagnosed with *G. duodenalis* were infected with *G. duodenalis* host-adapted assemblages C and D. The two dogs were identified with *Cryptosporidium* spp. and *C. felis*, respectively. The one cat isolate was found to be *Cryptosporidium* spp. and species determination failed. One human isolate was identified with *C. parvum* subfamily IIa, which is zoonotic as shown in Table 4.25.

4.5 Discussion

The response of students' participation to the survey was 37.2%. Compared to another study used mailed surveys and several follow up that achieved 19% response rate, our study response rate can be considered good.[39] Several journals in the USA and Canada, recommend survey response rates of at least 60.0% to prevent non-response bias.[40,41]

Studying the sample characteristics through the survey in combination with the examining each stool sample collected, helped in forming understanding of the risk factors that can lead to infection due to *G. duodenalis* and *Cryptosporidium* spp.. Regarding fecal sample collection, the logic of this emphasis was due to the length of the incubation period for *G. duodenalis* and *Cryptosporidium* spp.. Based on evidence from experimental infections, it has been estimated that the incubation period is between 5 and 7 days for human cryptosporidiosis [42,43,44] and from 1 to 45 days in human giardiasis. In most cases, the symptoms appear in 1-2 weeks.[45]

From the total of 51 respondents, 42 students (82.4%) provided fecal samples. From the laboratory result data of this study, five students out of 42 (11.9%) were positive to *G. duodenalis* and/or *Cryptosporidium* spp.. One student was positive to both *G. duodenalis* and *Cryptosporidium* spp. using the IFA test. Three students were positive to *G. duodenalis* and one student was positive to *Cryptosporidium* spp. using the PCR assays. Therefore, the prevalence of *G. duodenalis* was 9.5% (4/42) and prevalence of *Cryptosporidium* spp. was 4.8% (2/42) in human samples.

From the survey analysis of these positive cases, four students out of five were females (80.0%) and one student was male. From the students' demographic analysis, the majority of respondents were females (86.3%). Therefore, the number of positives were four females

compared to one male, in this study. According to the Morbidity and Mortality Weekly Report, 2011-2012 for human giardiasis and cryptosporidiosis, females are more likely to be infected with these protozoans due to their care giving to infected infants or toddlers. *Giardia duodenalis* cases are usually reported in younger populations that age range from 1-9 years old and younger adults from 35-39 years.[46] Positive females in this study, did not have children under 5 years old that have lived in the household. Also, all positive female students were not pregnant and have not been diagnosed with any health conditions that can suppress the immune system. It has been reported that the host immunity status can influence the severity of infection due to giardiasis in the host. For example, giardiasis is more frequently reported in immune-compromised individuals compared to immune-competent individuals.[47,48]

From the stratification by risk factor analysis conducted in this study, three students that were positive to *G. duodenalis*, were in the small animal track. A study conducted on Australian veterinarians where 63% of participants have worked in the small/companion animal practice. That study reported that 45% of respondents have contracted gastrointestinal conditions caused by different pathogens during their occupation as veterinarians.[49]

In this study, analysis of the laboratory results has shown that the dogs and cats owned by the five positive cases, were negative to either *Giardia* or *Cryptosporidium* except for one dog that was positive to both pathogens. The owner of this dog was *G. duodenalis* positive. Unfortunately, the DNA sequencing was not completed to this human isolate due to the low DNA concentration. Therefore, we were unable to determine which *Giardia* assemblage this isolate was. However, the DNA sequencing was completed in the dog isolate and that dog harbored host–adapted *Giardia* assemblage D. Few studies have suggested the zoonotic potential of *Giardia* transmission among dogs and cats and their owners or people live in close

proximity.[50-53] However, other studies reported that dogs were infected with *Giardia* either assemblage C or D and people were infected with assemblage A, i.e. independent transmission cycles among humans, dogs and cats.[54,55] Therefore, this finding is consistent with studies concluded that the contact with pets can be negatively associated with the risk of infection.[1,56,57]

All positive students, including the two *Cryptosporidium* spp. positive ones, one identified by the PCR and one identified by the IFA test have lived in a single-family residence where no cattle were housed. Regardless, all positive students have worked on an operation where cattle were raised from one year to more than five years. Some studies [58-60] suggested that contact with farm animals, especially cattle, are capable of transmitting *Cryptosporidium* spp. infections to farm workers and veterinary students. Also, C*ryptosporidium bovis* infections have been identified in few persons that were living and or working on cattle operations.[61,62] One report found out that *Cryptosporidium* infections occurred in approximately 90% of the USA dairy farms and about 20% pre-weaned heifers of any given day are shedding *Cryptosporidium* spp.[63]

DNA sequencing was completed in one of the two *Cryptosporidium* student cases. As suggested by chapter 3 of this dissertation, this isolate was assessed by the optimized gp60 assay. The data showed that this isolate was positive to *C. parvum* subfamily IIa. *Cryptosporidium parvum* possess complex epidemiology due to its ability to infect humans through zoonotic and anthroponotic transmission.[64] The demographic data indicated that the student was a 29 years old male in the large animal track, and has worked on an operation in adult cattle treatment for three to five years. In addition, this student had recently completed the dairy field service, large animal emergency medicine, and livestock medicine and surgery rotations. All these rotations involve intensive contact with cattle. However, it is unclear whether the student positive to this isolate

acquired it due to intensive exposure to cattle or from another human. However, if it was a human exposure, the species would have been more likely to be *C. hominis*. Farm exposure or majoring in large animal track can be considered an important risk factor for the infection due to *Cryptosporidium* since calves are frequently infected with a *C. parvum* subtype that is commonly found in humans in the same geographic areas[65] and epidemiologic studies have reinforced the occurrence of zoonotic transmission.[66] As part of the evaluation process for the optimized gp60 assay, this assay was compared to the IFA assay. The gp60 PCR *Cryptosporidium* spp. positive isolate was negative to the IFA assay. On the other hand, one *Cryptosporidium* oocyst was detected in one student by the IFA assay and negative to the gp60 PCR assay. This result can be interpreted as a false negative in the gp60 PCR assay. The demographics showed that this student is a 26-year-old female, in the small animal track, and all clinical rotations involved small animal medicine. This student worked on an operation in cattle milking for three to five years. The diagnostic sensitivity and specificity for the gp60 PCR assay and IFA was 50% and 100% respectively and this result was in accordance with another study.[67]

In terms of seasonality, all our *Giardia* cases had submitted their fecal samples in July to September. This finding is consistent with the *Giardia* surveillance in the United States that stated peak onset of this pathogen is in early summer through early fall.[5] Regarding, the one *C. parvum* case, the sample was submitted in February.

The one student that had dual infection of *Giardia* and *Cryptosporidium*, submitted her samples in July. In the *Cryptosporidium* surveillance work, the peak onset of the pathogen was in late summer months.[5]

Regarding the clinical rotations, most of the positive cases whether *Giardia* or *Cryptosporidium* as well as negative cases, were more likely to wear personal protective

equipment (PPE) and exam gloves when handling small or large animals with diarrhea. This finding is in accordance with a study conducted on Australian veterinarians that were more likely to use PPE for each case.[49]

Regarding pet positive cases in this study, all positive animals had female owners as shown in Table 4.26. All owners lived in a single-family residence with no cattle in the surroundings except for one student who lived in a single-family residence where cattle of any age were housed or fed. The dog of this student was positive to both pathogens. Also, all positive dog owners, except for two, had worked on a farm. It seems that dogs owned by students that worked on a farm or lived in a farm were positive to *Giardia* and *Cryptosporidium* using at least two diagnostic methods due to the intensive exposure to either pathogens.

In all seven positive dogs to any assay, four dogs (57.1%) dogs that were positive to either *Giardia* or *Cryptosporidium* or both, have attended the dog park at least once per week. Attending dog park was considered a risk factor in a study compared between dog and non-dog park attending dogs in the USA that concluded dog park attending dogs were more likely to be positive for *Giardia* or *Cryptosporidium* than non-dog park-attending dogs.[68] However, in this study, 14/26 (53.8%) of negative dogs also attended dog parks. Similarly, we had two out of seven (28.6%) positive dogs were used for herding or hunting at least once or twice per week. In this study, 11.5% of dogs that were negative for both protozoans were also used for herding or hunting. Additionally, the age range for positive dogs was from 1-5 years old. That means the risk of acquiring *Giardia* or *Cryptosporidium* increases as age decreases which agrees with the findings reported in Chapter 2 of this dissertation. The one hookworm egg identified in dog labeled 82 using the microscopic examination was most likely to belong to *Ancylostoma*

caninum. However, the egg size may overlap with *Uncinaria* spp. and confirmation was not available due to the very low number of eggs.

Regarding the two positive cats, cat #389 that was positive to *Cryptosporidium* spp. lived in the same household with a dog that was also positive to *C. felis.* While *Cryptosporidium* spp. can also be reported in cats, it is unusual to identify *C. felis* in dogs. The only justification for this can be that since the dog and the cat live together in the household a cross transmission may have occurred and because dogs are likely to eat cat feces. Cat #300 was positive for *Giardia* DNA by PCR. However, we were unable to determine which *Giardia* assemblage that cat was infected with due to the very low DNA concentration.

4.6 Conclusion

Giardia duodenalis and *Cryptosporidium* spp. isolates were detected in humans and their pets. Dogs that were positive to *G. duodenalis* harbored host-adapted genotypes. Gp60 PCR assay had high diagnostic specificity and low diagnostic sensitivity in our studies which were comparable to other studies. Additionally, veterinary students that completed the survey were more likely to work on farms that contain cattle regardless whether their track is small or large animals. The strength of this study is providing valuable information about veterinarians via the survey and identifying and characterizing isolates using highly sensitive assay. The main limitation of the study was the low participation and single fecal sample collected. It is recommended to continue working on the veterinary students' population and collect larger sample size to evaluate zoonosis amongst veterinary students and their pets.

4.7 Tables

Table 4.1 Respondents demographic descriptors of section I in the survey

Variable	Category	Number (%)	95% CI
Participation Preference	Yes	51/137 (37.23)	
	No	86/137 (62.77)	
Response Type	Survey Only	8/51 (15.69)	
	Survey and Pet Samples	1/51 (1.96)	
	Survey and Human Samples	5/51 (9.80)	
	Survey, Human, and Pet Samples	37/51 (72.55)	(58.0, 84.0)
Time of Participation: Month	February	4/51 (7.84)	
	April	9/51 (17.65)	
	May	12/51 (23.53)	(12.0, 37.0)
	July	6/51 (11.76)	
	September	9/51 (17.65)	
	October	3/51 (5.88)	
	November	2/51 (3.92)	
Year	2014	26/51 (50.98)	(36.0, 65.0)
	2015	25/51 (49.02)	(34.0, 63.0)
Respondents' gender	Female	44/51 (86.27)	(73.0, 94.0)
	Male	7/51 (13.73)	
Respondents' age	Average age in years	28 years	(27.08, 28.87)
Track Preference	Small Animal Track	23/51 (45.10)	(31.0, 59.0)
	General Track	15/51 (29.41)	
	Large Animal Track	13/51 (25.49)	
Residence Type	SFR*/No Animals	4/51 (7.84)	
	SFR/No Cattle	44/51 (86.27)	(73.0, 94.0)
	SFR/With Cattle	3/51 (5.88)	
	Other	0/51 (0)	

*SFR = Single Family Residence

Table 4.1 Continued

Variable	Category	Number (%)	95% CI
Number of Adults[a]	0	6/51 (11.76)	
	1	23/51 (45.1)	(31.0, 59.0)
	2	15/51 (29.41)	
	3	6/51 (11.76)	
	4	1/51 (1.96)	
Number of Children[b]	0	48/51 (94.12)	(83.0, 98.0)
	1	3/51 (5.88)	
Take Children to Daycare	N/A	48/51 (94.12)	(83.0, 98.0)
	Daily	2/51 (3.92)	
	Other[c]	1/51 (1.96)	
Respondent's Working on Farm[d]	Yes	32/51 (62.75)	(48.0, 75.0)
	No	19/51 (37.25)	

[a]Adults other than the respondent
[b]Children under 5 years and live within the household
[c]5 days/week
[d]In the last 10 years

Table 4.1 Continued (optional questions)

Variable	Category	Number (%)	95% CI
Respondent's Time Spent Working on Farm*	Within last year	21/51 (67.74)	(27.0, 55.0)
	1-2 Years	2/31 (6.45)	
	3-5 Years	5/31 (16.13)	
	>5 Years	3/31 (9.68)	
Type of Work on Farm	1. Adult cattle barn cleaning	8/29 (27.59)	
	2. Adult cattle barn bedding changing	8/29 (27.59)	
	3. Adult cattle feeding	13/29 (44.83)	
	4. Adult cattle birthing assistance	10/29 (34.48)	
	5. Adult cattle fecal disposal	8/29 (27.59)	
	6. Cattle milking	15/29 (51.72)	
	7. Cattle breeding	9/29 (31.03)	
	8. Adult cattle treatments	25/29 (86.21)	(68.0, 96.0)
	9. Adult cattle vaccinations	19/29 (65.52)	
	10. Newborn calf care	14/29 (48.28)	
	11. Newborn calf feeding	13/29 (44.83)	
	12. Calf treatments	21/29 (72.41)	(52.0, 87.0)
	13. Calf vaccinations	15/29 (51.72)	
	14. Care of recumbent cattle or calves	15/29 (51.72)	
	15. Cow/calf movement and transportation	10/29 (34.48)	
	16. Other	2/29 (6.90)	

*In the last 10 years

Table 4.2 Respondents health assessment of section II in the survey

Variable	Category	Number (%)	95% CI
Diagnosed conditions	None	49/51 (96.08)	(86.0, 99.0)
	Diabetes	0/51 (0)	
	Cancer	0/51 (0)	
	HIV/AIDS	0/51 (0)	
	Leukemia	0/51 (0)	
	Other[a]	3/51 (5.88)	
Pregnancy	Yes	3/51 (5.88)	
	No	41/51 (80.39)	(66.0, 90.0)
	Unsure	0/51 (0)	
	N/A if male	7/51 (13.73)	
Medications that suppress the immune system	None	49/51 (96.08)	(86.0, 99.0)
	Oral steroids	2/51 (3.92)	
	Azathioprine	0/51 (0)	
	Mycophenolate	0/51 (0)	
	Other	0/51 (0)	
Use of water filters before drinking water[b]	Yes	3/51 (5.88)	
	No	2/51 (3.92)	
	N/A	46/51 (90.20)	(78.0, 96.0)

[a]Hypothyroidism, Systemic lupus erythematosus
[b]Freshwater that not is chlorinated

Table 4.3 Water sources and number of times water swallowed accidentally by respondents

Source / Time	None	Once/3 mo.	Twice/3 mo.	3 times/3 mo.	>3 times/3 mo.	Total Respondents
Swimming pools	39/51 (76.47)	8/51 (15.69)	0/51 (0)	2/51 (3.92)	2/51 (3.92)	51
Water play	45/52 (88.24)	5/51 (9.80)	1/51 (1.96)	0/51 (0)	0/51 (0)	51
Hot tubs	40/51 (80.00)	9/51 (18.00)	0/50 (0)	0/50 (0)	1/50 (2.00)	50
Lakes	41/51 (80.39)	7/51 (13.73)	0/51 (0)	2/51 (3.92)	1/51 (1.96)	51
Rivers	44/51 (86.27)	5/51 (9.80)	0/51 (0)	1/51 (1.96)	1/51 (1.96)	51
Springs	48/51 (94.12)	0/51 (0)	1/51 (1.96)	0/51 (0)	2/51 (3.92)	51
Hot springs	45/51 (88.24)	5/51 (9.80)	0/51 (0)	0/51 (0)	1/51 (1.96)	51
Ponds	48/51 (94.12)	1/51 (1.96)	0/51 (0)	1/51 (1.96)	1/51 (1.96)	51
Streams	48/51 (94.12)	0/51 (0)	0/51 (0)	1/51 (1.96)	2/51 (3.92)	51
Other	27/28 (96.43)	0/28 (0)	0/28 (0)	1/28 (3.57)	0/28 (0)	28

Table 4.4 Water sources and number of times water swallowed intentionally by respondents

Source / Time	None	Once/3 mo.	Twice/3 mo.	3 times/3 mo.	>3 times/3 mo.	Total Respondents
Lakes	50/51 (98.04)	1/51 (1.96)	0/51 (0)	0/51 (0)	0/51 (0)	51
Rivers	50/51 (98.04)	1/51 (1.96)	0/51 (0)	0/51 (0)	0/51 (0)	51
Springs	48/51 (94.12)	2/51 (3.92)	0/51 (0)	0/51 (0)	1/51 (1.96)	51
Ponds	50/51 (98.04)	1/51 (1.96)	0/51 (0)	0/51 (0)	0/51 (0)	51
Streams	48/50 (96.00)	1/50 (2.00)	0/50 (0)	0/50 (0)	1/50 (2.00)	50
Other	32/33 (96.97)	1/33 (3.03)	0/33 (0)	0/33 (0)	0/33 (0)	33

Table 4.5 Frequency of watery non-bloody diarrhea self-reported by respondents

	Never	Once	Twice	3-5 times	>5 times	Total Respondents
In the last 12 months	31/51 (60.78)	8/51 (15.69)	5/51 (9.80)	4/51 (7.84)	3/51 (5.88)	51
In the last 6 months	37/49 (75.51)	7/49 (14.29)	1/49 (2.04)	3/49 (6.12)	1/49 (2.04)	49
In the last 3 months	35/49 (71.43)	10/49 (20.41)	2/49 (4.08)	1/49 (2.04)	1/49 (2.04)	49

Table 4.6 Owning dogs and cats – section III

Variable	**Category**	**Number (%)**	**95% CI**
Pet ownership	Dogs only	20/51 (39.22)	(25.0, 53.0)
	Cats only	7/51 (13.73)	
	Dogs and cats	20/51 (39.22)	(25.0, 53.0)
	Neither dogs nor cats	4/51 (7.84)	

Table 4.7 Dog demographics, health assessment, and husbandry – section IV

Variable	Category	Number of dogs per age group (0-20+)	Number of respondents (%)	95% CI
Dog age	< 1 mo.	0	0/20 (0)	
	1-6 mo.	0	0/20 (0)	
	< 1 yr.	1	2/20 (10.00)	
	1-5 yrs.	1	8/20 (40.00)	(19.0, 63.0)
		2	4/20 (20.00)	
		3	1/20 (4.00)	
		4	1/20 (5.00)	
	6-10 yrs.	1	4/20 (20.00)	
		2	3/20 (15.00)	
		3	1/20 (5.00)	
	>10 yrs.	1	1/20 (5.00)	
Dog source	Pet shop		0/20 (0)	
	Former research animal		0/20 (0)	
	Breeders		5/20 (25.00)	
	Shelter or rescue group		13/20 (65.00)	(40.0, 84.0)
	Friend		3/20 (15.00)	
	Other*		3/20 (15.00)	

*Guide dogs for the blind, south American street dog, released/retired from guide dog school

Table 4.7 Continued

Variable	Category	Number of respondents (%)	95% CI
Dog dewormers and heart worm preventatives	None	1/20 (5.00)	
	Advantage Multi®	3/20 (15.00)	
	Sentinel®	1/20 (5.00)	
	Heartgard®	13/20 (65.00)	(40.0, 84.0)
	Other[a]		
Dog health events	No health events	10/20 (50.00)	(27.0, 72.0)
	Acute conditions	7/20 (35.00)	
	Chronic conditions	3/20 (15.00)	
	Behavioral issues	1/20 (5.00)	
	Other[b]	3/20 (15.00)	
Dog use for herding or hunting	Never	16/20 (80.00)	(56.0, 94.0)
	Daily	1/20 (5.00)	
	Twice/wk.	0/20 (0)	
	Once/wk.	1/20 (5.00)	
	<Once/wk.	1/20 (5.00)	
	Other	1/20 (5.00)	

[a]Heartgard plus, Advantage multi, and fenbendazole all together, Sentinel and heartgard plus
[b]Tibial plateau leveling osteotomy, allergic dermatitis, eosinophilic folliculitis & furonculosis

Table 4.7 Continued

Variable	Category	Number of respondents (%)	95% CI
Taking dogs to dogs park	Never	7/20 (35.00)	(15.0, 59.0)
	Daily	1/20 (5.00)	
	Twice/wk.	2/20 (10.00)	
	Once/wk.	2/20 (10.00)	
	<Once/wk.	6/20 (30.00)	
	Other[a]	2/20 (10.00)	
Frequency of diarrhea in dogs[b]	None	16/20 (80.00)	(56.0, 94.0)
	Once/3 mo.	3/20 (15.00)	
	Twice/3 mo.	0/20 (0)	
	3 times/3 mo.	0/20 (0)	
	>3 times/3 mo.	1/20 (5.00)	
	I don't know	0/20 (0)	

[a]Once in past 3 months, no dog parks, but she has been to the beach in California about 3 times/week over the previous 6 weeks
[b]In the last 3 months

Table 4.8 Cat demographics, health assessment, and husbandry – section V

Variable	Category	Number of cats per age group (0-20+)	Number of respondents (%)	95% CI
Cat age	< 1 mo.	0	0/7 (0)	
	1-6 mo.	0	0/7 (0)	
	< 1 yr.	1	1/7 (14.28)	
	1-5 yrs.	1	2/7 (28.57)	
		2	1/7 (14.28)	
	6-12 yrs.	2	3/7 (42.85)	(9.0, 81.0)
	>12 yrs.	0	0/7 (0)	
Cat source	Pet shop		0/7 (0)	
	Former research animal		1/7 (14.29)	
	Breeders		0/7 (0)	
	Shelter or rescue group		5/7 (71.43)	(29.0, 96.0)
	Friend		1/7 (14.29)	
	Other[a]		1/7 (14.29)	
Cat dewormers and heart worm preventatives	None		6/7 (85.71)	(42.0, 99.0)
	Advantage Multi®		1/7 (14.29)	
Cat health events	No health events		4/7 (57.14)	
	Acute conditions		3/7 (42.86)	
	Other[b]		1/7 (14.29)	

[a]St. Kitts Stray
[b]Hairballs and intermittent vomiting

Table 4.8 Continued

Variable	Category	Number of respondents (%)	95% CI
Litter box cleaning frequency	Daily	3/7 (42.86)	(9.0, 81.0)
	3-4 times/wk.	1/7 (14.29)	
	Twice/wk.	2/7 (28.57)	
	Once/wk.	0/7 (0)	
	<Once/wk.	1/7 (14.29)	
	Other	0/7 (0)	
Washing hands frequency after handling the litter box	Always	6/7 (85.71)	(42.0, 99.0)
	Most of the time	0/7 (0)	
	Sometimes	0/7 (0)	
	Rarely	1/7 (14.29)	
	Never	0/7 (0)	
Frequency of diarrhea in cats*	None	6/7 (85.71)	(42.0, 99.0)
	Once/3 mo.	1/7 (14.29)	
	Twice/3 mo.	0/7 (0)	
	3 times/3 mo.	0/7 (0)	
	>3 times/3 mo.	0/7 (0)	
	Unsure	0/7 (0)	

*In the last 3 months

Table 4.9 Dog and cat demographics, health assessment, and husbandry – section VI

Variable	Category	Number of dogs per age group (0-20+)	Number of respondents (%)	95% CI
Dog age	< 1 mo.	0	0/20 (0)	
	1-6 mo.	0	0/20 (0)	
	< 1 yr.	2	1/20 (5.00)	
	1-5 yrs.	1	9/20 (60.00)	(23.0, 68.0)
		2	3/20 (15.00)	
		3	3/20 (15.00)	
		4	1/20 (5.00)	
	6-10 yrs.	1	3/20 (15.00)	
		2	3/20 (15.00)	
		3	2/20 (10.00)	
	>10 yrs.	1	2/20 (5.00)	
		2	1/20 (5.00)	
Cat age	< 1 mo.	0	0/7 (0)	
	1-6 mo.	0	0/7 (0)	
	< 1 yr.	1	1/20 (5.00)	
		2	1/20 (5.00)	
	1-5 yrs.	1	10/20 (50.00)	(27.0, 72.0)
		2	4/20 (20.00)	
	6-12 yrs.	1	2/20 (5.00)	
		2	3/20 (15.00)	
	>12 yrs.	1	4/20 (20.00)	
		2	1/20 (5.00)	

Table 4.9 Continued

Variable	Category	Number of respondents (%)	95% CI
Dog and cat source	Pet shop	0/20 (0)	
	Former research animal	1/20 (5.00)	
	Breeders	7/20 (35.00)	(15.0, 59.0)
	Shelter or rescue group	20/20 (100.00)	(83.0, 100.0) – One-sided CI (97.5%)
	Friend	4/20 (20.00)	
	Other	1/20 (5.00)	
Dog dewormers and heart worm preventatives	None	1/20 (5.00)	
	Advantage Multi	4/20 (20.00)	
	Sentinel	3/20 (15.00)	
	Heartgard	11/20 (55.00)	(31.0, 76.0)
	Other[a]	1/20 (5.00)	
Cat dewormers and heart worm preventatives	None	10/20 (50.00)	(31.0, 76.0)
	Advantage Multi	7/20 (35.00)	
	Revolution	1/20 (5.00)	
	Drontal	1/20 (5.00)	
	Other[b]	1/20 (5.00)	
Dogs and cats health events	No health events	7/20 (35.00)	(15.0, 59.0)
	Acute conditions	7/20 (35.00)	(15.0, 59.0)
	Chronic conditions	2/20 (10.00)	
	Diseases	4/20 (20.00)	
	Internal parasitic infections	2/20 (10.00)	
	Behavioral issues	5/20 (25.00)	

[a]Stray cats
[b]Pyrantel and praziquantel

Table 4.9 Continued

Variable	Category	Number of respondents (%)	95% CI
Dogs used for hunting/ herding	Never	18/20 (90.00)	(68.0, 98.0)
	Daily	0/20 (0)	
	Twice/wk.	1/20 (5.00)	
	Once/wk.	0/20 (0)	
	<Once/wk.	1/20 (5.00)	
	Other[a]	0/20 (0)	
Taking dogs to a dog park	Never	10/20 (50.00)	(31.0, 76.0)
	Daily	1/20 (5.00)	
	Twice/wk.	0/20 (0)	
	Once/wk.	2/20 (10.00)	
	<Once/wk.	7/20 (35.00)	
	Other	0/20 (0)	
Cleaning the litterbox frequency	Daily	6/20 (30.00)	
	3-4 times/wk.	3/20 (15.00)	
	Twice/wk.	5/20 (25.00)	
	Once/wk.	2/20 (10.00)	
	<Once/wk.	2/20 (10.00)	
	Other[b]	2/20 (10.00)	
Washing hands after handling the litterbox	Always	11/20 (55.00)	(31.0, 76.0)
	Most of the time	8/20 (40.00)	
	Sometimes	1/20 (5.00)	
	Rarely	0/20 (0)	
	Never	0/20 (0)	
Diarrhea in dogs and cats	None	15/20 (75.00)	(50.0, 91.0)
	Once/3 mo.	3/20 (15.00)	
	Twice/3 mo.	1/20 (5.00)	
	3 times/3 mo.	0/20 (0)	
	>3 times/3 mo.	1/20 (5.00)	
	I don’t know	0/20 (0)	

[a]Twice daily
[b]In the last 3 months

Table 4.10 Procedures frequency performed on senior practicum core, elective or externship rotations, have you performed for adult cattle and/or calves – section VII of the survey

Variable	Yes (%)	No (%)	Total
Cattle rectal palpation exams	26 (50.98)	25 (49.02)	51
Cattle physical exams	27 (52.94)	24 (47.06)	51
Cattle treatments	27 (52.94)	24 (47.06)	51
Diagnostic tests for cattle	27 (52.94)	24 (47.06)	51
Calving assistance	12 (23.53)	39 (76.47)	51
Breeding soundness exams	22 (43.14)	29 (56.86)	51
Calves physical exams	24 (47.06)	27 (52.94)	51
Calves treatments	25 (49.02)	26 (50.98)	51
Diagnostic tests for calves	25 (49.02)	26 (50.98)	51
Brucellosis vaccinations	20 (39.22)	31 (60.78)	51
Postmortem diagnosis	34 (66.67)	17 (33.33)	51

Table 4.11 Activities frequency after handling cattle and/or calves in the clinical rotations – section VII of the survey

Variable	**Category**	**Number of respondents (%)**	**95 % CI**
Washing hands	Always	31/51 (60.78)	(46.0, 74.0)
	Most of the time	9/51 (17.65)	
	Sometimes	6/51 (11.76)	
	Rarely	1/51 (1.96)	
	Never	4/51 (7.84)	
Eating/drinking	Always	6/51 (11.76)	
	Most of the time	8/51 (15.69)	
	Sometimes	25/51 (49.02)	(34.0, 63.0)
	Rarely	5/51 (9.80)	
	Never	7/51 (13.73)	
Washing coveralls/rubber boots	N/A	21/51 (41.18)	(27.0, 55.0)
	Daily	22/51 (43.14)	(29.0, 57.0)
	Twice/wk.	3/51 (5.88)	
	Once/wk.	0/51 (0)	
	<Once/wk.	3/51 (5.88)	
	Other		

Table 4.12 Use of personal protective equipment when handling cattle and/or calves in the clinical rotations – section VII of the survey

PPE	Always (%)	Most of the time (%)	Sometimes (%)	Rarely (%)	Never (%)	Total
PPE/Diarrheic cattle or calves						
Exam gloves	33 (64.71)	8 (15.69)	4 (7.84)	1 (1.96)	5 (9.80)	51
Coveralls	39 (76.47)	5 (9.80)	1 (1.96)	1 (1.96)	5 (9.80)	51
Rubber boots	39 (76.47)	4 (7.84)	2 (3.92)	0 (0)	6 (11.76)	51
Surgical mask or N95	3 (5.88)	0 (0)	6 (11.76)	8 (15.69)	34 (66.67)	51
PPE/Non-diarrheic cattle or calves						
Exam gloves	25 (49.02)	9 (16.65)	9 (17.65)	3 (5.88)	5 (9.80)	51
Coveralls	37 (72.55)	4 (7.84)	4 (7.84)	1 (1.96)	5 (9.80)	51
Rubber boots	37 (72.55)	3 (5.88)	5 (9.80)	0 (0)	6 (11.76)	51
Surgical mask or N95	3 (6.00)	0 (0)	6 (12.00)	7 (14.00)	34 (68.00)	50

Table 4.13 Procedures frequency performed on senior practicum core, elective or externship rotations, have you performed for dogs and cats – section VII of the survey

Variable	Yes (%)	No (%)	Total
Physical exams	51 (100.00)	0 (0)	51
Diagnostic tests	51 (100.00)	0 (0)	51
Taking animals to defecate or urinate	50 (98.04)	1 (1.96)	51
Change bedding	50 (98.04)	1 (1.96)	51
Cleaning exam rooms or tables	51 (100.00)	0 (0)	51
Cleaning cages, kennels or runs	49 (96.08)	2 (3.92)	51
Monitoring animals under sedation or anesthesia	48 (96.000	2 (4.000	50
Restraining animals for procedures	51 (100.00)	0 (0)	51
Post-operative care	50 (98.04)	1 (1.96)	51
Postmortem diagnosis	39 (76.47)	12 (23.53)	51

Table 4.14 Activities frequency after handling dogs and cats in the clinical rotations – section VII of the survey

Variable	Category	Number of respondents (%)	95% CI
Washing hands after procedures	Always	11/51 (21.57)	
	Most of the time	26/51 (50.98)	(36.0, 65.0)
	Sometimes	12/51 (23.53)	
	Rarely	2/51 (3.92)	
	Never	0/51 (0)	
Washing hands between patients	Always	9/51 (17.65)	
	Most of the time	28/51 (54.90)	(40.0, 68.0)
	Sometimes	9/51 (17.65)	
	Rarely	5/51 (9.80)	
	Never	0/51 (0)	
Eating/drinking	Always	7/51 (13.73)	
	Most of the time	14/51 (27.45)	
	Sometimes	28/51 (54.90)	(40.0, 68.0)
	Rarely	2/51 (3.92)	
	Never	0/51 (0)	
Washing clinic smocks or cloth surgical gowns	N/A	3/51 (5.88)	
	Daily	6/51 (11.76)	
	Twice/wk.	14 (27.45)	
	Once/wk.	21/51 (41.18)	(27.0, 55.0)
	<Once/wk.	7/51 (13.73)	
	Other	0/51 (0)	

Table 4.15 Use of personal protective equipment when handling diarrheic and non-diarrheic dogs and cats in the clinical rotations – section VII of the survey

PPE	Always (%)	Most of the time (%)	Sometimes (%)	Rarely (%)	Never (%)	Total
PPE/Diarrheic dogs and cats						
Exam gloves	25 (49.02)	14 (27.45)	8 (15.69)	3 (5.88)	1 (1.96)	51
Clinic smocks	39 (76.47)	8 (15.69)	4 (7.84)	0 (0)	0 (0)	51
Surgical gowns	4 (7.84)	2 (3.92)	15 (29.41)	21 (41.18)	9 (17.65)	51
Masks	0 (0)	0 (0)	3 (5.88)	22 (43.14)	26 (50.98)	51
Foot covers	0 (0)	1 (1.96)	8 (15.69)	20 (39.22)	22 (43.14)	51
PPE/Non-diarrheic dogs and cats						
Exam gloves	7 (13.73)	2 (3.92)	21 (41.18)	17 (33.33)	4 (7.84)	51
Clinic smocks	35 (68.63)	12 (23.53)	4 (7.84)	0 (0)	0 (0)	51
Surgical gowns	0 (0)	1 (1.96)	4 (7.84)	21 (41.18)	25 (49.02)	51
Masks	0 (0)	0 (0)	2 (3.92)	22 (43.14)	27 (52.94)	51
Foot covers	0 (0)	0 (0)	3 (5.88)	21 (41.18)	27 (52.94)	51

Table 4.16 Rotations completed from the beginning of the senior practicum until the time of the survey

Clinical Rotation	**Number of Respondents (%)**	**Total**
Dairy Field Service	19 (37.25)	51
Herd Management	5 (9.80)	51
Livestock Medicine any Surgery	0 (0)	51
Large Animal Emergency Medicine	20 (39.22)	51
Livestock Medicine and Surgery	21 (41.18)	51
Any senior practicum involving contact with cattle	20 (39.22)	51
Small Animal Internal Medicine	36 (70.59)	51
Community Practice	31 (60.78)	51
Critical and Emergency Care	29 (56.86)	51
Afterhours Small Animal Urgent Care	33 (64.71)	51
Postmortem Diagnosis (Large and small animals)	30 (58.82)	51

Table 4.17 Fecal flotation (FF) immuno-fluorescent assay (IFA), and polymerase chain reaction (PCR) results of *G. duodenalis* for human, dog and cat samples

Species	Diagnostic Test	Positives	Percentage	95% CI
Humans (42)	FF	0	N/A	N/A
Dogs[a] (31)	FF	1	3.23	(0.1, 16.7)
Cats (17)	FF	0	N/A	N/A
Humans (42)	IFA	1	2.38	(0.1, 12.6)
Dogs (31)	IFA	3	9.68	(2.0, 25.8)
Cats (17)	IFA	0	N/A	N/A
Humans (42)	PCR	3	7.14	(1.5, 19.5)
Dogs (31)	PCR	3	9.68	(2.0, 25.8)
Cats (17)	PCR	1	5.88	(0.1, 28.7)

[a]One dog was identified with hookworm egg that was *G. duodenalis* negative

Table 4.18 Polymerase chain reaction (PCR) results distributed by genes of *G. duodenalis* for human, dog and cat samples

Species	gdh	tpi	β-giardin
Humans (42)	0/42 (0%)	3/42 (7.14%)	0/42 (0%)
Dogs (31)	2/31 (6.45%)	3/31 (9.67%)	1/31 (3.22%)
Cats (17)	0/17 (0%)	0/17 (0%)	1/17 (5.88%)

Table 4.19 Fecal flotation (FF), immuno-fluorescent assay IFA, and polymerase chain reaction (PCR) results of *Cryptosporidium* spp. for human, dog and cat samples

Species	**Diagnostic Test**	**Positives**	**Percentage**	**95% CI**
Humans (42)	FF	0	N/A	N/A
Dogs (31)	FF	0	N/A	N/A
Cats (17)	FF	0	N/A	N/A
Humans (42)	IFA	1	2.38	(0.1, 12.6)
Dogs (31)	IFA	1	3.23	(0.1, 16.7)
Cats (17)	IFA	0	N/A	N/A
Humans (42)	PCR	1	2.38	(0.1, 12.6)
Dogs (31)	PCR	4	12.9	(3.6, 29.8)
Cats (17)	PCR	1	5.88	(0.1, 28.7)

Table 4.20 Polymerase chain reaction (PCR) results distributed by genes of *Cryptosporidium* spp. for human, dog and cat samples

Species	**18SrRNA**	**hsp70**	**gp60**
Humans (42)	0/42 (0%)	N/A	1/42 (2.38%)
Dogs (31)	2/31 (6.45%)	4/31 (12.9%)	0/31 (0%)
Cats (17)	0/17 (0%)	1/17 (5.88%)	0/17 (0%)

Table 4.21 Stratification of positive and negative human samples due to *G. duodenalis* and *Cryptosporidium* spp. by risk factors using the IFA test

Variable	Level	Positive (%)	Total	Fisher's exact P value	95% CI
Track Preference	SA	1 (5.0%)	20	0.65	(0.12, 25.0)
	GEN	0	14		
	LA	0	8		
Gender	M	0	6	0.86	(0.1, 14.5)
	F	1 (2.8%)	36		
Pet Ownership	Yes	1 (2.6%)	39	0.93	(0.1, 13.5)
	No	0	3		
Work on Farm	Yes	1 (3.8%)	26	0.61	(0.1, 19.6)
	No	0	16		

Table 4.22 Stratification of positive and negative human samples due to *G. duodenalis* by risk factors using the PCR test

Variable	Level	Positive (%)	Total	Fisher's exact P value	95% CI
Track Preference	SA	2 (10.0%)	20	0.59	(1.2, 32.0)
	GEN	1 (7.1%)	14		(0.2, 33.9)
	LA	0	8		
Gender	M	0	6	0.62	
	F	3 (8.3%)	36		(1.7, 22.5)
Pet Ownership	Yes	3 (7.7%)	39	0.79	(1.6, 20.9)
	No	0	3		
Work on Farm	Yes	3 (11.5%)	26	0.23	(2.4, 30.2)
	No	0	16		

Table 4.23 Stratification of positive and negative human samples due to *Cryptosporidium* spp. by risk factors using the PCR test

Variable	Level	Positive (%)	Total	Fisher's exact P value	95% CI
Track Preference	SA	0	20	0.32	
	GEN	0	14		
	LA	1 (12.5%)	8		(0.3, 52.7)
Gender	M	1 (16.7%)	6	0.14	(0.4, 64.1)
	F	0	36		
Pet Ownership	Yes	1 (2.6%)	39	0.93	(0.1, 13.5)
	No	0	3		
Work on Farm	Yes	1 (3.8%)	26	0.62	(0.1, 19.6)
	No	0	16		

Table 4.24 DNA sequencing results for *G. duodenalis*

Sample ID	Species	Age	Stool Character	Typed by gene	Parasite
242	Dog	1-5 yr.	Score = 4	tpi	*G. duodenalis* Assemblage D
				gdh	*G. duodenalis* Assemblage D
276	Dog	1-5 yr.	Score = 2	tpi	*G. duodenalis* Assemblage D
				gdh	*G. duodenalis* Assemblage D
204	Dog	<1 yr.	Score = 2	β-giardin	*G. duodenalis* Assemblage C
				gdh	*G. duodenalis* Assemblage C

Table 4.25 DNA sequencing results for *Cryptosporidium* spp.

Sample ID	Species	Age	Stool Character	Typed by gene	Parasite
242	Dog	1-5 yr.	Score = 4	18SrRNA	*Cryptosporidium* spp.
389	Cat	1-5 yr.	Score = 1	hsp70	*Cryptosporidium* spp.
389	Dog	6-10 yr.	Score = 4	hsp70	*C. felis*
575	Dog	1-5 yr.	Score = 3	hsp70	*Cryptosporidium* spp.
643	Human	29	Non-diarrheic	gp60	*C. parvum* (IIa Allele)

Table 4.26 Positive dog, cat and owner demographics

Animal ID	Pet Demographics	Owner Demographics
Dog #448	• *G. duodenalis* (IFA) • Age from <1 year and 6-10 years • Breeders • Chronic health conditions • Herding or hunting – once/wk. • Dog park – attended daily • Had diarrhea > 3 times /3 mo.	• *Giardia/Cryptosporidium* –negative • Female • Large animal track • Lived in single family residence/no cattle • Worked on a farm • Owned multiple dogs
Dog #242	• *G. duodenalis* (FF, IFA, PCR) • *Cryptosporidium* spp. (PCR) • Age 1-5 yrs. • Shelter • Acute health conditions • Behavioral issues • Never used for herding • Dog park – attended once/wk. • No diarrhea	• *Giardia/Cryptosporidium* –negative • Female • Small animal track • Lived in single family residence/no cattle • Worked on a farm • Owned dogs and cats
Dog #204	• *G. duodenalis* (IFA, PCR) • *Cryptosporidium* spp. (IFA, PCR) • Age <1 yr. • Breeder and shelter • No health events • Herding or hunting – twice/wk. • Dog park – not attended • No diarrhea	• *Giardia/Cryptosporidium* –negative • Female • Large animal track • Lived in single family residence/with cattle • Worked on a farm • Owned dogs and cats

Table 4.26 Continued

Animal ID	Pet Demographics	Owner Demographics
Dog# 276	• *G. duodenalis* (PCR) • *Cryptosporidium* spp. (PCR) • Age 1-5 yr. • Shelter • Acute conditions and diseases such as diabetes • Herding or hunting – never • Dog park – not attended • No diarrhea	• *G. duodenalis* (PCR) • Female • Small animal track • Lived in single family residence/no cattle • Worked on a farm • Owned dogs and cats

Table 4.26 Continued

Animal ID	Pet Demographics	Owner Demographics
Dog #575	• *Cryptosporidium* spp. (PCR) • Age 1-5 yrs. • Shelter • No health events • Herding or hunting – never • Dog park – once/wk. • No diarrhea	• *Giardia/Cryptosporidium* –negative • Female • Small animal track • Lived in single family residence/no cattle • Have not worked on a farm • Owned one dog only
Dog #389	• *Cryptosporidium* spp. (PCR) • Age 6-10 yrs. • Shelter • No health events • Herding or hunting – never • Dog park – not attended • No diarrhea	• *Giardia/Cryptosporidium* –negative • Female • Small animal track • Lived in single family residence/no cattle • Have not worked on a farm • Owned dogs and cats
Dog#82	• Hookworm • *Giardia/Cryptosporidium* – negative • Age 1-5 yrs. • Breeder/shelter • No health events • Herding or hunting – never • Dog park – <1/wk. • No diarrhea	• *Giardia/Cryptosporidium* –negative • Female • Small animal track • Lived in single family residence/no cattle • Have not worked on a farm • Owned dogs and cats

Table 4.26 Continued

Animal ID	Pet Demographics	Owner Demographics
Cat #389	• *Cryptosporidium* spp. (PCR) • Age 1-5 yrs. • Shelter • No health events No diarrhea	Same owner as Dog #398
Cat #300	• *G. duodenalis* (PCR) • Age 1-5 yrs. • Breeder and Shelter • Acute conditions Had diarrhea > 3 times /3 mo.	• *Giardia/Cryptosporidium* –negative • Female • Small animal track • Lived in single family residence/no cattle • Worked on a farm • Owned dogs and cats

REFERENCES

1. Hunter PR, Thompson RCA. The zoonotic transmission of *Giardia* and *Cryptosporidium*. *Int J Parasitol.* 2005;35:1181-1190. doi: 10.1016/j.ijpara.2005.07.009.

2. Roy SL, DeLong SM, Stenzel SA, et al. Risk factors for sporadic cryptosporidiosis among immunocompetent persons in the United States from 1999–2001. *J Clin Microbiol.* 2004;42: 2944–2951. doi: 10.1128/JCM.42.7.2944-2951.2004

3. Robertson B, Sinclair MI, Forbes AB, et al. Case-control studies of sporadic cryptosporidiosis in Melbourne and Adelaide, Australia. *Epidemiol Infect.* 2002;128(3):419–431. doi: 10.1017}S0950268802006933.

4. Hunter PR, Hughes S, Woodhouse S, et al. Sporadic cryptosporidiosis case-control study with genotyping. *Emerg Infect Dis.* 2004;10:1241-1249. doi: 10.3201/eid1007.030582.

5. Painter JE, Hlavsa MC, Collier SA, Yoder JS. Cryptosporidiosis surveillance — United States, 2011–2012. Giardiasis surveillance — United States, 2011–2012. *Morb Mortal Wkly Rep*. 2015;64(3):1-28.

6. Isaac-Renton JL, Philion JJ. Factors associated with acquiring giardiasis in British Columbia residents. *Can J Public Health.* 1992;83:155–158. http://www.jstor.org/stable/41990762.

7. Warburton AR, Jones PH, Bruce J. Zoonotic transmission of giardiasis: a case control study. *Commun Dis Rep CDR Rev.* 1994;4:R32–R36.

8. Hoque ME, Hope VT, Kjellstrom T, Scragg R, Lay-Yee R. Risk of giardiasis in Aucklanders: a case-control study. *Int J Infect Dis.* 2002;6:191–197. doi:10.1016/S1201-9712(02)90110-4.

9. Hoque ME, Hope VT, Scragg R. Children at risk of giardiasis in Auckland: a case-control analysis. *Epidemiol Infect.* 2003;131:655–662. doi: 10.1017/S0950268803008598.

10. Stuart JM, Orr HJ, Warburton FG, et al. Risk factors for sporadic giardiasis: a case-control study in southwestern England. *Emerg Infect Dis.* 2003;9(2):229–233. doi: 10.3201/eid0902.010488.

11. Chappell CL, Okhuysen PC, Langer-Curry R, et al. *Cryptosporidium hominis*: experimental challenge of healthy adults. *Am J Trop Med Hyg.* 2006;75(5):851-857.

12. Yoder JS, Gargano JW, Wallace RM, Beach MJ. Giardiasis Surveillance — United States, 2009–2010. *Morb Mortal Wkly Rep.* 2012;61(SS05):13-23.

13. Uga S, Matsumura T, Ishibashi K, Yatomi K, Kataoka N. Cryptosporidiosis in dogs and cats in Hyogo Prefecture, Japan. *Jpn J Parasitol*. 1989;38:139-143.

14. Kirkpatrick CE, Farrell JP. Feline giardiasis: observations on natural and induced infections. *Am J Vet Res*. 1984;45:2182-2188.

15. Tangtrongsup S, Scorza V. Update on the diagnosis and management of *Giardia* spp. infections in dogs and cats. *Top Companion Anim Med*. 2010;25(3):155-62. doi: 10.1053/j.tcam.2010.07.003.

16. Grinberg A, Pomroy WE, Squires RA, Scuffham A, Pita A, Kwan E. Retrospective cohort study of an outbreak of cryptosporidiosis caused by a rare *Cryptosporidium parvum* subgenotype. *Epidemiol Infect.* 2011 Oct;139(10):1542-50. doi: 10.1017/S0950268810002499.

17. Gait R, Soutar RH, Hanson M, Fraser C, Chalmers R. Outbreak of cryptosporidiosis among veterinary students. *Vet Rec.* 2008;162(26):843-5.

18. Traub RJ, Monis PT, Robertson I, Irwin P, Mencke N, Thompson RC. Epidemiological and molecular evidence supports the zoonotic transmission of *Giardia* among humans and dogs living in the same community. *Parasitol.* 2004;128:253-262. doi: http://dx.doi.org/10.1017/S0031182003004505.

19. Minvielle MC, Molina NB, Polverino D, Basualdo JA. First genotyping of *Giardia lamblia* from human and animal feces in Argentina, South America. *Mem Inst Oswaldo Cruz.* 2008;103(1):98-103. http://dx.doi.org/10.1590/S0074-02762008000100015.

20. Liang CH, Tsaihong JC, Cheng YY, Peng SY. Occurrence and genotype of *Giardia* cysts isolated from faecal samples of children and dogs and from drinking water samples in an aboriginal area of central Taiwan. *Exp Parasitol.* 2012;131(2):204-9. doi: 10.1016/j.exppara.2012.04.002.

21. Marangi M, Berrilli F, Otranto D, Giangaspero A. Genotyping of *Giardia duodenalis* among children and dogs in a closed socially deprived community from Italy. *Zoonoses Public Health.* 2010;57(7-8):e54-e58. doi: 10.1111/j.1863-2378.2009.01304.x.

22. Eligio-García L, Cortes-Campos A, Jiménez-Cardoso E. Genotype of *Giardia intestinalis* isolates from children and dogs and its relationship to host origin. *Parasitol Res.* 2005; 97(1):1-6. doi: 10.1007/s00436-005-1368-9.

23. Yoder JS, Harral C, Beach MJ. Cryptosporidiosis Surveillance-- United States, 2006-2008. *Morb Mortal Wkly Rep.* 2010;59(SS06):1-14.

24. Weigler BJ, Di Giacomo RF, Alexander S. A national survey of laboratory animal workers concerning occupational risks for zoonotic diseases. *Comp Med.* 2005;55(2):183-91.

25. Salman MD, New JG, Scarlett JM, Kris PH, Ruch-Gallie R, Hetts S. Human and animal factors related to the relinquishment of dogs and cats in selected animal shelters in the United States. *J Appl Anim Welfare Sci.* 1998;J(3):207-226.

26. Scorza AV, Ballweber LR, Tangtrongsup S, Panuska C, Lappin MR. Comparisons of mammalian *Giardia duodenalis* assemblages based on the β-giardin, glutamate dehydrogenase and triose phosphate. *Vet Parasitol.* 2012;189:182-188. doi: 10.1016/j.vetpar.2012.04.032.

27. O'Handley RM, Olson ME, Fraser D, Adams P, Thompson RCA. Prevalence and genotypic characterisation of Giardia in dairy calves from Western Australia and Western Canada. *Vet Parasitol.* 2000;90:193-200. http://dx.doi.org/10.1016/S0304-4017(00)00235-1.

28. Da Silva AJ, Bornay-Llinares FJ, Moura NS, Slemenda SB, Tuttle JL, Pieniazek NJ. Fast and reliable extraction of protozoan parasite DNA from fecal specimens. *Mol Diag.*1999;4(1):57-64. doi:10.1016/S1084-8592(99)80050-2.

29. Scorza AV, Brewer MM, Lappin MR. Polymerase chain reaction for the detection of *Cryptosporidium* spp. in cat feces. *J Parasitol.* 2003;89:423-426. doi: http://dx.doi.org/10.1645/0022-3395(2003)089[0423:PCRFTD]2.0.CO;2

30. Vasilopulos RJ, Rickard LG, Mackin AJ, Pharr GT, Huston CL. Genotypic analysis of *Giardia duodenalis* in domestic cats. *J Vet Intern Med.* 2007;21:352–355. http://dx.doi.org/10.1016/j.vetpar.2012.04.032

31. Read CM, Monis PT, Thompson AT. Discrimination of all genotypes of *Giardia duodenalis* at the glutamate dehydrogenase locus using PCR-RFLP. *Infect Genet Evol.* 2004;4:125-130. doi:10.1016/j.meegid.2004.02.001.

32. Lalle M, Pozio E, Capelli G, Bruschi F, Crotti D, Cacciò SM. Genetic heterogeneity at the β-giardin locus among human and animal isolates of *Giardia duodenalis* and identification of potentially zoonotic subassemblages. *Int J Parasitol.* 2005;35:207–213. doi:10.1016/j.ijpara.2004.10.022.

33. Sulaiman IM, Fayer R, Bern C, et al. Triosephosphate isomerase gene characterization and potential zoonotic transmission of *Giardia duodenalis*. *Emerg Infect Dis*. 2003;9(11):1444-1452.

34. Lebbad M, Mattsson JG, Christensson B, et al. From mouse to moose: Multilocus genotyping of *Giardia* isolates from various animal species. *Vet Parasitol.* 2010;168(3-4):231–239. doi:10.1016/j.vetpar.2009.11.003.

35. Rochelle PA, De Leon R, Stewart MH, Wolfe RL. Comparison of primers and optimization of PCR conditions for detection of *Cryptosporidium parvum* and *Giardia lamblia* in water. *Appl Environ Microbiol.* 1997;63:106–114.

36. Xiao L, Bern C, Limor J, et al. Identification of 5 types of *Cryptosporidium* parasites in children in Lima, Peru. *J Infect Dis.* 2001;183(3):492-497. doi: 10.1086/318090.

37. Alves M, Xiao L, Sulaiman IM, Lal AA, Matos O, Antunes F. Subgenotype analysis of *Cryptosporidium* isolates from humans, cattle, and zoo ruminants in Portugal. *J Clin Microbiol.* 2003;41(6):2744–2747. doi: 10.1128/JCM.41.6.2744-2747.2003.

38. Morgan UM, Monis PT, Xiao L, et al. Molecular and phylogenetic characterisation of *Cryptosporidium* from birds. *Int J Parasitol.* 2001;31:289-296. http://dx.doi.org/10.1016/S0020-7519(00)00164-8.

39. Hocking JS, Lim MS, Read T, Hellard M. Postal surveys of physicians gave superior response rates over telephone interviews in a randomized trial. *J Clin Epidemiol.* 2006;59(5):521-524. doi: 10.1016/j.jclinepi.2005.10.009.

40. Wiebe ER, Kaczorowski J, MacKay J. Why are response rates in clinician surveys declining? *Can Fam Physician*. 2012;58(4):e225–e228.

41. Burns KE, Duffett M, Kho ME, et al. A guide for the design and conduct of self-administered surveys of clinicians. *Can Med Assoc J.* 2008;179(3):245-52. doi: 10.1503/cmaj.080372.

42. Okhuysen PC, Chappell CL, Kettner C, Sterling CR. *Cryptosporidium parvum* metalloaminopeptidase inhibitors prevent in vitro excystation. *Antimicrob Agents Chemother.* 1996;40(12):2781–2784.

43. Chappel C, Okhuysen PC, Langer-Curry RC et al. *Cryptosporidium hominis*: experimental challenge of healthy adults. *Am J Trop Med Hyg.* 2006;75(5):851–857.

44. Chappell CL, Okhuysen PC, Langer-Curry RC, Akiyoshi DE, Widmer G, Tzipori S. *Cryptosporidium meleagridis*: infectivity in healthy adult volunteers. *Am J Trop Med Hyg.* 2011;85(2):238–242. doi:10.4269/ajtmh.2011.10-0664.

45. Giardiasis. The Center for Food Security and Public Health. Institute for International Cooperation in Animal Biologies Web site http://www.cfsph.iastate.edu/Factsheets/pdfs/giardiasis.pdf Published on 2012. Accessed on September 5, 2015.

46. Cacciò SM, Thompson RC, McLauchlin J, Smith HV. Unravelling *Cryptosporidium* and *Giardia* epidemiology. *Trends Parasitol.* 2005;21:430–437. doi:10.1016/j.pt.2005.06.013.

47. Faubert G. Immune response to *Giardia duodenalis*. *Clin Microbiol Rev.* 2000;13:35–54.

48. Dwivedi KK, Prasad G, Saini S, Mahajan S, Lal S, Baveja UK. Enteric opportunistic parasites among HIV infected individuals: associated risk factors and immune status. *Jpn J Infect Dis*. 2007;60:76–81.

49. Dowd K, Taylor M, Toribio JA. Zoonotic disease risk perceptions and infection control practices of Australian veterinarians: Call for change in work culture. *Prev Vet Med*. 2013; 111: 17– 24. http://dx.doi.org/10.1016/j.prevetmed.2013.04.002.

50. Volotão AC, Costa-Macedo LM, Haddad FS, Brandão A, Peralta JM, Fernandes O. Genotyping of *Giardia duodenalis* from human and animal samples from Brazil using beta-giardin gene: a phylogenetic analysis. *Acta Trop.* 2007;102:10–19. doi:10.1016/j.actatropica.2007.02.010.

51. Traub RJ, Monis PT, Robertson I, Irwin P, Mencke N, Thompson RC. Epidemiological and molecular evidence supports the zoonotic transmission of *Giardia* among humans and dogs living in the same community. *Parasitology*. 2004;128:253–262. doi: 10.1017/S0031182003004505.

52. Traub RJ, Inpankaew T, Reid SA, 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; 111:125–132. doi:10.1016/j.actatropica.2009.03.006.

53. Inpankaew T. Traub RJ, Thompson A, Sukthana Y. Canine parasitic zoonoses in Bangkok temples. *Southeast Asian J Trop Med Public Health*. 2007;38:247–255.

54. Souza SL, Gennari SM, Richtzenhain LJ, 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;149:258–264. doi:10.1016/j.vetpar.2007.08.019.

55. van der Giessen JW, de Vries A, Roos M, Wielinga P, Kortbeek LM, Mank TG. Genotyping of *Giardia* in Dutch patients and animals: a phylogenetic analysis of human and animal isolates. *Int J Parasitol*. 2006;36: 849–858. doi:10.1016/j.ijpara.2006.03.001.

56. Robertson B, Sinclair MI, Forbes AB, et al. Case control studies of sporadic cryptosporidiosis in Melbourne and Adelaide, Australia. *Epidemiol Infect.* 2002;128(3):419-431.

57. Chacín-Bonilla L, Barrios F, Sanchez Y. Environmental risk factors for *Cryptosporidium* infection in an island from Western Venezuela. *Mem Inst Oswaldo Cruz, Rio de Janeiro.* 2008;103(1):45-49.

58. Bern C, Ortega Y, Checkley W, et al. Epidemiologic differences between cyclosporiasis and cryptosporidiosis in Peruvian children. *Emerg Infect Dis.* 2002; 8:581-585. doi: 10.3201/eid0806.010331.

59. Anderson BC, Donndelinger T, Wilkins RM, et al. Cryptosporidiosis in a veterinary student. *J Am Vet Med Assoc.* 1982;180(4):408-409.

60. Levine JF, Levy MG, Walker RL, Crittenden S. Cryptosporidiosis in veterinary students. *J Am Vet Med Assoc. 1988*;193(11):1413-1414.

61. Khan SM, Debnath C, Pramanik AK, Xiao L, Nozaki T, Ganguly S. Molecular characterization and assessment of zoonotic transmission of *Cryptosporidium* from dairy cattle in West Bengal, India. *Vet Parasitol*. 2010;171(1–2):41–47. doi: 10.1016/j.vetpar.2010.03.008.

62. Ng JS, Eastwood K, Walker B, et al. Evidence of *Cryptosporidium* transmission between cattle and humans in northern New South Wales. *Exp Parasitol.* 2012;130(4):437–441. doi: 10.1016/j.exppara.2012.01.014.

63. *Cryptosporidium* is Common in Dairy Calves. National Dairy Heifer Evaluation Project (NDHEP) 1991-1992. USDA–APHIS Web site http://www.aphis.usda.gov/animal_health/nahms/dairy/downloads/ndhep/NDHEP_Crypto.pdf. Published on 1993. Accessed September 1, 2015.

64. Alves M, Xiao L, Antunes F, Matos O. Distribution of *Cryptosporidium* sub-types in humans and domestic wild ruminants in Portugal. *Parasitol Res.* 2006;99:287–292. doi: 10.1007/s00436-006-0164-5.

65. Xiao L. Overview of *Cryptosporidium* presentations at the 10th international workshops on opportunistic protists. *Eukaryot Cell*. 2009;8(4):429–436. doi:10.1128/EC.00295-08.

66. Hunter PR, Hadfield SJ, Wilkinson D, Lake IR, Harrison FCD, Chalmers RM. Subtypes of *Cryptosporidium parvum* in humans and disease risk. *Emerg Infect Dis.* 2007;13:82–88.

67. Peng MM., Matos O, Gatei W, et al. A comparison of *Cryptosporidium* subgenotypes from several geographic regions. *J Eukaryot Microbiol.* 2001;(Suppl.):28S–31S. doi: 10.1111/j.1550-7408.2001.tb00442.x.

68. Wang A, Ruch-Gallie R, Scorza V, Lin P, Lappin MR. Prevalence of *Giardia* and *Cryptosporidium* species in dog park attending dogs compared to non-dog park attending dogs in one region of Colorado. *Vet Parasitol.* 2012;184: 335–340. doi:10.1016/j.vetpar.2011.08.019.

CHAPTER 5: CONCLUSIONS AND FUTURE DIRECTIONS

5.1 Conclusions

Giardia spp. and *Cryptosporidium* spp. are the leading causal agents of parasitic diarrhea in humans, dogs and cats. Our studies highlight that these two protozoans can be isolated from all three species. Both pathogens contain host-adapted and zoonotic strains. Dogs and cats can be infected with both strains. Assessing exposure factors that are associated with the increased probability of infection to these two pathogens as well as identifying and characterizing the isolates that infect dogs, cats and humans could help in understanding which factors are significantly associated with the infection due to these two pathogens that will ultimately aid in disease management and control. Furthermore, molecular characterization of the human, dog or cat fecal isolates identifies zoonotic genotypes in these species which may point out to the transmission routes of infection or disease among humans, dogs and cats.

Our study results showed that all *G. duodenalis* assemblages were host-adapted in dogs and was not identified in cats. In addition, most cats in the national study were infected with *C. felis*. This finding could indicate that pet dogs and cats are not potential reservoirs for zoonotic transmission in humans.

Even though the senior veterinary student track preference was not significantly associated with the increased probability of infection or disease of *Giardia* or *Cryptosporidium*, *C. parvum* was identified in cats and dogs who their owners have previously worked in farms contained cattle. There is baseline exposure to these two pathogens due to intensive contact with cattle. Thus, humans can potentially transmit the infection to their pets. Regardless, this conclusion needs further investigation by collecting more samples from veterinary students who have previously worked in the farm.

Even though the majority of dogs or cats owned by senior veterinary students that participated in this study were adopted from the shelter, we did not identify any other protozoan or intestinal parasites. This indicates that these dogs are well taken care of by their owner, especially, the majority of students have used the dewormers or heartworm preventative programs for their dogs, but not their cats. Regardless, we have not identified other intestinal parasites in cats as well.

In one research study conducted for this dissertation, molecular genotyping to *Cryptosporidium* spp. using gp60 locus was more specific for samples of human origin and not of dog or cat origin.

The importance of the timely processing of fecal samples was noted to reserve as much pathogen DNA as possible for detection. Another technique followed for diagnostic tests conducted in this dissertation, the PCR assay was applied to all samples regardless whether they are tested positive or negative to the IFA assay that is the gold standard. Additionally, it is recommended to use multilocus PCR protocol to characterize *Giardia* spp. isolates due to the divergent agreement between genes in the *Giardia* genome.

It is worth noting from conducting research that involved human subjects, that students' participation was lower than expected most likely because of the requirement to collect fecal samples. That could be attributed to cultural perspectives or recruitment methods. Therefore, it is suggested that multiple face-face recruiting approach can be more efficient than recruiting via email alone. Also, students were more willing to take the anonymous online survey without submitting fecal samples.

5.2 Future Directions

To continue investigating potential risk factors that can associate with the infection of *G. duodenalis* and *Cryptosporidium* spp. in veterinary students, research is needed to identify those factors in both junior and senior veterinary students in collaboration with other universities in the USA. This will allow for a larger sample size and to compare the risk in these two segments of populations. In addition, it will be helpful to identify a baseline exposure of the faculty that work on large animal rotations. More particularly, it is helpful to determine zoonotic or anthroponotic transmission of *Cryptosporidium* spp. in large animal track veterinarians, those who have worked or are working on a dairy farm and faculty veterinarians and their pet dogs or cats.

As a follow up of research conducted in Chapter 2 of this dissertation, an evaluation for zoonotic transmission can be determined. This evaluation can be conducted for associations of positive test results tested by the PCR assays with the clinical findings and determine the probability of dogs or cats are carrying the zoonotic species of *Giardia* and *Cryptosporidium*.

APPENDIX 1: QUESTIONNAIRE

Senior Veterinary Students Survey

Instructions

Evaluation of Zoonotic Giardia duodenalis and Cryptosporidium spp. Infection amongst Veterinary Students and their Dogs and Cats

Welcome to the CSU Veterinary Students Survey. Please read the informed consent carefully in the following page and let us know if you have questions. Please answer all questions. Please use the survey browsers (e.g. Prev and Next). Do not use the computer browsers.

Senior Veterinary Students Survey

INFORMED CONSENT

This research project is designed to investigate the potential for disease transmission between humans and their companion animals. As veterinary students are often pet owners and are occupationally exposed to enteric zoonotic pathogens, we believe you are an excellent group to gather information regarding the zoonoses of *Giardia duodenalis* and *Cryptosporidium* spp. The project team consists of: Dr. Michael Lappin (Principal Investigator), Dr. Francisco Olea-Popelka (Co-Principal Investigator), Dr. Valeria Scorza (Co-Investigator), and Hanaa Thigeel (Co-Investigator). A medical doctor will also be involved in the research team for human cases consultations.

As participants, you will be asked to do the following:

• Take a 5-10 minute online survey.
<u>Optional:</u>
• Collect a single fecal specimen from yourself
• Collect a single fecal specimen from your pets (if pets are owned) residing within the home (1 dog and 1 cat)

If you are willing to submit a fecal sample, you will receive a UNIQUE RANDOM ID provided to you in the survey package as well as fecal sample containers and instructions regarding sample collection. We will match your survey ID with the ID on your fecal containers; therefore, please USE THE SAME ID IN THE ONLINE SURVEY AS INDICATED. We will not collect names or personal identifiers from you; therefore, the SURVEY AND ALL SAMPLE COLLECTIONS WILL BE ANONYMOUS. Positive laboratory results for Giardia duodenalis and Cryptosporidium spp. should be reported to the Colorado Department of Public Health and Environment (CDPHE) or a local health agency within 7 days of diagnosis. ONCE AGAIN, no names or identifiers will be used in reporting.

Instructions on sample collection are as follows:

• Strictly follow instructions on sample collection and submission procedures provided in your SOP.

• Submit fecal samples between 10 AND 21 DAYS AFTER STARTING A ROTATION THAT INVOLVES INTENSIVE ANIMAL HANDLING.

All fecal samples will be processed in the Center for Companion Animal Studies. We will isolate and genotype *Giardia duodenalis* and *Cryptosporidium* spp. and compare genotypes isolated from humans, dogs and cats.

It is not possible to identify all potential risks, but the researcher(s) have taken reasonable safeguards to minimize any known risks to the participants. There is no cost for participation. A stipend of $100 will be provided to students that provide a fecal sample and the completed survey. The stipend will be $50 for those that complete the survey and submit feces from one of their dogs and/or cats. Also, you will receive $25 for only completing the survey. You can contact Jennifer Hawley: Jennifer.Hawley@COLOSTATE.EDU to receive the compensation once the survey is completed and the forms and samples are submitted. We will provide a free medical consultation for positive cases and a free veterinary consultation for regarding pet positive cases. Because samples are anonymous, please inform us if you would like to receive the results of your specimen analysis and if you wish to seek the free medical consultation. CONSULTATION WILL BE PROVIDED VOLUNTARY ONLY TO STUDENTS WHO REQUEST IT.

Your participation in this research is voluntary. If you decide to participate in the study, you may withdraw your consent and stop participating at any time without penalty or loss of benefits to which you are otherwise entitled. This consent form was approved by the CSU Institutional Review Board for the protection of human subjects in research on June 26, 2014. Completing this online survey is your consent to participate. If you have any questions about this research, please contact Hanaa Thigeel at thhanaa@lamar.colostate.edu. If you have any questions about your rights as a volunteer in this research, please contact the CSU IRB at: RICRO_IRB@mail.colostate.edu; 970-491-1553.

* 1. Please specify your participation preference:

- ◯ I agree to participate
- ◯ I do not agree to participate

Senior Veterinary Students Survey

SECTION I - Veterinary Students Demographics

1. Please provide the ID you received in your package in the text box below if you have it:

* 2. What is your gender?

- ◯ Male
- ◯ Female

* 3. Please provide your age in the text box below:

* 4. Please indicate your track preference.

- ◯ Large Animal Track
- ◯ General Track
- ◯ Small Animal Track

* 5. What type of residence do you have in Colorado?

- ◯ Single family residence where cattle of any age are housed or fed.
- ◯ Single family residence where animals (other than cattle) of any age are housed or fed.
- ◯ Single family residence where no animals are housed or fed
- ◯ Other

If you answered "Other", please specify what type:

* 6. In the last 12 months, how many adults (other than yourself) have lived in your household?

Number of adults

* 7. In the last 12 months, how many children under 5 years old have lived in your household?

Number of children

* 8. If one or more children under 5 years old live(d) in your household in the last 12 months, how often do children go to the daycare facilities?

- ◯ N/A if no children live(d) in your household
- ◯ N/A if your children don't go to the daycare facilities
- ◯ Daily
- ◯ Twice per week
- ◯ Once per week
- ◯ Less than once per week
- ◯ Other

If you answered "Other", please specify how often:

* 9. In the last 10 years, have you worked on an operation (yours or owned by others) on which cattle of any age are housed or fed?

- ◯ Yes (please answer questions 10-12)
- ◯ No (please skip to Section II)

10. How long ago did you last work on an operation (yours or owned by others) on which cattle of any age are housed or fed in the last 10 years?

- ◯ Within the last year
- ◯ 1-2 years
- ◯ 3-5 years
- ◯ More than 5 years

11. In the last 12 months, has your work on the operation involved any of the following activities? Please select all that apply.

- [ ] Adult cattle barn cleaning
- [ ] Adult cattle barn bedding changing
- [ ] Adult cattle feeding
- [ ] Adult cattle birthing assistance
- [ ] Adult cattle fecal disposal
- [ ] Cattle milking
- [ ] Cattle breeding
- [ ] Adult cattle treatments
- [ ] Adult cattle vaccinations
- [ ] Newborn calf care
- [ ] Newborn calf feeding
- [ ] Calf treatments
- [ ] Calf vaccinations
- [ ] Care of recumbent cattle or calves
- [ ] Cow/calf movement and transportation
- [ ] Other

If you answered "Other", please specify what service:

12. In the last 12 months, how much time have you spent in each activity listed in question 11?

	1-5 hr/wk	5-10 hr/wk	10-20 hr/wk	More than 20 hr/wk	N/A
Adult cattle barn cleaning	○	○	○	○	○
Adult cattle barn bedding changing	○	○	○	○	○
Adult cattle feeding	○	○	○	○	○
Adult cattle birthing assistance	○	○	○	○	○
Adult cattle fecal disposal	○	○	○	○	○
Cattle milking	○	○	○	○	○
Cattle breeding	○	○	○	○	○
Adult cattle treatments	○	○	○	○	○
Adult cattle vaccinations	○	○	○	○	○
Newborn calf care	○	○	○	○	○
Newborn calf feeding	○	○	○	○	○
Calf treatments	○	○	○	○	○
Calf vaccinations	○	○	○	○	○
Care of recumbent cattle or calves	○	○	○	○	○
Cow/calf movement and transportation	○	○	○	○	○
Other	○	○	○	○	○

Senior Veterinary Students Survey

SECTION II - Veterinary Students Health Assessment

* 1. Have you ever been diagnosed with any of the following conditions? Please select all that apply.

- [ ] None
- [ ] Diabetes
- [ ] Cancer
- [ ] HIV/AIDS
- [ ] Leukemia
- [ ] Other

If you answered "Other", please specify what condition:

* 2. If you are female, are you pregnant?

- ◯ Yes
- ◯ No
- ◯ Unsure
- ◯ N/A If you are male

* 3. In the last three months, have you taken any medications listed below that suppress your immune system? Please select all that apply.

- [ ] None
- [ ] Oral steroids (e.g. Deltasone ®, Hydrocortone ®,)
- [ ] Azathioprine (e.g. Imuran ®, Azasan ®)
- [ ] Mycophenolate Mofetil (e.g. CellCept ®)
- [ ] Other

If you answered "Other", please specify what medication:

* 4. In the last three months, how many times have you accidentally swallowed water from the following sources? Please select all that apply.

	None	Once per 3 months	Twice per 3 months	Three times per 3 months	More than 3 times per 3 months
Swimming pools	☐	☐	☐	☐	☐
Water play areas (e.g. Interactive fountains, wet deck,etc.)	☐	☐	☐	☐	☐
Hot tubs	☐	☐	☐	☐	☐
Lakes	☐	☐	☐	☐	☐
Rivers	☐	☐	☐	☐	☐
Springs	☐	☐	☐	☐	☐
Hot springs	☐	☐	☐	☐	☐
Ponds	☐	☐	☐	☐	☐
Streams	☐	☐	☐	☐	☐
Other	☐	☐	☐	☐	☐

If you answered "Other", please specify:

* 5. In the last three months, how many times have you intentionally drunken water from the following sources? Please select all that apply.

	None	Once per week	Twice per week	Three times per week	More than 3 times per week
Lakes	☐	☐	☐	☐	☐
Rivers	☐	☐	☐	☐	☐
Springs	☐	☐	☐	☐	☐
Ponds	☐	☐	☐	☐	☐
Streams	☐	☐	☐	☐	☐
Other	☐	☐	☐	☐	☐

If you answered "Other", please specify:

* 6. If you had intentionally drunken water from sources listed in question 5, did you use water filters before drinking?

- ◯ Yes
- ◯ No
- ◯ N/A If you never drink from these sources

* 7. How many times have you had watery, non-bloody and intermittent diarrhea that persisted from 1-2 weeks?

	Never	Once	Twice	3-5 times	More than 5 times
In the last 12 months (Between 6-12 months ago)	◯	◯	◯	◯	◯
In the last 6 months (Between 3-6 months ago)	◯	◯	◯	◯	◯
In the last 3 months (Between 1-3 months ago)	◯	◯	◯	◯	◯

Senior Veterinary Students Survey

SECTION III - Pet Ownership

* 1. Do you own dogs or cats?

- ◯ Dogs only
- ◯ Cats only
- ◯ Dogs and cats
- ◯ Neither dogs nor cats

Senior Veterinary Students Survey

SECTION IV - Dog Demographics, Health Assessment, Husbandry

* 1. Which category best describes your dog age? Please indicate how many dogs per age group if you have multiple dogs.

	Number of dogs per age group
Less than 1 year	
1-5 years	
6-10 years	
More than 10 years	

* 2. What was the source of your dog? Please select all that apply if you have multiple dogs.

- [ ] Pet shop
- [ ] Former research animal
- [ ] Breeders
- [ ] Shelter or rescue group (e.g. Owner relinquishment, stray, unknown)
- [ ] Friend
- [ ] Other

If you answered "Other", please specify:

* 3. In the last six months, have any dewormers and heart worm preventatives listed below been administered to any of your dogs?

- ( ) None
- ( ) Advantage Multi ® (Imidacloprid and Moxidectin)
- ( ) Sentinel ® (Milbemycin Oxime and Lufenuron)
- ( ) Revolution ® (Selamectin)
- ( ) Heartgard ® Plus (Ivermectin and Pyrantel)
- ( ) Panacur ® C (Fenbendazole)
- ( ) Drontal ® Plus (Praziquantel, Pyrantel Pamoate and Febantel)
- ( ) Droncit ® (Praziquantel)
- ( ) Other

If you answered "Other", please specify what medication:

* 4. In the last six months, have any of your dogs experienced any of the following health events? Please select all that apply.

- [ ] No health events
- [ ] Acute conditions (e.g. Diarrhea, gastroenteritis)
- [ ] Chronic conditions (e.g. Diarrhea, inflammatory bowel disease, food intolerance)
- [ ] Diseases (e.g. Cancer, diabetes, endocrine diseases, etc.)
- [ ] Internal parasitic infections
- [ ] Blood disorders (e.g. Hemolytic anemia)
- [ ] Behavioral issues (e.g. Pica, coprophagia, house soiling)
- [ ] Other

If you answered "Other", please specify:

* 5. In the last three months, how many times have any of your dogs been used for herding or hunting?

- ◯ Never
- ◯ Daily
- ◯ Twice per week
- ◯ Once per week
- ◯ Less than once per week
- ◯ Other

If you answered "Other", please specify how many:

* 6. In the last three months, how many times have you taken any of your dogs to a dog park?

- ◯ Never
- ◯ Daily
- ◯ Twice per week
- ◯ Once per week
- ◯ Less than once per week
- ◯ Other

If you answered "Other", please specify how many:

* 7. In the last three months, how many times have any of your dogs experienced watery, non-bloody diarrhea that persisted from 1-2 weeks?

- ◯ None
- ◯ Once per 3 months
- ◯ Twice per 3 months
- ◯ Three times per 3 months
- ◯ More than 3 times per three months
- ◯ I don't know

Senior Veterinary Students Survey

SECTION V- Cat Demographics, Health Assessment, Husbandry Practices

* 1. Which category best describes your cat age? Please indicate how many cats per age group if you have multiple cats.

	Number of cats per age group
Less than 1 year	
1-5 years	
6-12 years	
More than 12 years	

* 2. What was the source of your cat? Please select all that apply if you have multiple cats.

- ☐ Pet shop
- ☐ Former research animal
- ☐ Breeders
- ☐ Shelter or rescue group (i.e. Owner relinquishment, stray, unknown)
- ☐ Friend
- ☐ Other

If you answered "Other", please specify:

* 3. In the last six months, have any dewormers and heart worm preventatives listed below been administered to any of your cats?

- ◯ None
- ◯ Advantage Multi ® (Imidacloprid and Moxidectin)
- ◯ Revolution ® (Selamectin)
- ◯ Drontal ® (Praziquantel and Pyrantel Pamoate)
- ◯ Profender ® (Emodepside and Praziquantel)
- ◯ Other

If you answered "Other", please specify what medication:

* 4. In the last six months, have any of your cats experienced any of the following health events? Please select all that apply.

- ☐ No health events
- ☐ Acute conditions (e.g. Diarrhea, gastroenteritis)
- ☐ Chronic conditions (e.g. Diarrhea, inflammatory bowel disease, food intolerance)
- ☐ Diseases (e.g. Cancer, diabetes, endocrine diseases, etc.)
- ☐ Internal parasitic infections
- ☐ Blood disorders (e.g. Hemolytic anemia)
- ☐ Behavioral issues (e.g. Pica, coprophagia, house soiling)
- ☐ Other

If you answered "Other", please specify:

* 5. How often do you clean the litter box?

- ◯ Daily
- ◯ Three to four times per week
- ◯ Twice per week
- ◯ Once per week
- ◯ Less than once per week
- ◯ Other

If you answered "Other", please specify how many times:

* 6. How often do you wash your hands after cleaning the litter box?

- ◯ Always
- ◯ Most of the time
- ◯ Sometimes
- ◯ Rarely
- ◯ Never

* 7. In the last three months, how many times have any of your cats experienced watery, non-bloody diarrhea that persisted from 1-2 weeks?

- ◯ None
- ◯ Once per 3 months
- ◯ Twice per 3 months
- ◯ Three times per 3 months
- ◯ More than 3 times per three months
- ◯ Unsure

Senior Veterinary Students Survey

SECTION VI- Dog/Cat Demographics, Health Assessment, Husbandry Practices

* 1. Which category best describes your dog age? Please indicate how many dogs per age group if you have multiple dogs.

	Number of dogs per age group
Less than 1 year	
1-5 years	
6-10 years	
More than 10 years	

* 2. Which category best describes your cat age? Please indicate how many cats per age group if you have multiple cats.

	Number of cats per age group
Less than 1 year	
1-5 years	
6-12 years	
More than 12 years	

* 3. What was the source of your dogs and cats? Please select all that apply if you have multiple pets.

- [ ] Pet shop
- [ ] Former research animal
- [ ] Breeders
- [ ] Shelter or rescue group (i.e. Owner relinquishment, stray, unknown)
- [ ] Friend
- [ ] Other

If you answered "Other", please specify:

* 4. In the last six months, have any dewormers and heart worm preventatives listed below been administered to any of your dogs?

- ( ) None
- ( ) Advantage Multi ® (Imidacloprid and Moxidectin)
- ( ) Sentinel ® (Milbemycin Oxime and Lufenuron)
- ( ) Revolution ® (Selamectin)
- ( ) Heartgard ® Plus (Ivermectin and Pyrantel)
- ( ) Panacur ® C (Fenbendazole)
- ( ) Drontal ® Plus (Praziquantel, Pyrantel Pamoate and Febantel)
- ( ) Droncit ® (Praziquantel)
- ( ) Other

If you answered "Other", please specify what medication:

* 5. In the last six months, have any dewormers and heart worm preventatives listed below been administered to any of your cats?

- ◯ None
- ◯ Advantage Multi ® (Imidacloprid and Moxidectin)
- ◯ Revolution ® (Selamectin)
- ◯ Drontal ® (Praziquantel and Pyrantel Pamoate)
- ◯ Profender ® (Emodepside and Praziquantel)
- ◯ Other

If you answered "Other", please specify what medication:

* 6. In the last six months, have any of your dogs or cats experienced any of the following health events? Please select all that apply.

- ☐ No health events
- ☐ Acute conditions (e.g. Diarrhea, gastroenteritis)
- ☐ Chronic conditions (e.g. Diarrhea, inflammatory bowel disease, food intolerance)
- ☐ Diseases (e.g. Cancer, diabetes, endocrine diseases, etc.)
- ☐ Internal parasitic infections
- ☐ Blood disorders (e.g. Hemolytic anemia)
- ☐ Behavioral issues (e.g. Pica, coprophagia, house soiling)
- ☐ Other

If you answered "Other", please specify:

* 7. In the last three months, how many times have any of your dogs been used for herding or hunting?

- ◯ Never
- ◯ Daily
- ◯ Twice per week
- ◯ Once per week
- ◯ Less than once per week
- ◯ Other

If you answered "Other", please specify how many:

* 8. In the last three months, how many times have you taken any of your dogs to a dog park?

- ◯ Never
- ◯ Daily
- ◯ Twice per week
- ◯ Once per week
- ◯ Less than once per week
- ◯ Other

If you answered "Other", please specify how many:

* 9. How often do you clean the litter box?

- ◯ Daily
- ◯ Three to four times per week
- ◯ Twice per week
- ◯ Once per week
- ◯ Less than once per week
- ◯ Other

If you answered "Other", please specify how many times:

* 10. How often do you wash your hands after cleaning the litter box?

- ◯ Always
- ◯ Most of the time
- ◯ Sometimes
- ◯ Rarely
- ◯ Never

* 11. In the last three months, how many times have any of your dogs or cats experienced watery, non-bloody diarrhea that persisted from 1-2 weeks?

- ◯ None
- ◯ Once per 3 months
- ◯ Twice per 3 months
- ◯ Three times per 3 months
- ◯ More than 3 times per three months
- ◯ I don't know

Senior Veterinary Students Survey

SECTION VII - Clinical Rotations

* 1. On your senior practicum core, elective or externship rotations, have you performed the following procedures for adult cattle and or calves?

	Yes	No
Cattle rectal palpation exams	○	○
Cattle physical exams	○	○
Cattle treatments (e.g. Injections)	○	○
Diagnostic tests for cattle (e.g. abdominocentesis, blood or fecal sample collection)	○	○
Calving assistance	○	○
Breeding soundness exams (For bulls)	○	○
Calves physical exams	○	○
Calves treatments	○	○
Diagnostic tests for calves (e.g. blood or fecal sample collection)	○	○
Brucellosis vaccinations	○	○
Postmortem diagnosis	○	○

* 2. How often do you wash your hands after performing any of the procedures listed in question 1?

- ○ Always
- ○ Most of the time
- ○ Sometimes
- ○ Rarely
- ○ Never

* 3. How often do you eat or drink during or within 1 hour after performing any of the procedures listed in question 1?

- ◯ Always
- ◯ Most of the time
- ◯ Sometimes
- ◯ Rarely
- ◯ Never

* 4. How often do you wear the following personal protective equipment when working with diarrheic cattle or calves?

	Always	Most of the time	Sometimes	Rarely	Never
Exam gloves	◯	◯	◯	◯	◯
Coveralls	◯	◯	◯	◯	◯
Rubber boots	◯	◯	◯	◯	◯
Surgical mask or N95	◯	◯	◯	◯	◯

* 5. How often do you wear the following personal protective equipment when working with non-diarrheic cattle or calves?

	Always	Most of the time	Sometimes	Rarely	Never
Exam gloves	◯	◯	◯	◯	◯
Coveralls	◯	◯	◯	◯	◯
Rubber boots	◯	◯	◯	◯	◯
Surgical mask or N95	◯	◯	◯	◯	◯

* 6. How often do you wash your coveralls and rubber boots after working with cattle or calves or between farms?

- ◯ N/A if you small animal tracker
- ◯ Daily
- ◯ Twice per week
- ◯ Once per week
- ◯ Less than once per week
- ◯ Other

If you answered "Other", please specify how many times:

* 7. On your senior practicum core, elective or externship rotations, have you performed the following procedures for dogs and or cats of any age?

	Yes	No
Physical exams (e.g. Rectal exams, expression of anal glands, etc.)	◯	◯
Diagnostics (e.g. Cystocentesis, aspiration of masses, blood draw, fecal sample collection, etc.)	◯	◯
Taking animals to defecate or urinate in the designated areas	◯	◯
Change bedding	◯	◯
Cleaning exam rooms or tables	◯	◯
Cleaning cages, kennels or runs	◯	◯
Monitoring animals under sedation or anesthesia	◯	◯
Restraining animals for procedures	◯	◯
Post operative care	◯	◯
Postmortem diagnosis	◯	◯

* 8. How often do you wash your hands after performing any of the procedures listed in question 7?

- ◯ Always
- ◯ Most of the time
- ◯ Sometimes
- ◯ Rarely
- ◯ Never

* 9. How often do you wash your hands between patients?

- ◯ Always
- ◯ Most of the time
- ◯ Sometimes
- ◯ Rarely
- ◯ Never

* 10. How often do you eat or drink during or within 1 hour after performing any of the procedures listed in question 7?

- ◯ Always
- ◯ Most of the time
- ◯ Sometimes
- ◯ Rarely
- ◯ Never

* 11. How often do you wear the following personal protective equipment when working with diarrheic dogs or cats?

	Always	Most of the time	Sometimes	Rarely	Never
Exam gloves	◯	◯	◯	◯	◯
Clinic smocks	◯	◯	◯	◯	◯
Surgical gowns	◯	◯	◯	◯	◯
Masks	◯	◯	◯	◯	◯
Foot covers	◯	◯	◯	◯	◯

* 12. How often do you wear the following personal protective equipment when working with non-diarrheic dogs or cats?

	Always	Most of the time	Sometimes	Rarely	Never
Exam gloves	◯	◯	◯	◯	◯
Clinic smocks	◯	◯	◯	◯	◯
Surgical gowns	◯	◯	◯	◯	◯
Masks	◯	◯	◯	◯	◯
Foot covers	◯	◯	◯	◯	◯

APPENDIX 2: IRB LETTER OF APPROVAL I

Colorado State University
Knowledge to Go Places

Research Integrity & Compliance Review Offic
Office of the Vice President for Researc
321 General Services Building - Campus Delivery 2011 Fort Collin
C
TEL: (970) 491-15
FAX: (970) 491-22

NOTICE OF APPROVAL FOR HUMAN RESEARCH

DATE:	July 02, 2014
TO:	Lappin, Michael, Clinical Sciences
	Orton, Chris, Olea-Popelka, Francisco, Thigeel, Hana'a, Scorza, Valeria, 1678 Clinical Sciences
FROM:	Swiss, Evelyn, Coordinator, CSU IRB 2
PROTOCOL TITLE:	Evaluation of zoonotic Giardia duodenalis and Cryptosporidium spp. infection amongst veterinary students and their d and cats
FUNDING SOURCE:	Other Funding
PROTOCOL NUMBER:	14-4820H
APPROVAL PERIOD:	Approval Date: June 26, 2014 Expiration Date: April 17, 2015

The CSU Institutional Review Board (IRB) for the protection of human subjects has reviewed the protocol entitled: Evaluation of zoonotic Giardia duodenalis and Cryptosporidium spp. infection amongst veterinary students and their dogs and cats . The project has been approved for the procedures and subjects described in the protocol. This protocol must be reviewed for renewal on a yearly basis for as long as the research remains active. Should the protocol not be renewed before expirat all activities must cease until the protocol has been re-reviewed.

If approval did not accompany a proposal when it was submitted to a sponsor, it is the PI's responsibility to provide the sponsor with the approval notice.

This approval is issued under Colorado State University's Federal Wide Assurance 00000647 with the Office for Human Research Protections (OHRP). If you have questions regarding your obligations under CSU's Assurance, please do not hesitate to contact us.

Please direct any questions about the IRB's actions on this project to:

IRB Office - (970) 491-1553; RICRO_IRB@mail.Colostate.edu
Evelyn Swiss, IRB Coordinator - (970) 491-1381; Evelyn.Swiss@Colostate.edu

Swiss, Evelyn

Evelyn Swiss

Swiss, Evelyn

Approval is to recruit up to 131 participants with the approved recruitment and consent material. Because of the nature of this research, it will not be necessary to obtain a signed consent form. However, all subjects must receive a copy of the approved cover letter printed on department letterhead. The requirement of documentation of a consent form is waived under § _ _.117(c)(2).

Approval Period:	June 26, 2014 through April 17, 2015
Review Type:	EXPEDITED
IRB Number:	00000202
Funding:	Center for Companion Animal Studies

Page: 1

APPENDIX 3: IRB LETTER OF APPROVAL II

Colorado State University
Knowledge to Go Places

Research Integrity & Compliance Review Office
Office of the Vice President for Research
321 General Services Building - Campus Delivery 2011 Fort Collins, CO
TEL: (970) 491-1553
FAX: (970) 491-2293

NOTICE OF APPROVAL FOR HUMAN RESEARCH

DATE:	April 01, 2015
TO:	Lappin, Michael, Clinical Sciences Orton, Chris, Olea-Popelka, Francisco, Thigeel, Hana'a, Scorza, Valeria, 1678 Clinical Sciences
FROM:	Swiss, Evelyn, Coordinator, CSU IRB 1
PROTOCOL TITLE:	Evaluation of zoonotic Giardia duodenalis and Cryptosporidium spp. infection amongst veterinary students and their dogs and cats
FUNDING SOURCE:	Other Funding
PROTOCOL NUMBER:	14-4820H
APPROVAL PERIOD:	Approval Date: April 17, 2015 Expiration Date: April 16, 2016

The CSU Institutional Review Board (IRB) for the protection of human subjects has reviewed the protocol entitled: Evaluation of zoonotic Giardia duodenalis and Cryptosporidium spp. infection amongst veterinary students and their dogs and cats . The project has been approved for the procedures and subjects described in the protocol. This protocol must be reviewed for renewal on a yearly basis for as long as the research remains active. Should the protocol not be renewed before expiration, all activities must cease until the protocol has been re-reviewed.

If approval did not accompany a proposal when it was submitted to a sponsor, it is the PI's responsibility to provide the sponsor with the approval notice.

This approval is issued under Colorado State University's Federal Wide Assurance 00000647 with the Office for Human Research Protections (OHRP). If you have any questions regarding your obligations under CSU's Assurance, please do not hesitate to contact us.

Please direct any questions about the IRB's actions on this project to:

IRB Office - (970) 491-1553; RICRO_IRB@mail.Colostate.edu
Evelyn Swiss, IRB Coordinator - (970) 491-1381; Evelyn.Swiss@Colostate.edu

Evelyn Swiss

Swiss, Evelyn

Approval is to recruit the remaining 99 participants with the approved recruitment and consent material. Because of the nature of this research, it will not be necessary to obtain a signed consent form. However, all subjects must receive a copy of the approved cover letter printed on department letterhead. The requirement of documentation of a consent form is waived under § _ _.117(c)(2).

Approval Period:	April 17, 2015 through April 16, 2016
Review Type:	EXPEDITED
IRB Number:	00000202
Funding:	Center for Companion Animal Studies