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PARAZİTOLOJİ ANABİLİM DALI

Investigation of the prevalence of giardia infection in dogs in çankırı municipality stray animals treatments and rehabilitation centers

Yüksek Lisans Tezi

ALHAN M SHEET

Çankırı 2021

INVESTIGATION OF THE PREVALENCE OF *GIARDIA* INFECTION IN DOGS IN ÇANKIRI MUNICIPALITY STRAY ANIMALS TREATMENTS AND REHABILITATION CENTERS

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Master's Thesis

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THESIS APPROVAL

ALHAN M SHEET, graduate student of Çankırı Karatekin University, Institute of Health Sciences, has prepared her thesis titled **"Investigation of the prevalence of *Giardia* infection in dogs in Çankırı municipality stray animals treatments and rehabilitation centers"** which she prepared after fulfilling all the necessary conditions determined by the relevant regulations. She successfully presented in front of the jury with their signatures.

Thesis Defense Date : 19.08.2021

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It is stated that the thesis **"Investigation of the prevalence of *Giardia* infection in dogs in Çankırı municipality stray animals treatments and rehabilitation centers"** which was prepared in accordance with the Çankırı Karatekin University Graduate Education-Teaching and Examination Regulations, is my own and original work; To make the preparations suitable for preparation, data collection, analysis and use, to be suitable for all use, to develop the production of the thesis and to get the performance from another place, to see the thesis as a source according to Çankırı, I declare that I gave it to another scientific community for academic purposes and to take flour, and that it was screened by the "Plagiarism Detection Program" used by Çankırı University, "plagiarism free". If it is found to be contrary to this statement about my work, it will appear and can be directed in a positive way. I request that necessary actions be taken in accordance with the relevant articles of Çankırı Karatekin University Postgraduate Education and Examination Regulation.

19.08.2021

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PREFACE AND ACKNOWLEDGEMENTS

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ALHAN M SHEET

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LIST OF ACRONYMS

PCR	:	Polymerase chain reaction
kg	:	Kilogram
g	:	Gram(s)
mg	:	Miligram
mL	:	Milliliter
mm	:	Millimetre
μm	:	Micrometre(s)
μl	:	Microlitre(s)
%	:	Percent
VO	:	Orally
pH	:	Power of hydrogen
°C	:	The degree Celsius
NaCl	:	Sodium chloride
FLUTAX-2	:	Fluorescent taxoid
DNA	:	Deoxyribonucleic acid
ELISA	:	Enzyme-Linked ImmunoSorbent Assay
WSPA	:	World Society for Animal Protection
FDA	:	Food and Drug Administration
CDC	:	Center for Disease Control

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ABSTRACT

Investigation of the prevalence of *Giardia* infection in dogs in Çankırı municipality stray animals treatments and rehabilitation centers

The objective of the present study was to investigate the prevalance of infection with *Giardia* parasite in stray dogs in Cankırı Municipality Stray Animals Treatment and Rehabilitation Center from February to April 2021. One hundred fresh samples were collceted from dogs of different ages and of both gender.

In laboratory of parasitology, Veterinary Control Central Research Institute of Minister of Agriculture and Foresty, different traditional methods were used to diagnose of the parasite. In addition, Giemsa dye and Trichrome dye were also used.

The prevalence of infection by traditional methods was 43% in males and 57% in females. In the examination of pigments, the study showed that the rates of infection in young ages less than a year 71% and in ages between 1-3 years ranged between 25.8%, and at older ages, it ranged between 3.2%.

Key Words: Cankırı, Stray dog, *Giardia*, Prevalence.

2021, 48 pages

ÖZET

Çankırı Belediyesi Sokak Hayvanları Tedavi ve Rehabilitasyon Merkezindeki Köpeklerde *Giardia* Enfeksiyonun Prevalansının Araştırılması

Bu çalışmanın amacı, Şubat-Nisan 2021 tarihleri arasında Çankırı belediyesi rehabilitasyon merkezlerinde sokak köpeklerinde *Giardia* paraziti ile enfeksiyon prevalansının araştırılmasıdır. Farklı yaşlardaki ve her iki cinsiyetteki köpeklerden yüz taze örnek toplandı.

Tarım ve Orman Bakanlığı Veteriner Kontrol Merkez Araştırma Enstitüsü parazitoloji laboratuvarında parazit teşhisi için farklı geleneksel yöntemler kullanıldı. Ayrıca Giemsa boyası ve Trikrom boyası da kullanılmıştır.

Geleneksel yöntemlerle enfeksiyon prevalansı erkeklerde %43, dişilerde %57 idi. Pigmentlerin incelenmesinde, çalışma bir yaşından küçük genç yaşlarda %71 ve 1-3 yaş arası çocuklarda enfeksiyon oranlarının %25,8, daha ileri yaşlarda ise %3,2 arasında değiştiğini göstermiştir.

ANAHTAR KELİMELER: Çankırı, Sokak köpeği, *Giardia*, Prevalans.

2021, 48 sayfa

1. INTRODUCTION

Human-animal-life, which has influenced each other since the first periods of history, the van relationship continued depending on all the changing conditions. In the cities where modern life is formed over time; human-animal continued together under the same roof. But on the streets stray animals living in these cities also face the difficulties of life. They have faced. Owners living on the streets in Turkey the solution to the difficulties and problems that you animals face carried out under the responsibility of municipalities. (Healy *et al.* 2019).

Until 1991 in Turkey, only the duties and studies on stray animals that fall within the scope of responsibility after the establishment of the Ministry of Environment, preparation of the raft framework, strategy for stray animals and while assigning duties on making plans and auditing; local governments, in cooperation with voluntary organizations, animal for the protection of idle and weakened animals were asked to establish nursing homes and hospitals (Gurler and Osmanagaoglu 2009).“Temporary Animal Care Home” within the scope of municipal services Institutions named as gives. Animal that is very closely related to public health; animals living and breeding uncontrollably on the streets very important in terms of control. municipality animal shelters in general; uncontrolled in animals that are in the appropriate age range to prevent reproduction sterilization of animals, diseases transmitted from animals to humans to take protective-rescue measures to prevent Treatment of internal and external parasites of adult cats and dogs-What to do, ear tagging of adult cats and dogs to serve with the aim of ownership transactions is working (Capar and Demir, 2017). These aforementioned service areas have increased in recent years. Attention in society in the care of animal health and animal rights has become attractive. In this regard, share at the therapeutic point veterinarian, who stands in the first place with his presence among his “Implementing Regulation on the Protection of Animals” major in both legal and professional practice duties and responsibilities.

Street animals; homeless, living on the street, born on the street or stray animals thrown out of the house (Kandır and Hesna, 2014). Both the news on social media, eyes on nursing homes translated it. As a matter of fact, with stray animals living in cities, by keeping the interaction between people at a healthy level, it is the state’s responsibility to secure animal health is one of them. Today, with the urbanization process, the derelict

street where the living spaces of the animals are narrowed, regular and sufficient they cannot be fed and in terms of both public health and environmental health. It can be said that the problems increase because of this (Yiğit *et al.* 2016). In their study; derelict, weakened in Turkey housing and rehabilitation of abandoned animals about 200 animal shelters, rehabilitation centre or (temporary) nursing homes, and it has been reported that the number of animals coming here is increasing day by day Truck. In this study, animals coming to temporary care homes when evaluated with the data of the last four years, the number of it was determined that there was a 2.7-fold increase in 2018.

1.1. What are Stray Animals Rehabilitation Services in Turkish Municipalities?

In Turkey, as in most developing countries, the keeping of dogs as pets is not subject to the requirement, mandatory in other nations, that the interested party inform the competent authority of the decision to become the owner of the animal. This leads to the non-existence of an official record of possession of affectionate animals. There isn't an animal welfare law applicable to the various contexts that this concept encompasses. At the institutional level, the importance and social need for animal welfare and protection legislation is not recognized, nor has animal welfare been conceptualized in the current Cuban context. Consequently, stray dogs are already a daily image in the main cities of the country. (Istanbul Metropolitan Municipality Food, 2016).

According to the statement made by Kırklareli Municipality Veterinary Services Directorate, Turkey, with the aim of informing our citizens and raising awareness on the subject; Law No. 5199 has determined how the activities of controlled (Demirbaş and Emre 2015). Controlled animals and stray animals, inspection, control, registration and rehabilitation services will be carried out and to which institutions and persons the duties and authorities are assigned. Duties and authorities on combating epidemic diseases are carried out in accordance with the provisions of the Animal Health Police Law No. 3285 until the implementation regulations of the Veterinary Services, Plant Health, Food and Feed Law No. 5996 are published. be hosted until they are rehabilitated or adopted. It is not included in the relevant legislation that As stated in the definitions section of the Animal Protection Law No. 5199 Implementation Regulation; the centres opened by municipalities to carry out rehabilitation services for stray animals are called Temporary Nursing Homes. These are places where stray animals will animals are taken from the streets, closed in facilities structured with open and closed projects called shelters, and in

a sense, keeping them in prison. In addition, forcing animals to be kept in confinement is scientifically and ethically wrong in terms of protecting their mental and physical health.

In the efforts to solve the stray animal problem, the fact that animals are collected from the streets and housed by incarceration does not cause the problem to be solved, but causes the problem to increase to an unsolvable level. As a result of the studies carried out in the world and in our country, the problem of stray animals can be partially solved by creating the conditions for living together with people and other living things by making the animals harmless and compatible with their environment in the environment where they live, not with imprisonment under the name of shelter. To sum up, some of our citizens constantly complain about stray animals and demand that these animals be recalled, and it is known that the stray animal problem will not be solved even if their requests are fulfilled, although it is not legal in this direction. Stray animals roam the streets in search of food and shelter. They stay where they find shelter and food. If three stray dogs live in a street and they do not leave this street, it means that there is enough leftover food and shelter for three dogs in that street. A fourth dog will not have a chance to live on this street. After a certain period of time, these animals recognize the street residents and protect the street and the people living there (Ilgar, 2007).

They become harmless after they are sterilized and vaccinated by the municipality. If these animals are taken away from here, many stray dogs will come here to eat the food leftovers and to shelter. A fierce struggle begins between them for food and shelter. They pose a danger to the environment and people until they get used to it, both because of their fights among themselves and because they are strangers to the environment and street residents. Instead of the three dogs that were sheltered here first, more dogs will make this street their home. The way to find solutions to problems and to make stray animal movements harmless for the environment and human health, to live with animals, not to disturb the ecological balance and to eliminate the harmful effects of animals, the society, in an organized way, According to these provisions, rehabilitation services of municipalities consist of leaving stray animals to the place where they were taken after observation, treatment, sterilization, rabies vaccination, adoption and registration of stray animals in temporary care homes. Municipalities can perform these services to the extent that the physical conditions of the facilities they create allow.

Municipalities cannot perform the requested services within the scope of opportunities they do not have. “We will continue to continue our rehabilitation services

as far as our means allow. ”Since our physical conditions are not suitable for other species, our studies only cover stray dogs for now. However, we also treat other types of stray animals that will be brought for treatment by animal lovers if an appointment is made in advance, on the condition that they are taken by the people who bring them after the treatment. ”It is mandatory by law for pet owners who have animal caregivers to register their animals with our municipality. ”There are penal provisions for those who do not register the animals and will be applied if necessary. In today's world where the living conditions are getting worse every day, animal owners must register their animals with the Veterinary Directorate of our Municipality as soon as possible in order not to be punished for this reason. As a result of the reflection of the problems among the neighbors to the animals and the events arising from the neglect of the rights of the neighbors, many complaints about the owners of the animals are received by our Agency. In order to minimize these complaints, the responsibilities imposed on animal owners by the Animal Protection Law No. 5199 in order to inform animal owners about the issues that must be followed and paid attention to are stated below. In the penal provisions section of the same law, administrative fines and other legal sanctions to be imposed on animal owners are specified in case of unlawful acts. In order not to be punished, animal owners must take responsibility for their responsibilities. Some of our citizens want and expect our municipality to act as a court about the complaints of owned animals and to impose penalties on those responsible. Sanctions of the authorized units of our municipality, registration of animals with the municipality, environmental and public health issues cannot go beyond warning and implementation on the issues specified in the law on misdemeanors. For this reason, it is necessary for our citizens to solve such problems in legal terms. Duties and responsibilities of animal owners Article 10(1). Owners of domestic and ornamental animals or controlled animals and those who will adopt new animals;

a) In order to ensure that all transactions made to animals such as cats and dogs can be traced back and forth and kept under control; by identifying and registering the animal by veterinarians with microchip application,
b) In cases where microchip application cannot be made; By registering with the relevant municipality, including the animal and his/her own information, in the Owner Animal Registry in Annex-1,
c) To meet the ethological needs of the animal in accordance with its species; to have

the necessary vaccinations and treatments done by the veterinarian, to take care of their health,

d) In order to prevent uncontrolled reproduction, by sterilizing the cats and dogs that are fed and housed in communal areas, and in case of wanting to breed the animal, notifying the municipality to register the puppies to be born, taking care of them and/or distributing them,
e) In case of the death of owned animals such as cats and dogs, submitting the information and documents of the animal to the municipality within seven days
f) Notifying the municipality within seven days of the disappearance of an animal such as a cat or dog,
g) When tying pets and controlled animals, if necessary; paying attention to the tools and equipment used in such a way that they do not harm the animal, ensuring that the tools and equipment give the animals sufficient freedom of movement,
h) The environmental conditions in which the animals are cared for and housed; by paying attention to their being in optimum conditions suitable for their ethological and species characteristics,
i) The animal owner or the institution and organization that keeps animals, in order to control the sound level of the animal they own, the sound pressure level for a bedroom in the house closest to the living environment of the animal or animals in question, and the acceptable sound level for living rooms. By providing the values in the Regulation on the Evaluation and Management of Environmental Noise (2002/49/EC) published in the Official Gazette dated 1/7/2005 and numbered 25862, regarding the pressure level) Cleaning the feces of animals circulated outside the residence) By walking around the pet and ornamental animal or controlled animal in public places under control,
j) Dangerous animals adopted before the entry into force of this Regulation; It is responsible and responsible for wearing the mouthpiece and circling it with a leash under control, in a way that will prevent the damage to the environment during its circulating in public places (Savaşan et al, 2020).

According to the World Society for Animal Protection (WSPA), stray dogs are unsupervised animals that are not vaccinated and problematic situations arise from this. From a humanitarian point of view, abandonment reduces the quality of life of animals due to poor diet, exposure to diseases and the dangers of the public highway, where they

can be victims of accidents or be the cause of accidents traffic. From another point of view, the canine population that roams the streets causes various disorders to the public, including the possibility of being bitten or falling ill due to fecal materials spread in parks and streets that constitute potential risks of infectious or zoonotic diseases, such as parasitosis (Mentes and Ayse, 2007).

A determining factor in the dynamics of stray dog populations is human behavior. Therefore, it is important to recognize and assume that promoting responsible and rewarding interactions between animals and humans will lead to both an improvement in animal welfare and a reduction in many sources of stray dogs. In the province of Ciego de Ávila, the number of dogs with a home is estimated at 42,700 and the vagrant canine population at 2,177, according to data from the Provincial Center for Hygiene, Epidemiology and Microbiology at the end of 2015. Stray dogs, like all living things, they can get sick and require care by veterinary doctors. For this reason, diseases with risk of zoonotic transmission represent a public health problem in Avilanian. Canids are hosts of various parasites, among which the most common and widely disseminated gastroenteric nematodes, cestodes and protozoa are identified. In the excrement of dogs there are many parasite eggs in different stages of development that contaminate the environment. When the excrement dries, it is carried by the wind and thus pathogens enter the respiratory system or the digestive system of humans when consuming food contaminated with excreta (Robertson et al,. 2002).

Municipalities carry out their services based on legal article: The first of these is the Metropolitan Municipality Law, which was published in the Official Gazette dated 23.07.2004 and numbered 25531. In the relevant paragraph of this law article; In the integrity served by the Metropolitan Municipalities; It has defined the duties, powers and responsibilities of building, having, operating, and operating regional parks, zoos, animal shelters and similar places. In the relevant section of the “Regulation on Voluntary Participation in Special Provincial Administration and Municipal Services”, which was published in the Official Gazette dated 09.10.2005 and numbered 25961 within the scope of the Turkish Union of Municipalities Legislation, municipalities carry out their services on the basis of providing services for stray and stray animals or employing volunteers. Again, the Union of Municipalities of Turkey, which entered into force by being published in the Official Gazette dated 11.04.2007 and numbered 26490; Health-related duties are also included in the relevant section of the Municipal Police Regulation4. Based on the

Animal Health and Police Law No. 3285 and the relevant regulations, in case of disease or animal deaths of unknown origin, to temporarily cordon off these places and inform the authorities in this regard. It has been determined as providing all kinds of aid, helping the culling of animals that need to be destroyed, and having them destroyed in a way that will not harm human health. Another legal basis is; it is the use of the powers given to municipalities within the scope of their duties by the Law No. 5199 on the Protection of Animals, which is included in the Municipal Police Regulation4.

1.2. A brief view stray animal rehabilitation centres and their services in Turkey

Çankaya Municipality Unattended Stray Animal Care and Rehabilitation Center found a warm home for over a thousand stray animals in 2016 as well as every year. The Center which found lifetime human friends for a total of 1 thousand 473 stray animals, 188 of which were cats and 285 of which were dogs has brought the animals together with their new warm homes. An average of 52 animals were treated per day in Çankaya Municipality Unattended Stray Animal Care and Rehabilitation Center. The total number of treatments provided within the center during the year was 19 thousand 78. In addition to simple injuries and illnesses, the diseases that require special care such as cancer are being treated within the center.

Küçükçekmece Municipality Stray Animals Rehabilitation Center provides treatment-oriented services with modern and advanced technological methods. Our new center, which became operational in 2020, was established on an 8-decare land. 2 acres of the area is designed as indoor and 6 acres of open space. Many diseases can be diagnosed in this laboratory, which is designed to monitor blood and biochemical parameters for our hospitalized patients with systemic disease who are treated in our hospital. A microscope with an LCD screen is used for detailed diagnosis of skin lesions. In this hospital, dental calculus cleaning and tooth extractions are performed for our animals that have nutritional difficulties due to dental problems.

The aims of the Stray Animal Neutering and Rehabilitation Center established within Beşiktaş Municipality are: Adopting animals, - Reducing the number of stray animals with humane methods, - and Preventing zoonotic diseases caused by stray animals, - Preventing complaints from animals and following bite cases. Stray Animals Rehabilitation Center evaluates citizen complaints or catches animals as a result of routine capture studies. Afterwards, the animals are brought to the healing center where

they are kept under observation. In the rehabilitation center; sterilization operation, micro-chip, numbered ear tag insertion, adoption, rabies vaccination and withdrawal of animals are carried out.

Stray Animals and Rehabilitation Center of Konya Metropolitan Municipality, which is Turkey's largest and most modern animal shelter with an area of 1 million square meters, is adopted after health checks. Konya Metropolitan Municipality Mayor Uğur İbrahim Altay stated that they have adopted more than 4,500 animals treated in the shelter with the Volunteer Animal Friends project for the first time in Turkey, and that they support individuals and families who undertake to adopt stray animals with 125 TL per dog per month.

Stray Animals Rehabilitation Center located in Kapıçam area is 18459,30 m It has a capacity of 250 dogs established on the area and serves with 4 Veterinarians and 11 Shelter Staff. After the routine examinations of the stray animals brought to our shelter by our teams, they are taken into cages according to their health status and the treatment process is started. Earrings that write Kahramanmaraş Metropolitan Municipality and registration number are attached to the ears of our dogs. After rabies vaccination is done to our stray animals, which are kept in quarantine for ten days, ovariohysterectomy is performed for female animals and castration surgery is performed for male animals in order to control reproduction. After the neutering activity, our stray cats and dogs are given postoperative treatment and care-feeding. Kadıköy Municipality launched the Stray Animals Rehabilitation and Training Center. In the center opened in Merdivenköy, full care is provided for stray animals

In Stray Animals Rehabilitation and Training Center under our Directorate, services such as examination, diagnosis, treatment, vaccination, surgery, recording, microchipping are provided to all animals within the scope of rehabilitation of stray animals and combating zoonotic diseases (diseases transmitted from animals to animals and humans). Only municipality registration is carried out for owned animals.(Babazadeh and Daryoush, 2014).

Van Metropolitan Municipality opened the doors of the Animal Care and Rehabilitation Center to animal lovers on October 4, World Animal Day, and made internal and external parasite vaccinations of 180 stray animals. Veterinarians working within the Department of Health Affairs administered internal and external parasite vaccinations of 180 stray animals identified throughout the province. The teams that

bring animals in need of care to the center for treatment also routinely leave food at 75 feeding points throughout the province.

Due to the close contact of dogs with their owners, the possibility of a cross infection arises, which is why it is important to determine the presence of *Giardia* spp., in pets, since it constitutes the increase in the possibilities of contagion towards their owners; which is a causal agent of various gastrointestinal and diarrhoeal conditions. The greatest risk of contracting the disease from this protozoan occurs in dogs that have not followed up on their deworming scheme due to lack of commitment on the part of the owners to preserve their health; and by contact with infected dogs where there is a greater opportunity for direct and indirect transmission of the parasite; because as it is an aggressive agent at the gastrointestinal level, it favors relapses in patients as they remain asymptomatic carriers. Dogs that present *Giardia* spp., promote a zoonotic risk; therefore, it is necessary to establish educational programs to prevent the possibility of contagion. In general, a good sanitary, nutritional and immunological state prevents to a certain extent the appearance of parasitosis. Similarly, stressful situations and/or pathological processes favor the settlement of the parasite and its subsequent development (ESCCAP, 2011). In Valle de Bravo there is no history of reports of contagion of *Giardia* spp., because it usually goes unnoticed and is classified as a gastrointestinal disease. Therefore, this study was carried out to diagnose the presence of this protozoan and inform veterinary clinics about the presence of this parasite and be able to present an alternative to the veterinarian and dog owners to prevent, identify and control this disease. The importance of this study will develop a greater knowledge of this parasitosis, preventing its spread to other dogs, providing information on the results to the health authorities to reduce the problem detected through animal health campaigns; as well as the dissemination in schools about this parasite that allows its identification; since the aim of the veterinarian is to maintain the health of human beings through good animal health. General position of animal shelters/care centres in the Aegean Region reveal the situation and identify existing problems made for the purpose.

The objective of the present study was to investigate the prevalance of infection with *Giardia* parasite in stray dogs in Cankırı Municipality Stray Animals Treatment and Rehabilitation Center.

2. LITERATURES REVIEW

Gastrointestinal problems caused by parasites are the most common queries in veterinary clinics. Many of these diseases are zoonotic, such as giardiasis; which constitutes parasitizes of great epidemiological and clinical importance due to its high pathogenicity in animals and humans (Tamer et al, 2015). Giardiasis is a parasitic disease of man and some animals, caused by the flagellated protozoan *Giardia* spp., Located in the intestinal tract; causing diarrhoeal disease. Companion animals are capable of transmitting a number of zoonotic diseases to their owners, including giardiasis, but the magnitude of this risk is not well known; because it is currently classified into genotypes from A to G. Being A and B infective for man. In other countries, 10% have been identified in well-cared adult dogs, rising to 36–50% in puppies and up to 100% in farm animals. In stool tests, it is advisable to take into account the detection of *Giardia* spp., to avoid risks to the health of dogs and human health at the time of being in contact with animals infected with this parasite. Because animals, in this case dogs, represent a source of contagion for humans; The purpose of this study was carried out in order to identify the presence of *Giardia* spp., in dogs from the central area of Valle de Bravo; using two methods of laboratory diagnosis for the detection of this intestinal parasite that threatens the life of the known faithful companion (Einarsson *et al.* 2016).

2.1. History of *Giardia* spp.

The protozoan *Giardia* spp., was described shortly after the invention of the microscope in 1681 by scientist Anton van Leeuwenhoek. Leeuwenhoek verified that he was infected by the parasite himself and, when observing his diarrhoeal feces under the microscope, he found that they contained mobile “animalunculi”. In 1859 the Czech physician Vilém Dusan Lambl presented the first morphological description. He described in detail the protozoan he found when analyzing stool from children who had symptoms of diarrhoea. Thinking that it was a non-pathogenic organism, Lambl named it Cercomas intestinalis. Giovanni Batista Grassi, in 1879, reported for the first time the occurrence of the parasite in animals. He observed the protozoan in rodents, suggesting the name Dimorphus muris, unaware of the description already made by Lambl. Grassi also found that the form of the parasite found in man was identical to other findings in domestic animals. However, it was Kunstler in 1882 who first used the name *Giardia*

when describing a parasite flagellated in the intestine of anuran amphibians tadpoles, thus creating the genus *Giardia*. Throughout the research, several names were proposed by different scientists: *Lamblia intestinalis*, *Giardia duodenalis*, *Giardia enterica* (Fig 2.1).

In 1915, the team of zoologist Charles Stiles created the name *Giardia lamblia* to honor Professor Alfred Mathieu Giard of Paris and Dr. Vilém Dusan Lambl of Prague. The morphological description of the genus was published by Francis Filici in 1952 who classified the species into three distinct groups: *G. lamblia*, *Giardia muris* and *Giardia agilis* (Leung *et al.* 2019). With the advent of electron microscopy, it was possible to distinguish new species that until then were thought to be *Giardia lamblia*. They were *Giardia psittaci*, *Giardia ardeae* and *Giardia microti*. The World Health Organization (WHO) in 1979 recognized the zoonotic potential of *Giardia* spp. and in 2004 it was included by the WHO as part of the "Neglected Diseases Initiative" Nowadays, *Giardia lamblia* is also called *Giardia intestinalis* and *G. duodenalis* (Tauber and Joseph, 2016).

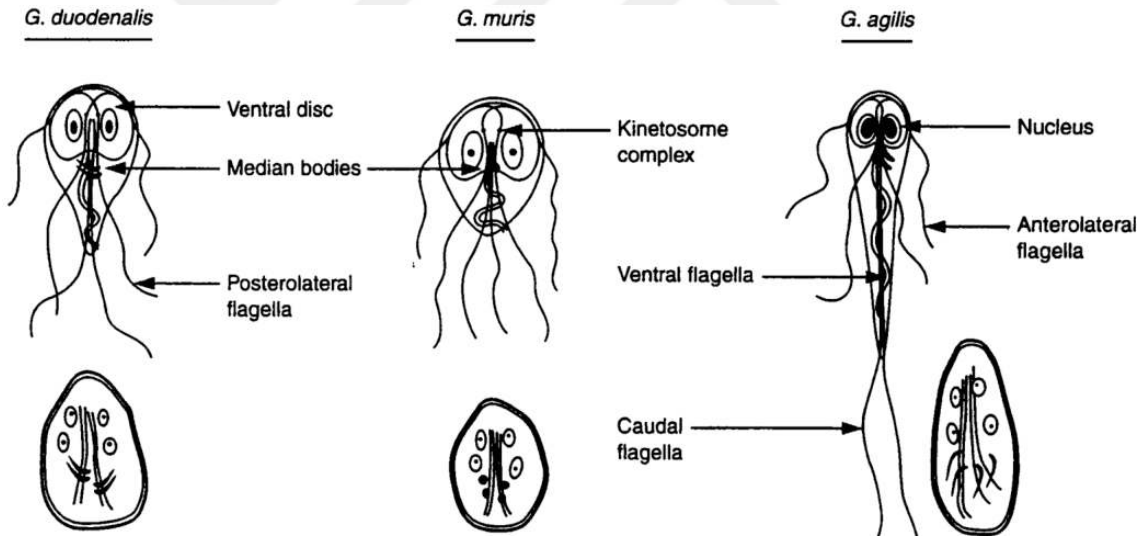

Figure 2.1. Three species of *Giardia*: *Giardia duodenalis*, *G. muris* and *G. agilis* (Tauber and Joseph, 2016).

2.1.1. Infectious agent

It is a protozoan with a pyriform appearance, provided with eight flagella and a pair of suction cups that cause serious damage to the intestinal mucosa of its hosts. Characterized by two phases or stages: (Fig 2.2)

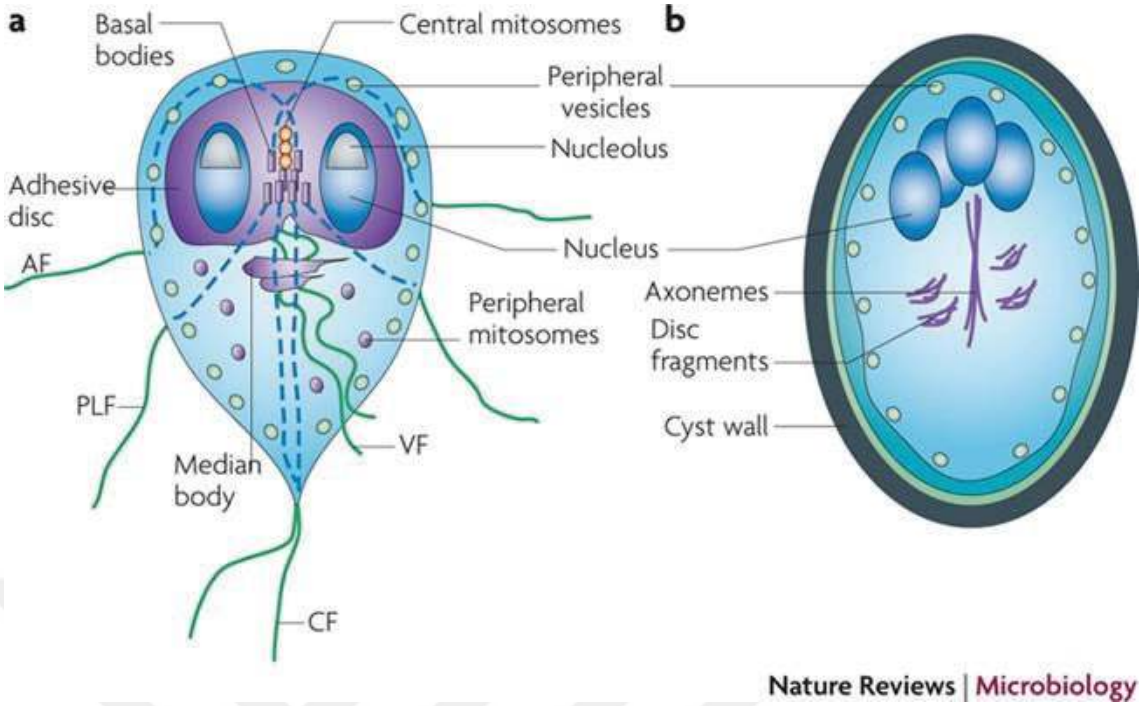

Figure 2.2. Stages *Giardia* spp. Trophozoit form (a) Cyst form (b) (Plutzer *et al.* 2010).

2.1.2. Taxonomy and classification

For a long time, the genus *Giardia* was classified based on its morphology, which included it as part of the Phylum Sarcomastigophora, Subphylum Mastigophora (flagellates), Class Zoomastigophorea, Order Diplomonadida and Family Hexamitidae.(Martínez *et al.* 2014).

However (Plutzer *et al.* 2010). reported a new nomenclature based on genetic, structural and biochemical data with a new classification: the Phylum Metamonada, Subphylum Trichozoa, Superclass Eophanryngia, Class Trepomonadea, Subclass Diplozoa, Order Giardiida and Family Giardiidae. This peculiar parasite has attracted attention not only for its medical and veterinary importance, but also because of its "primitive nature", having been described as a biological fossil, as it is a true eukaryote which, however, retained peculiarities of prokaryote ancestors such as the absence of mitochondria, peroxisomes and a traditional golgi apparatus. The genus *Giardia* thus remains a key organism in the attempt to understand the evolution of eukaryotic cells.

2.2. General morphology

Protozoa of the genus *Giardia* are eukaryotic, unicellular, binucleate, flagellate and facultative anaerobes, tolerant to small amounts of oxygen. However, genes from proteins considered mitochondrial markers have been identified. These proteins, IscU

and IscS, were found in organelles called mitosomes, considered remnant mitochondria. The microorganism's life cycle has two stages; trophozoite, a vegetative form that adheres to the intestinal microvilli, and the cyst, an infective form, capable of persisting in the environment under different conditions (Fig 2.3 - Fig 1.4).

This parasite has a microtubule-structured cytoskeleton complex, composed of tubulins, with the function of providing maintenance of shape, cell organization, cytoplasmic transport, motility and cell division. The cytoskeleton of *Giardia* spp. is mainly represented by the ventral adhesive disc, flagella, funnels, nuclei and by the medial bodies. The golgi complex is only visible during the encystment phase, and its presence in the trophozoite is not confirmed, although the observation of stacked membranes suggests its existence (Sulaiman *et al.* 2006).

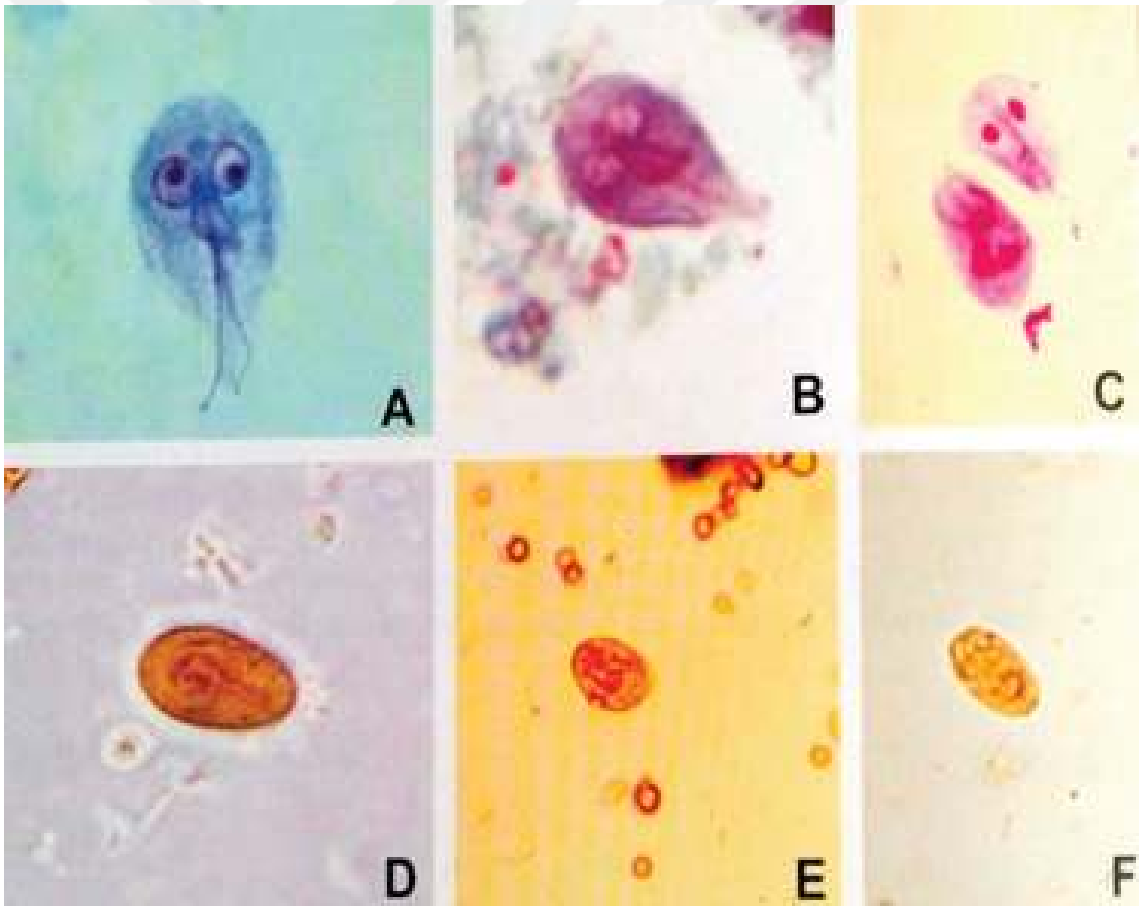

Figure 2.3. *Giardia lambia* trophozoites and cysts seen under a microscope . A and B- Trichrome-stained trophozoites; C- Giemsa-stained trophozoites: D, E and F. Cysts stained by iodine solution.

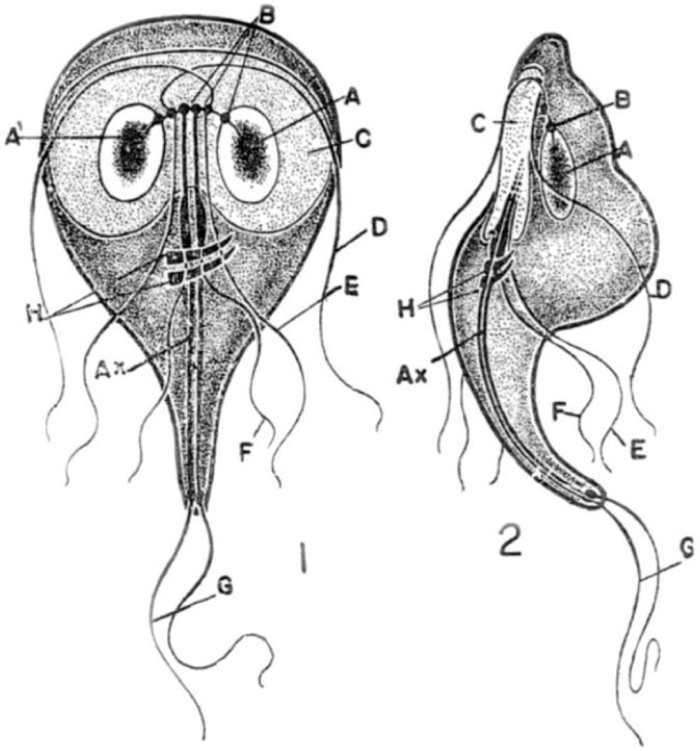

Figure 2.4. Several structures of the *Giardia lambia* trophozoite 1.Front view 2.Side view A and A. Nuclei, B. Blepharoplasts, C. Ventral disc D. Anterior flagellum E - Median flagellum F Ventral flagellum G. Caudal flagellum H. Parabasal bodies Ax- Axonemas

2.2.1. Trophozoites

The trophozoites (Fig 2.4) are the active form of the parasite, are mobile and adapted to fixation on the epithelial cells of the mucosa of the small intestine. They are associated with the clinical presentation of the disease and are occasionally found in stools, especially if these are diarrhoeal and, due to the increase in peristalsis, are expelled before encystment. It does not survive for long outside the host and, therefore, does not cause infection. Trophozoites usually give rise to infective cysts before being eliminated in the faeces. They have a teardrop shape, with a compressed face forming the adhesive disk. Inside the cell are two nuclei, each with a large endosome, which makes the organism look like a tennis racket with eyes when viewed from above under a microscope. (Pessoa and Samuel, 1967), described the body of *Giardia* spp. as pear-shaped, rounded anteriorly and slender posteriorly. According to (Ivanov and Andrey, 2010), this protozoan is piriform, with bilateral symmetry and its dimensions

vary from 12 to 15μm in length by 6 to 8μm in width. The flexible dorsal surface can be lifted upwards towards the convex surface. On the ventral surface, on each side, there is a disk, in the shape of a suction cup that allows the flagellate to attach itself to the epithelial cells. The perception of differences between species is detectable at the molecular and morphological level. In particular through the shape of the trophozoite, the medial bodies and the size of the ventral disc (Adam and Rodney, 2001).

2.2.2. Ventral adhesive disc

It is a rigid and indispensable structure for the parasite's survival as it allows its adherence to the intestinal villi (Fig 2.5). The ventral disc (Fig 2.4 – C) is specific to *G. lambia* and is very important for the constitution of the cytoskeleton. It has a concave shape that covers the entire ventral surface with a maximum depth of 0.4 mm. Formed by a spiral arrangement of microtubules interconnected by small filaments/ribbons. Each microtubule has three accessory structures: two short lateral filaments connecting it to the ventral membrane and a bandlike projection (called a ribbon) that extends to the dorsal region of the disc cytoplasm. The center of the adhesive disk has a region that does not have cytoskeletal proteins, called the uncovered area or bare zone. Occasionally, a protrusion is seen in the bare zone, containing glycogen and cytoskeletal proteins, but with no known function. In 1981, Holberton and Ward, identified the proteins that were part of the disc, having been designated as giardins. The giardins (a, b and g) are found in the dorsal ribbons of the microtubules. According to (Adam and Rodney, 2001), 23 forms of giardins have already been identified and some of them, such as a1, a2, b and g, were submitted to genome cloning and sequencing. Knowledge of the genes that encode these proteins enabled the development of techniques based on molecular biology (PCR, Polymerase Chain Reaction) for the specific detection of the *Giardia* protozoan, present in human, animal and water feces. Only in the microtubules of the ventral disc are other proteins called tubulins found, but not in the ribbons or flagella. It is suspected that drugs used to treat giardiasis, react with these proteins tubulin and giardin, preventing the adhesion of the parasite to the mucosa and causing rupture of the ventral disc (Terra, 2008). The disc has a horseshoe shape, with a dense fibrous structure at its outer end called the lateral crest. During parasite adhesion, the ventral flagella beat fast and draw fluid from under the ventral disc. The lateral crest seals this set in the substrate, creating a low pressure zone, which would cause the parasite to adhere to the intestinal epithelium.

(House and Susan, 2012) identified proteins from the giardin family (Alpha-1, Beta, Delta and Gamma) and two isoforms of tubulin (Alpha-2 tubulin and Beta-tubulin), in addition to a new protein, SALP-1, that are part of the constitution of the ventral disc (Terra, 2008), in his master's thesis, identified 13 proteins in the ventral disc by mass spectrometry. Of these 13 proteins, 9 have a known function and location, which indicates the importance of studies related to the parasite. During the encystment process the ventral disc undergoes some modifications. It was observed that the ventral disc breaks, forming a set with four fragments that are stored inside the cyst.

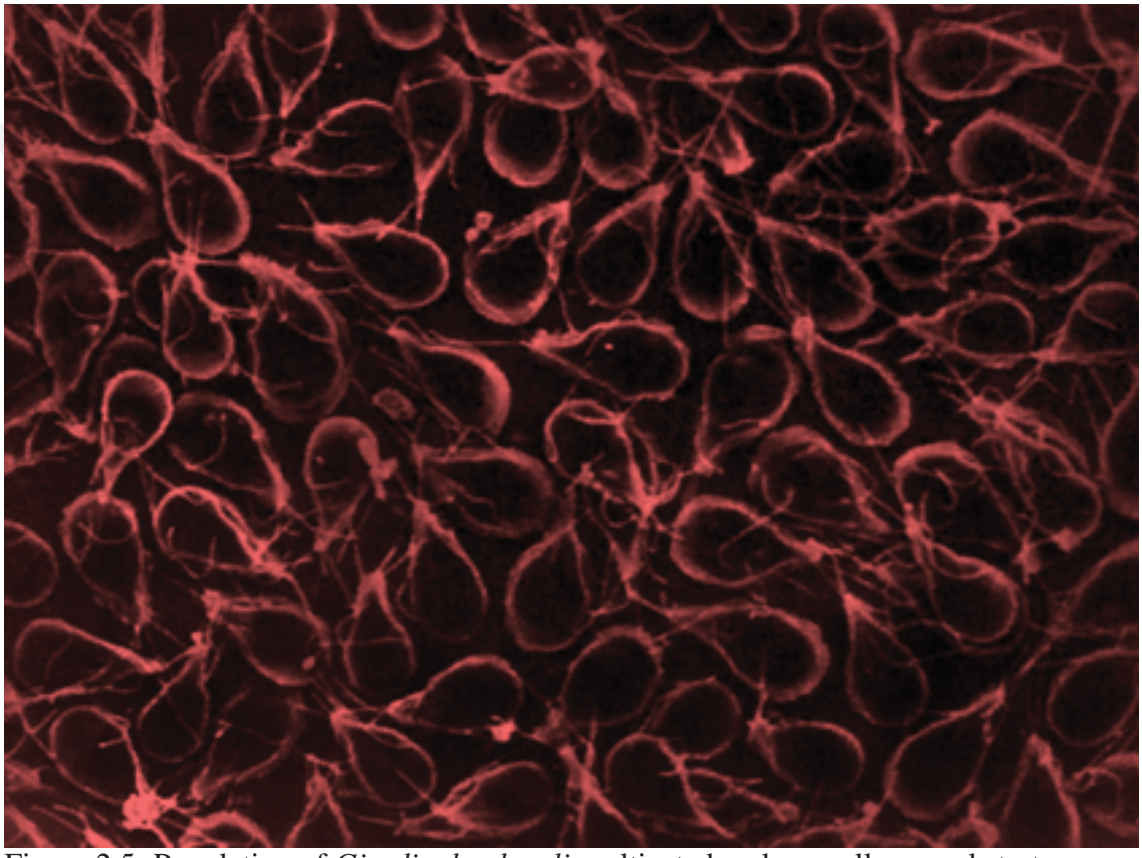

Figure 2.5. Population of *Giardia duodenalis* cultivated under a collagen substrate, where the trophozoites attach themselves through the ventral disc (Carranza *et al.* 2009).

2.2.3. Nuclei

Nuclei (Fig 2.4 – A) are located inside the cytoplasm, where structures are almost always double and symmetrical. Trophozoites have two oval nuclei, having a large central karyosome, which can appear as a mass of granules or as a solid, oval mass. There are two parallel axonemes, which originate at a distance from the anterior end and end at the posterior end in two blepharoplasts, while the anterior end of these axonemes

has (each) a blepharoplast, in connection with a structure called the centrosome, in the anterior pole of the nucleus. The nuclear membrane is double, perforated by nuclear pores that allow transport between membranes. Actin filaments from the cytoskeleton appear important for spatial rigidity, for the position of nuclei and possibly also for their segregation on cell division. Each nucleus contains five chromosomes, with equal amounts of deoxyribonucleic acid (DNA), which replicate at almost the same time. Furthermore, diploid nuclei have practically identical transcriptional activity (Terra, 2008; Carranza *et al.* 2009).

2.2.4. Flagella

The flagella, eight in number, originate from the basal bodies located close to the nuclei and emerge from the anterior, posterior, ventral and caudal regions of the trophozoite (Fig 2.4-D,E,F,G). Their function is related to mobility, but they can also prevent trophozoites from being dragged by intestinal peristaltic movements, however, they are not involved in adhesion to the host's intestine (Terra, 2008). The activity of the flagellum gives them a rapid and irregular movement, however, they adhere in large quantities to the mucosal surface, thanks to the ventral adhesive disc, forming an extensive coating, thus interfering with the absorption of fats and fat-soluble vitamins, especially vitamin A. It is assumed that scourges can play an important role in the disenchantment phase, as they appear early in this process (Terra, 2008).

2.3. *Giardia* species

Over the years, *Giardia* species have been described based on hosts, which has led to the description of more than fifty species (Ivanov and Andrey, 2010). However, in 1952, Filice proposed a new assessment based on morphological characteristics, setting aside the criterion related to the host. Six species of *Giardia* (Fig 2.6) were distinguished and characterized based on light microscopy, by observing the shape of the trophozoite and the median body, and also by electron microscopy in relation to the flagella and ventral disc. Five of these species refer to isolates obtained from amphibians (*G. agilis*), birds (*G. ardeae*, *Giardia psittaci*), rodents (*G. muris*) and mice (*G. microti*). The other species, G. duodenalis (Table 2.1) infects humans as well as dogs, cats, cattle, pigs, sheep and horses (Fekete *et al.* 2021).

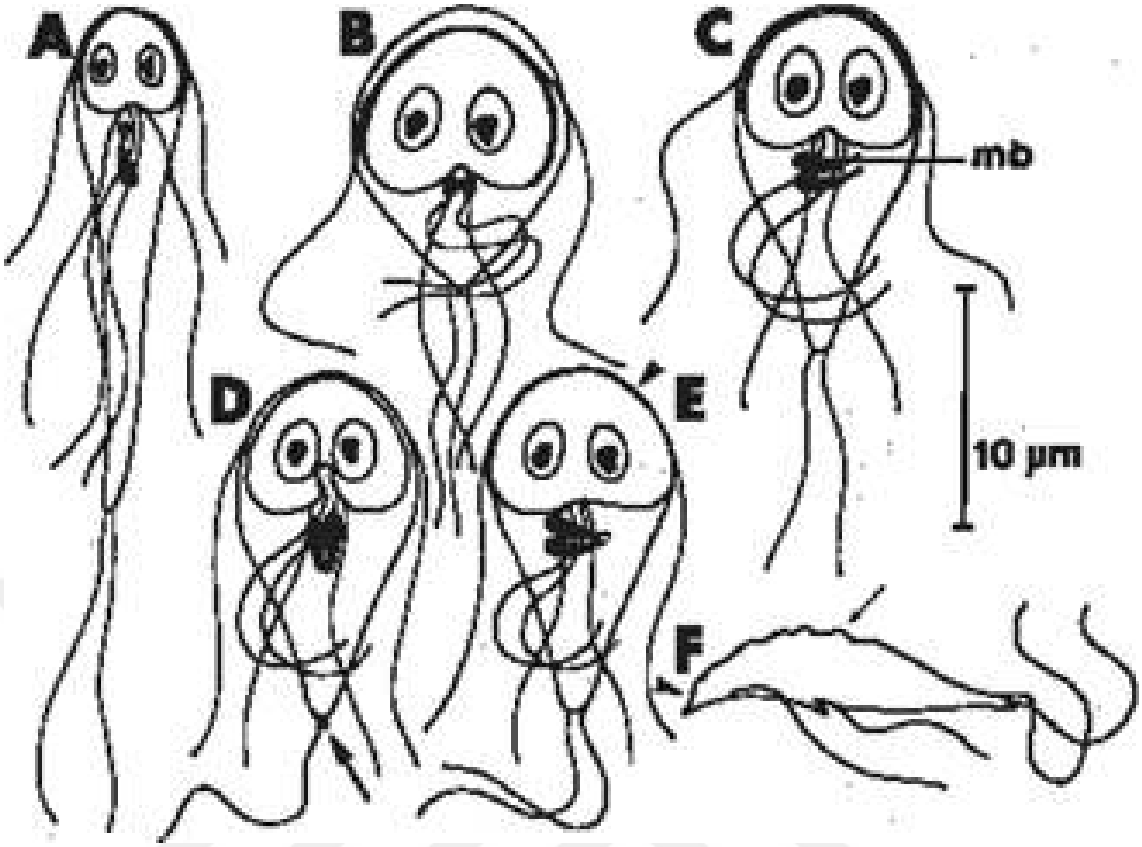

Figure 2.6. *Giardia species* -(A) *G. agilis* (B) *G. muris* (C) *G. duodenalis* (D) *G. ardeae* (E - F) *G. Psittaci*.

Table 2.1 Species of *Giardia* spp. and their hosts (Quispe and Antonio, 2017)

Species	Hospedeiros	Morphological characteristics	*DImensions of the trophozoite*
Giardia duodenalis	Domestic mammals, wild e or homem	Pear-shaped trophozoite with hook-shaped medial bodies.	***12-15µm 6-8µm***
Giardia agilis	Amphibians	Long, slender trophozoite with medium bodies in the form of an elongated drop.	***20-30µm 4-5µm***
Giardia muris	Rodents	Rounded trophozoite with small, rounded median bodies.	***9-12µm 5-7µm***
Giardia ardeae	Birds	Rounded trophozoite, with prominent notch on ventral disc and rudimentary caudal flagellum. claw-shaped oval medial bodies.	***10µm 6,5µm***
Giardia psittaci	Birds	Pear-shaped trophozoite and claw-shaped medial bodies.	***14µm 6µm***
Giardia microti	Rodents	A trophozoite similar to G. duodenalis. The cyst contains trophozoites fully differentiated.	***12-15µm 6-8µm***

2.4. Life cycle

Giardia spp., has a direct biological cycle, with the asexual production of trophozoites (active and mobile forms) that adhere to epithelial cells in the small

intestine in which they evolve into cysts (forms of resistance) that arrive in large numbers to the feces along with those that will be released intermittently. Ingestion of these cysts restarts the cycle of this protozoan. The prepatency period is 4-16 days and the patency period is usually several weeks or even months. Cyst excretion has been observed both in healthy animals and in animals with clinical signs. An initial infection induces partial immunity reducing the severity of the clinical picture and sometimes even the elimination of the pathogen, however there is limited resistance to reinfection. The infection is of the fecal-oral type due to the ingestion of the cysts present in the fur, water, food or in the environment (Fig 2.7): only a few oocysts are necessary to infect the animal. Although oocysts are sensitive to desiccation and low temperatures (their number decreases in the environment during winter), they can survive outdoors for several months. In addition to other vertebrates, including wild animals, man can also be a host (Montoya *et al.* 2007).

Therefore the infection process includes two phases:

- Trophozoite: (Vegetative form). It lives in the small intestine, being responsible for the clinical manifestations

- Cyst: (Form of resistance). Capsule that allows you to live in harsh environments for a long time. Responsible for the transmission of the parasite. The dog is infected by ingesting the cyst.

The *Giardia* cycle is monoxene (Fig 2.8), European Scientifique Companion Animal Parasites, and starts with the ingestion of cysts present in water, contaminated food or infected feces. However, it is known that direct transmission through the fecal-oral route, from animal to animal or from animal to man can also occur. Upon exposure to gastric juice, acidic pH and pancreatic enzymes, the cyst ruptures. This process is known as decystment and occurs in the anterior region of the small intestine. A cyst turns into two trophozoites (Esch *et al.* 2013).

The trophozoites adhere to and colonize the mucosal surface of the small intestine, causing the disease that can often be asymptomatic. At this moment, they multiply asexually by binary division. There are factors that influence trophozoite fixation, which can be inhibited by temperatures below 37°C, increased oxygen levels or decreased cysteine concentration. *In vitro* studies have shown that the optimum pH is 4.0 and that pancreatic proteases facilitate the process, occurring quickly and taking about 10 to 15 minutes. The action of bile salts and host immune response mechanisms, responsible for

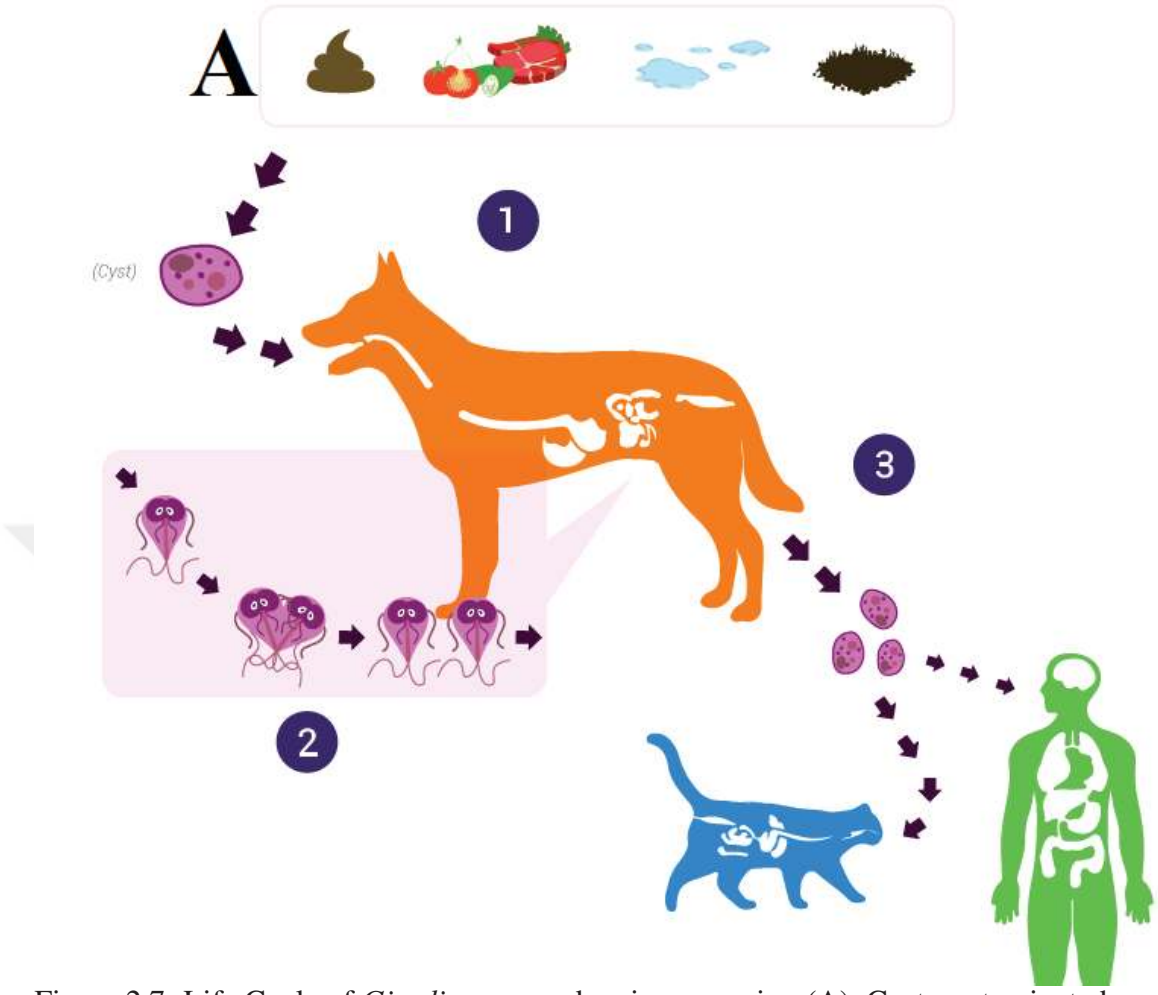

Figure 2.7. Life Cycle of *Giardia* spp., and main reservoirs. (A). Cyst-contaminated waste, food, water, soil or other sources. 1. If a dog or other animal or human comes into contact with a giardia cyst, they ingest it and the body becomes infected with giardiasis. 2. *Giardia* cyst is excreted in the body. It survives for months in the environment with the potential to infect others. 3. *Giardia* is now in the form of trophozoites in the small intestine. The host absorbs its nutrients and reproduces (Yulia, 2021).

the detachment of trophozoites from the mucosa, induce encystment (Fig 2.8) thus enabling the release of cysts along with feces into the environment. When the encystment process is complete, the outer portion of the trophozoites becomes rounded and filamentous, they lose mobility and are no longer attached to the intestinal mucosa. The elimination of cysts by an infected host is not constant and can disappear from the feces for periods of 7 to 10 days. The number of cysts present in infected feces varies from 300 million to 14 billion. In case of severe diarrhoea, the trophozoites are released along with the cysts into the environment through the faeces. However, trophozoites do not survive in the external environment. The cysts found in feces can contaminate water,

food, people and animals, thus closing the cycle. The pre-patent period of *Giardia* in dogs and cats is 5-16 days, 7-8 days in cattle, 6-10 days in goats and 10-21 days in sheep, with cyst release being cyclical. Temperature interferes with the biological cycle of the protozoan, where the highest incidence is observed in seasons when the temperature is colder. Seasonal variations are one of the most common factors for the prevalence of parasites in dogs (Quispe and Antonio, 2017).

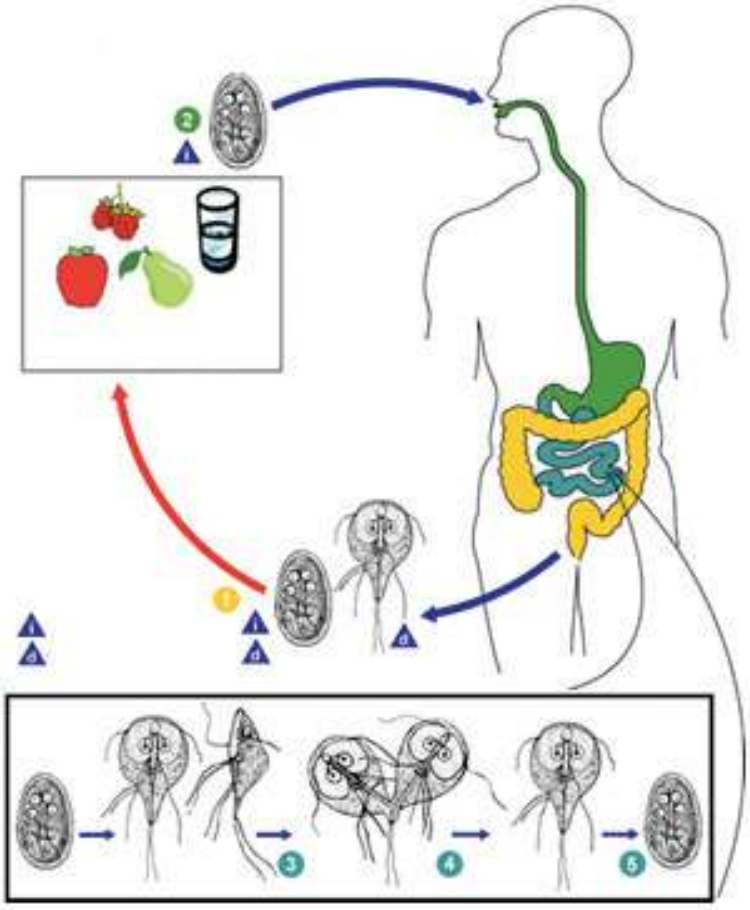

Figure 2.8. *Giardia duodenalis* cycle (1). The cysts are resistant and can survive for several months in cold water. Infection occurs through ingestion of the cysts via water, contaminated food or fecal-oral contamination (2). In the small intestine, the excystment process occurs in which each cyst produces two trophozoites (3). The trophozoites multiply by binary longitudinal division and remain in the lumen of the proximal small intestine, where they adhere to the mucosa through the ventral disc (4). Encryption occurs when parasites transit to the colon. The cyst is the most common stage in non-diarrheic stools, being the infective stage (5). (Adapted from: CDC-USA Center for Disease Control)

2.5. Clinical signs

Commonly asymptomatic. Acute, chronic and intermittent diarrhoea. In most cases, the infection is subclinical, but in immunocompromised animals and in puppies and kittens co-infected with other digestive pathogens (viruses or bacteria), *Giardia* spp., can cause intermittent mucus diarrhoea or persistent diarrhoea (Fig 2.9) with steatorrhea, anorexia, vomiting, loss of appetite and apathy. The presence of *Giardia* licking in the duodenal-jejunal mucosa, inserted into the cells through their ventral adhesive discs, can induce irritation or aggravate existing inflammatory conditions, thus causing chronic diarrhoea. When trophozoites or *Giardia* spp. they are present in small numbers, do not produce clinical disease, however, in large quantities or in a host with low immunological resistance, diarrhoea may occur (Erdem *et al.* 2006).

The number of ingested cysts plays an important role in the pathogenesis of the disease. In humans, 10 to 100 cysts are needed to establish an infection, whereas in animals, ingesting only 10 cysts is capable of causing the infection. Inanimate objects such as food bowls and cages in catteries and kennels can serve as a reservoir for infective cysts. The pathophysiology is believed to involve enterocyte apoptosis, villous atrophy, intestinal barrier dysfunction and lymphocyte activation leading to poor absorption of nutrients in the small intestine and increased intestinal transit. Mechanical disturbances, determined by a large number of parasites, in the duodenal mucosa interfere with the absorption of fat, leading the patient to develop a deficiency of vitamins, especially fat-soluble ones, and the onset of diarrhoea with steatorrhea, abdominal cramps, frequent bowel movements and weight loss. Steatorrhea is due to decreased lipase activity, increased mucin production, and decreased intestinal absorption. Due to reduced intestinal absorption, some dermatological signs caused by deficiency of fat-soluble vitamins are also common. Large numbers of parasites inhibit the absorption of simple sugars and disaccharides that are fermented by bacterial flora, creating intestinal gas. Sugars have an osmotic effect and attract water to the intestinal light. The result is distension of the small intestine by fluids and gases. (Erdem *et al.* 2006) reported that *Giardia* infection can cause hypersecretion of hydrochloric ions. This change is caused by the combination of the parasite, toxins and host immune factors such as CD8+ lymphocyte cells. The clinical signs of giardiasis range from mild to severe and may be persistent, intermittent or self-limiting.

They can be confused with other parasitic, viral or bacterial gastrointestinal

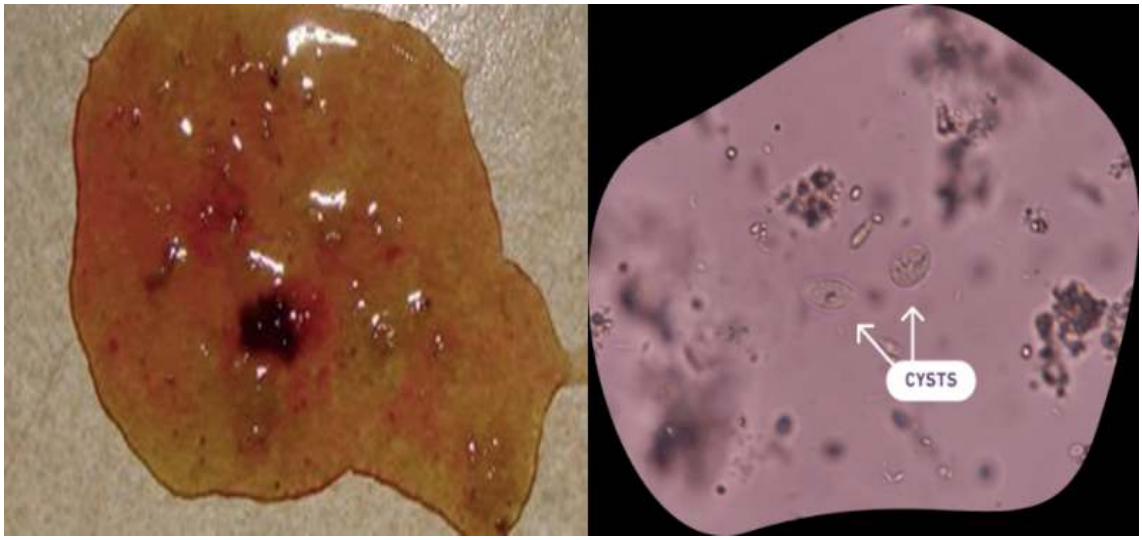

Figure 2.9. Diarrhoea caused by *Giardia* (Cavalini and Zapa, 2011).

infections. They also resemble food-borne allergies, malabsorption disease, drug-induced gastroenteritis, and allergic diseases. Giardiasis is considered the main cause of diarrhoea in young farm animals, over 30 days of age, leading to decreased feed efficiency and animal development with repercussions on carcass weight, having a great economic impact on production systems. Symptoms in humans differ from individual to individual and may depend on several factors, such as inoculum size, duration of infection, individual host factors and interference from factors related to the parasite. The disease can present itself subclinical, mainly in ruminants, dogs and cats. Infection in animals such as chinchillas and birds have been reported as fatal cases. (Gultekin et al, 2017), described as the main clinical signs of animals infected with the parasite, chronic pasty diarrhoea, weight loss, lethargy and insufficient development in puppies. In dogs, diarrhoea may start as early as five days after exposure to the agent. Being acute or chronic, causing lethargy, loss or decrease in weight gain despite these maintaining normal appetite. In cats, trophozoites are usually found in the jejunum and ileum rather than the duodenum. The main clinical sign is persistent diarrhoea resulting in intestinal malabsorption. The stools are mucoid, pale, soft and fetid (Fig 2.9). In a case report of a cat infected with *Giardia* spp., the authors concluded that feline giardiasis is a disease that can cause different clinical manifestations, including hemorrhagic diarrhoea. In calves, it was associated with chronic diarrhoea marked by high morbidity. In lambs, infected animals show a delay in reaching slaughter weight. Clinical signs are present, mainly in young animals, while in adults the disease can be subclinical, unless there are conditions such as overcrowding, increased stress and dietary problems (Halliez *et al.*

2013). On physical examination, animals may be normal or show evidence of diarrhoea, dehydration, and weight loss.

2.6. Diagnosis

The detection of *Giardia* spp. it is not only important from the point of view of clinical treatment of the disease, but also to limit infection by cysts and prevent zoonosis. The diagnosis is generally underestimated, despite the fact that the genus *Giardia* is one of the most prevalent parasites in dogs and cats. Failure to disregard it as a differential diagnosis, error in recognizing the microorganism, the use of inappropriate methods for fecal analysis and the intermittent excretion of protozoa in the feces of infected hosts are factors that make diagnosis difficult. There are elements that interfere with the results of the coprological exams and, for this reason, they must be taken into account when analyzing the samples. These factors are: the consistency of the stool, the pattern of excretion of the cysts, the use of preservatives in the stool and products that can mask the results of the analysis. In formed stools, cysts are usually present and in stools with a pasty or liquid consistency the presence of trophozoites, which do not remain for more than 30 minutes in the environment. There are hosts with different patterns of cyst clearance. Some with higher frequency than others, and thus can be a barrier to diagnosis.. Many authors indicate the need to perform the coprological examination with three fecal samples on consecutive or alternate days. It is often not possible to examine the newly eliminated feces, for this reason, some substances are used in order to preserve the structures of the parasite. Among them are formalin, polyvinyl alcohol and merthiolate-iodo-formaldehyde. Some products may mask the analysis results. (Balcioglu *et al.* 2003) cited antacids, antibiotics, laxative oils or enema preparations as potential causes of morphological changes or the disappearance of the parasite in the feces. Diagnosis is based on confirming the presence of the parasite through visualization of the cyst, trophozoite or both elements. Another form of diagnosis is the identification of its genetic material or antigens using fecal or intestinal samples from the host. The absence of the cyst or trophozoite in the fecal samples does not exclude giardiasis. According to (Sursal *et al.* 2020), all dogs and cats presenting diarrhoea and/or vomiting should be evaluated for the pesence of parasites, including *Giardia* spp. There are different diagnostic methods, which have advantages and disadvantages. Standard diagnostic tests used in any clinical setting should include fresh saline smears

and zinc sulfate float. Direct fecal smears can be used in the clinic to assess for the presence of *Giardia* trophozoites. The superficial layer of stool or mucus should be used if present as trophozoites are normally found in these areas. Applying lugol's solution or methylene blue to the smears helps to visualize the internal structures of the trophozoites. A refrigerated sample or a sample that is analyzed several hours after collection is likely to have no living organisms. The zinc sulfate flotation technique is the most reliable coprological test for demonstrating *Giardia* spp. in a fecal sample, being used as the standard test in screening for intestinal parasites in universities and veterinary clinics. In the case of dogs, 70% of positive animals can be identified with a single zinc sulfate fluctuation test, as opposed to 40% of dogs after three saline smears are performed. It is important that the density of the solution used in the zinc sulphate fluctuation test is 1.18 as the protozoan tends to shrink and become contorted, preventing its identification. Due to the fact that animals eliminate the cysts intermittently, several authors recommend that several concentration tests with zinc sulfate be carried out during 3 to 5 days, to maximize the possibilities of diagnosis or exclude the disease. A positive result justifies treatment.

A fresh stool with saline solution is made by mixing one drop of liquid stool with one drop of the solution. When viewed under a microscope, trophozoites are pear-shaped, have a concave ventral disc, and show an oscillating motion. It is of paramount importance to differentiate the *Trichomonas* parasite from *Giardia*. This differentiation is based on the movement pattern. The *Trichomonas* parasite has a forward-directed movement and the absence of a concave disk. The advantages of these methods, such as direct fecal smear, fresh smear with saline solution and zinc sulphate flotation, are based on the low cost of the consumables used and for being non-invasive methods for sample collection. However, diagnostic success depends on the experience and skills of the microscopy technician. The detection of antigens in the stool can be performed using three different techniques: indirect immunofluorescence, Enzyme-Linked ImmunoSorbent Assay (ELISA) and immunochromatography, all of which use monoclonal antibodies against cyst and trophozoite wall proteins. According to (Hooshyar *et al.* 2019), the reference method for diagnosis is the detection of specific fecal antigens for *Giardia*. For this, numerous kits are available on the market. The advantage of this test over microscopy for observation of cysts results in the possibility of diagnosis, even with interruption of their excretion. The immunochromatography test

according to (Pitães *et al.* 2015), which uses monoclonal antibodies directed against specific proteins in the wall of cysts and trophozoites, are quick to prepare and read, and can be used both for companion animals and for production animals. The fecal ELISA test detects the *Giardia* parasite antigen produced by dividing trophozoites. It is very sensitive in humans, with 30% more cases of *Giardia* being reported than when using the zinc sulfate flotation method. By identifying the antigens in the feces, this method avoids the problem of obtaining false negatives due to the intermittent excretion of the cysts, and can be performed in the clinic or in a laboratory. A study by (Saleh *et al.* 2019), with the objective of determining the prevalence in dogs and cats symptomatic for the protozoan *Giardia* through the Snap Test Kit ELISA and fresh stool smears, concluded that the ELISA was the reference method with the best sensitivity and specificity. The sensitivity of this test is 95% and the specificity 99% compared to direct immunofluorescence and 85% and 100% compared to the zinc sulfate flotation method. This proves to be a simple method, easy and clear to interpret and above all fast, when compared to other methods.

The investigation of serum antibodies to *Giardia* spp. it can also be performed using indirect immunofluorescence. However, its diagnostic value is limited, being more advantageous in epidemiological studies as it does not distinguish between a recent infection and an established one. Duodenal biopsy is indicated in cases of negative coprological exams in which the animals continue to show clinical signs that affect health. It is an expensive method, requires specific equipment and the procedure duration is generally long, which makes it impractical in clinical routine. The use of PCR provides information about the genotype, allowing the identification of different species, being useful for epidemiological and taxonomic studies. The biggest advantage is the easy execution and interpretation of results. Its speed allows the analysis of numerous samples at the same time. This method has a high sensitivity and it is necessary to take into account inhibitors present in faecal samples that can interfere with molecular techniques. PCR methods are increasingly used to control water quality with the aim of identifying the source of contamination (Gardner *et al.* 2001; Saleh *et al.* 2019). Frasson and collaborators used a fluorescent derivative of Taxol to investigate the distribution of microtubules in the cytoskeleton of G. lamblia and consequently identified trophozoites and cysts. The results obtained in this work show that the constitution of the cytoskeleton of this parasite can be investigated using FLUTAX-2 and indicate

perspectives for the development of a new diagnostic method for the disease. Ovoid cysts excreted with feces measure 8-17 x 7-10 µm, and can be observed directly in fresh feces or after a concentration process by flotation. It is important to take into account that these are deformed if a saline flotation is carried out. In freshly excreted feces from animals with clinical signs, cysts can be detected with a pyriform shape and a size of 9-21 x 5-12 µm. Due to intermittent excretion, it is recommended to collect feces for 3-5 days to increase the possibility of detecting them. The detection of *Giardia* spp. Antigen in fecal samples is possible through the use of rapid immunodiagnostic tests that are currently commercialized, although the results obtained are not comparable due to the great antigenic variability between individuals. The direct immunofluorescence technique is very sensitive and is used in many reference laboratories. Some of them are:

a) Direct fecal smear: Quick, inexpensive, easy diagnostic method that requires a small amount of stool sample; but with limited sensitivity and specificity.
b) Zinc sulfate.: Ideal for the diagnosis of cysts and protozoa with good sensitivity and specificity, allowing a large amount of stool for diagnosis
c) Quantitative method in chamber of Mac Master: Determine the number of eggs per gram of stool. Also used for the observation of coccidean nematode larvae and oocysts
d) ELISA test for diagnosis of coproantigens: Laboratory technique that identifies small particles (antigens) and germs that cause diseases
e) SNAP *Giardia* Test: Rapid enzyme immunoassay, allowing the clinic to obtain a simple and accurate diagnosis in just eight minutes. Having a sensitivity of 92% and a specificity of 99%

   One advantage of this is that a single sample is required.

2.7. Treatment

Metronidazole is an old drug for the treatment of canine giardiasis. It acts as an antibacterial and a protozoacidal. A dose of 22-25 mg/kg is used, twice a day for five days). In recent times, some benzimidazole derivatives (albendazole, mebendazole, fenbendazole) have shown high efficacy against *Giardia* spp. Albendazole for giardiasis; it is recommended to administer every 12 hours: 25 mg / kg for two days. Fenbendazole is currently used for this treatment; 50-55 mg/kg, once a day for five days by mouth. Treatment can be repeated as long as clinical signs or cyst excretion persist.

Fenbendazole is registered for the treatment of giardiasis in dogs in most countries. Other drugs sensitive to giardiasis are the combination of Febantel/Pyrantel / Praziquantel at a dose of 15 mg / kg, 14.4 mg / kg and 5 mg / kg respectively, once a day for three days. This treatment is registered in several countries outside the European community. Many times the treatments are not effective because reinfections, co-infections or other latent diseases are frequent or because the antiparasitic treatment is not completed. Resistance to antiparasitics has been described in isolated cases in humans. The success of the treatment is linked to the strong pressure of reinfection from the contaminated environment. Therefore, it is essential to apply supplementary measures: the use of baths in the dog (products with chlorhexidine digluconate) at the beginning and at the end of the antiparasitic treatment to contribute to the reduction of reinfections. Treatment of infected, sick or clinically healthy dogs, cats and other animals is strongly recommended due to the possible zoonotic risk. The first goal of treatment is to stop diarrhoea and then eliminate the infection (Díaz and Víctor, 2017). Different drugs were used to treat *Giardia* spp. including metronidazole, tinidazole, ipronidazole, ronidazole, febendazole, albendazole, pyrantel/praziquantel/febantel association, quinacrine, furozolidone and nitazoxanide (Table 2.2).

Table 2.2 Medicines used to treat *Giardia* spp. in dogs and cats.

Medicines	Species	Dose
Metronidazole	B	15 - 20 mg/kg, 12 - 24 hours for 7 days
Tinidazole	D	44 mg/kg, PO, 24 hours for 6 days
Ipronidazole	D	126 mg/L, PO, for 7 days
Fenbendazole	B	50 mg/kg, PO, 24 hours for 3 days
Albenazole	B	25 mg/kg, VO, 12 hours for 2 days
Praziquantel	D - C	Label VO dose for 3-5 days 56 mg/kg based on VO febantel 24 hours a day
Febantel	D - C	9 mg/kg, PO, 24 hours for 6 days
Quinacrine	C	11 mg/kg, PO, 24 hours for 12 days
B: Dogs and cats; C: Cats; D: Dogs; VO: Orally.		

Drugs from the group of nitroimidazoles and benzimidazoles are efficient in the treatment of infection in humans and some pets. Nitroimidazoles are a group of drugs with antibacterial and antiprotozoal activity. This group includes metronidazole and tinidazole, which are frequently used to treat animals positive for *Giardia* and ronidazole used to treat *Giardia* in humans in the United States.

The best known compound in this group is metronidazole, being the most used in

veterinary medicine. Although this drug is not approved by several regulatory agencies such as the Food and Drug Administration (FDA), metronidazole is one of the most used drugs in the treatment of giardiasis in dogs. However, unwanted carcinogenic and teratogenic effects associated with high toxicity restrict its use, and cases of resistance have also been described. Resistance to this drug in humans has been described, possibly due to the mechanism that allows the protozoan to tolerate the oxidative stress caused by this drug. Metronidazole has its resulting action in the association of intermediate products, originating from its intracellular bond with DNA, forming a complex that inhibits replication and inactivates DNA. According to (Díaz and Víctor, 2017), the bactericidal mechanism of metronidazole is not completely understood. Metronidazole is believed to act directly on bacterial DNA topoisomerase and nucleic acid synthesis. It acts against obligate anaerobic bacteria and protozoa, such as *Trichomonas*, *Entamoeba* and *Giardia*. High doses or prolonged treatments can cause neurotoxicity (nystagmus, ataxia and tremors). Plumb in his book recommended a dose of 10 mg/kg, orally, two to three times a day for prolonged treatments. A case report of metronidazole poisoning in a six-and-a-half-year-old dog was described by (Hajek *et al.* 2017). In this study, the animal had ataxia, tremors, nystagmus and weakness of the hind limbs and was treated with metronidazole for 5 weeks, at a dose higher than that indicated in the literature 15-20 mg/kg, 12 - 24 hours for 7 days (Petri, 2005).

Nitroimidazole should not be used in pregnant women due to its teratogenicity. It can also cause loss of appetite, nausea, vomiting and reversible neutropenia. Cats experience sialorrhea when taking metronidazole, mainly in suspension form. (Coura *et al.* 2002), carried out a study in which the treatment of giardiasis in dogs and cats was used at a dose of 22-25 mg/kg, orally, BID, for 5 days. (Petri *et al.* 2005) . indicated the dose of 15-20 mg/kg, orally, SID or BID, for 5 to 7 days. European Scientific Counsel Companion Animal Parasites (ESCCAP 2011), proposed a dose of 25 mg/kg, orally, BID for five days. Silymarin, which in clinical practice is used for the treatment of liver diseases, has an effect on both humans and animals, being a potent antioxidant, anti-inflammatory and anti-fibrogenic agent for the liver. A study evaluated the effect of silymarin in the treatment of giardiasis in asymptomatic dogs receiving the antimicrobial drug metronidazole. The results show that silymarin had no direct effect on the treatment of the disease, but the animals treated with metronidazole associated with silymarin had less weight loss and better clinical status than those treated with metronidazole alone.

The administration of probiotics orally can be beneficial in the control of *Giardia* and associated with the treatment. The use of *Lactobacillus casei* makes it difficult for trophozoites to adhere to the intestinal wall. (Solaymani *et al.* 2010) found that the co-administration of albedazol with the probiotic exerts a synergism by inhibiting the fixation and growth of the parasite, thus leading to a reduction in the excretion of cysts and trophozoites. (Borehman *et al.* 1985) demonstrated the efficacy of ronidazole in vitro compared to metronidazole. This drug had an effect five times greater than metronidazole. Another active principle derived from nitroimidazoles is secnidazole, used in the treatment of *Giardia* in humans. Secnidazole was tested on cats naturally infected by the protozoan, showing 100% efficacy. One of the reasons this drug is not used for treatment in animals is the fact that it is only marketed for humans. Quinacrine was a drug frequently used in cases of metronidazole resistant dogs and cats. Although quinacrine is more effective than metronidazole, it causes more side effects and is therefore no longer used today. Chloroquine is an old but promising drug for the treatment of *Giardia*. It is a synthetic compound, modified from quinacrine, in which its acridine ring was replaced by a quinoline ring. For many years, chloroquine has been used as the first option for the vast majority of human malaria treatments. A recent study in dogs infected with *Giardia* spp. and treated with chloroquine at a dose of 2.5 mg/kg, orally, BID, for 5 days, associated with environmental disinfection significantly reduced the excretion of cysts to 99.8% on day 3 and 99.9% on days 7 and 10, being another low-cost option for treating the disease in dogs.

Fenbendazole is a benzimidazole widely used in human and veterinary medicine for the control of nematodes and *Giardia* spp. It is a safe drug and does not have a teratogenic effect, making it the drug of choice for the treatment of *Giardia* in pregnant animals and cats. Its combined use with metronidazole appears to reduce diarrhoea. (Saleh *et al.* 2019) conducted a study in which they developed a protocol for the treatment of *G. duodenalis* in a group of dogs from a college of veterinary medicine in Virginia, United States, where 34 dogs were treated with fenbendazole at a dose of 50 mg/kg, BID , orally for 10 days, associated with bathing on the fifth day, treatment and disinfection of the quarters with quaternary ammonia. At the end of the study, all positive animals had a negative result. Another treatment option recommended by (ESCAP, 2011), is the association of 15 mg/kg of febantel, 14.4 mg/kg of pyrantel pamoate and 5 mg/kg of praziquantel, SID for three days. Albendazole has similar efficacy to fenbendazole but

is not recommended due to side effects: leukopenia and lethargy. In a case report of an 8-month-old dog, presenting *Giardia* cysts in the feces and treated with azithromycin at a dose of 10 mg/kg/day for 5 days, the result was satisfactory. To assess the effectiveness of the treatment, direct stool smears were performed on days 2.4 and 6 with negative results confirmed by polymerase chain reaction (PCR). This active principle can be considered as a promising antibiotic for the treatment of *Giardia* in dogs (Sursal et al,. 2020). Recent research where nitazoxanide was used showed efficacy at a dose of 75 mg/kg every 14 days in dogs infected with *Giardia*. This study helps in the development of new treatments for the disease, mainly in shelters (Bouzid *et al.* 2015).

2.8. Prophylatic measures

Giardia infection control involves not only therapeutic treatment, but also the prevention of contamination of food and water by infected faeces through sanitation and environmental disinfection. The most effective way to prevent infection is to avoid ingesting the cysts that contaminate the environment and lead to possible reinfection. Prevention involves boiling or filtering water used for human and animal consumption. Faeces from infected animals must be promptly removed from the environment, followed by thorough steam cleaning (> 65°C) or disinfection with quaternary ammonia compounds for 1 minute of contact time. In a study carried out in Turkey, by (Kucuk *et al.* 2019) , through questionnaires, it was found that 8.1% of respondents assumed that they did not periodically collect cat feces from litters. Also as prophylactic measures, food bowls, drinking fountains and toys should be washed every day with boiling water or placed in the dishwasher at a temperature above 65°C. It is also recommended to clean all blankets, pillows and bedding that the animal uses at a minimum temperature of 65°C. Cat litters should be disinfected daily with boiling water and then dried, as well as cat scratchers cleaned with a vacuum cleaner. The animals must be treated, and bathing is recommended for sick animals and for all animals that are in the same environment and could possibly be infected and also cut the hair of the anal region. Other studies show the importance of bathing sick animals and disinfecting the environment in order to prevent reinfection by *Giardia* cysts. These are supplementary recommendations given by (ESCAP, 2015) to be followed in kennels and catteries, such as informing employees who deal directly with infected animals of the risk of zoonosis, performing tests to identify carriers in all arriving animals, examining animals that have diarrhoea, if

necessary, establish a quarantine and keep all damp areas dry.

Vaccination helps to interrupt the spread of cysts to the environment and therefore the possibility of contagion; managing to reduce the zoonotic transmission of this parasites of importance in public health. There is a laboratory that manages this vaccine against the *G. lamblia* parasite, which is an inactivated liquid vaccine that prevents the disease and spread caused by the infestation caused by *G. lamblia* in healthy canines. The recommended dose is 1 ml subcutaneously; where the suggested vaccination schedule should begin at 8 weeks of age, repeating the dose at 2 or 4 weeks. In animals older than eight weeks, two doses should be applied with an interval of 2 or 4 weeks. Recommending annual revaccination with one dose. Vaccination of parasitized, weakened puppies or pregnant females should be avoided. The production of antibodies has been demonstrated in vaccinated animals, where they have a cytolytic effect on the cysts or inactivation of the trophozoites during the decystisation process. The variability in the prevalence of this parasitosis is probably explained by zoonotic transmission in such a way that vaccination in animals could prevent intraspecies transmission. Another important advantage of vaccination is from the epidemiological point of view, since it could be used in endemic regions where there are poor sanitary conditions, as well as avoiding its pathogenic effect, not only at the level of the intestinal mucosa, but also the effect produced for its toxins. Although it is true, the vaccine has been accredited in the United States of America, so it was necessary to add it to the line of existing vaccines for dogs, to help veterinarians in the prevention of this disease; decreasing the incidence and clinical severity due to gastroenteric affections of the pathological process of *Giardia* spp. In the United States, a vaccine for dogs and cats against *G. duodenalis*, produced from trophozoites isolated from sheep, has been available since 1999. This vaccine is also indicated to stimulate immunity in patients with chronic infections, as well as to treat and prevent the disease. Clinical studies developed to demonstrate the effectiveness of this vaccine have shown that subcutaneous administration in young animals reduces cysts in the feces, eliminates or reduces trophozoites in the intestine, prevents clinical disease and leads to weight gain. However, other research has failed to demonstrate the vaccine's effect on infected animals (Serradell et al,. 2016).

In a recent study by Serradell et al. (2016). an oral vaccine was tested as a way to prevent *Giardia* in dogs and cats. It showed positive results, both in experimental infections and in natural infections. As a result, it was observed a reduction in cases

of animals with chronic giardiasis and avoided new infections in young dogs and cats. Notably, immunization of animals in a highly endemic area decreased the percentage of infected children, suggesting that this vaccine would block zoonotic disease transmission. Vaccination for animals against *Giardia* spp. it is not available in Europe (ESCCAP, 2011).

2.9. Prevention

To prevent infection with this disease in dogs it is advisable to:

a) Bathing the animals to remove the fecal remains of cysts.
b) Use clean utensils for food and water.
c) Clean and disinfect the environment; as well as the dog's rest space.
d) Remove and destroy feces.
e) Although there are no registered disinfectants to remove cysts from surfaces, some studies indicate that they can be removed with quaternary ammonium compounds.
f) Good animal hygiene is essential to avoid the spread of cysts.
g) It is advisable to carry out an in situ test for the presence of cysts in case a puppy arrives at a home if there are already other animals in it.
h) In animals with diarrhoea and clinically healthy animals, it is recommended to place them in quarantine and be well diagnosed, especially those that come from kennels or shelters.

According to Papini et al. (2009); emphasizes that there is a high risk of dog-to-dog transmission of *Giardia* spp., and it occurs in common areas where high amounts of infected dog feces are allowed to accumulate on the ground.

3. MATERIALS AND METHODS

3.1. Reagents and equipment

No.	Materials	Amount
1	Light microscope	1
2	Centrifuge	1
3	Plastic gaita containers (25mL)	200
4	Slides and cover slides	200
5	Disposable gloves	200
6	Gieamsa stain	1
7	Trichrome stain	1
8	Parasite concentration tube	150
9	Normal saline (0.85%NaCl)	1
10	Lugol's iodine 5%	1
11	Plastic conical tube (15mL)	150

3.2. Animals

The study permit was obtained from Cankırı municipality to carry out the study in Cankırı Municipality Stray Animal Treatment and Rehabilitation Center (06.11.2020/9332).The study is outside the scope of animal experiments ethics committee approval. Therefore, approval was obtained from the Animal Experiments Central Ethics Committee of the Ministry of Agriculture and Forestry (15.10.2020/60). Cankırı Municipality Stray Animals Treatment and Rehabilitation Center from February to April 2021, 100 stray dogs were included in this study. Animals were daily examined by Center's veterinary doctor. Center records were used for the age and information of the animals.

3.3. Samples

Stool sample was collected using gloves from the rectum of the animals and fine care were followed during sample collection. Stool was kepet in a clean sterile plastic container for laboratory works. The sample was alauqated into three parts, part one sample were used for the direct method, part two sample was used of special staine using flotation method, part three were employed for molecular work.

3.4. Detection of *Giardia*

For detection of *Giardia* parasite through the different stage of infection using following routin laboratory investigation.

3.4.1. Direct saline/ iodine wet mount protocol

Through this technique a saline solution was used modified diluteLugol's iodin staining method was used according to (Leland et al,. 2010). Briefly as follows; the stool is the most common specimen submitted to the diagnostic laboratory; the most commonly performed procedure in parasitology is the trophozoites of parasite examination. Gastro-intestinal infections by parasites (protozoan) are primarily diagnosed by detecting live motile trophozoites (for protozoans); cyst (inactive dormant stage of protozoa) in the stool. Microscope slides made from the fecal specimen of the patients can be examined under low and high power. These etiological agents are identified based on their morphological/staining characteristics Protocol; apply the patient's sample to a small area on a clean microscope slide. Remove any gross fibers and particles. Immediately before the specimen dries, add 1 or 2 drops of saline with a pipette. Mix with a pipette tip. Cover the specimen with a cover slip (Fig 3.1). If desired the coverslip(s) can be sealed using petroleum jelly and paraffin oil or other suitable sealing preparations. Sealing the coverslip keeps organisms from moving when using oil immersion objectives and prevents the preparation from drying out. Examine the specimen with the low power objective (10X) and low light. Begin at one corner of the smear and systematically examine successive adjacent swaths with the low power microscope. Low power examination includes an entire area of 22 by 22 mm coverslip preparation (both saline and iodine). When a parasite-like object comes into view, it should be more closely examined and identified under high power (40X) objective. High dry power examination should include at least one-third of the coverslip area (both saline and iodine).

3.4.2. Dilute lugol's iodine

Protocol; dilute lugol's iodine 1:5 with sterile de-ionized water before use. Prepare a direct smear of the specimen by mixing a small portion (2 mg) of feces / specimen with a drop of sterile physiological (0.85%) saline on a clean dry glass slide. Place a coverslip over the sample and examine the wet mount preparation for the presence of motile protozoa. The organisms are very pale and transparent and are more easily observed under low light intensity. After a thorough examination of the wet mount has been a drop of lugol's iodine (working solution) can be placed at the edge of the coverslip, or a new mount can be prepared using iodine alone. The prepared slides can be sealed if desired.

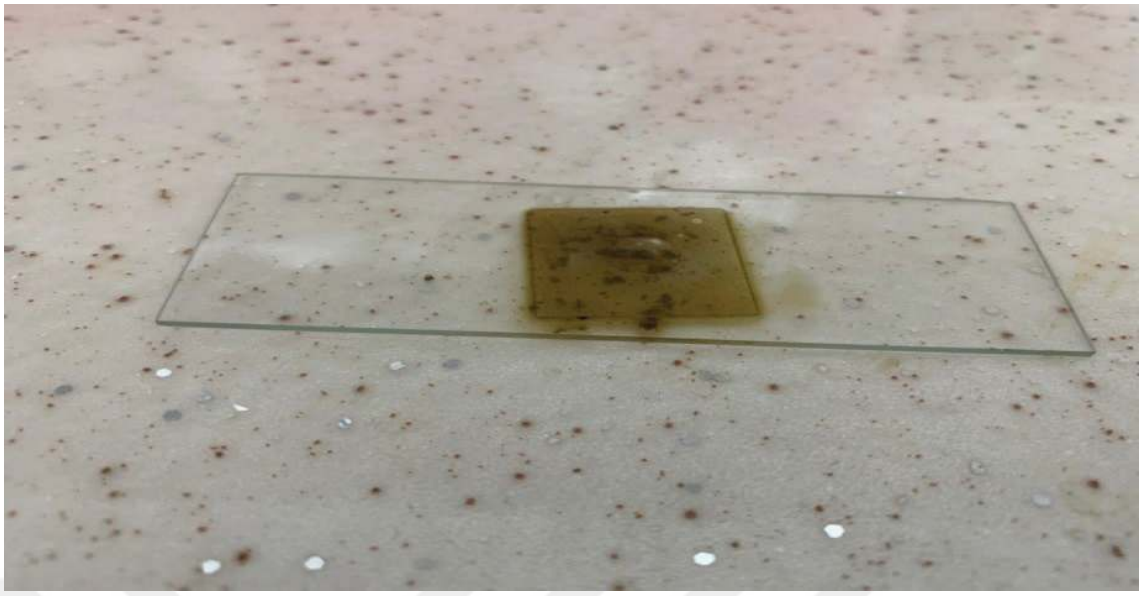

Figure 3.1. Wet mount slide.

Examine the slides for the presence of brown parasitic structure (Charles *et al.* 2012).

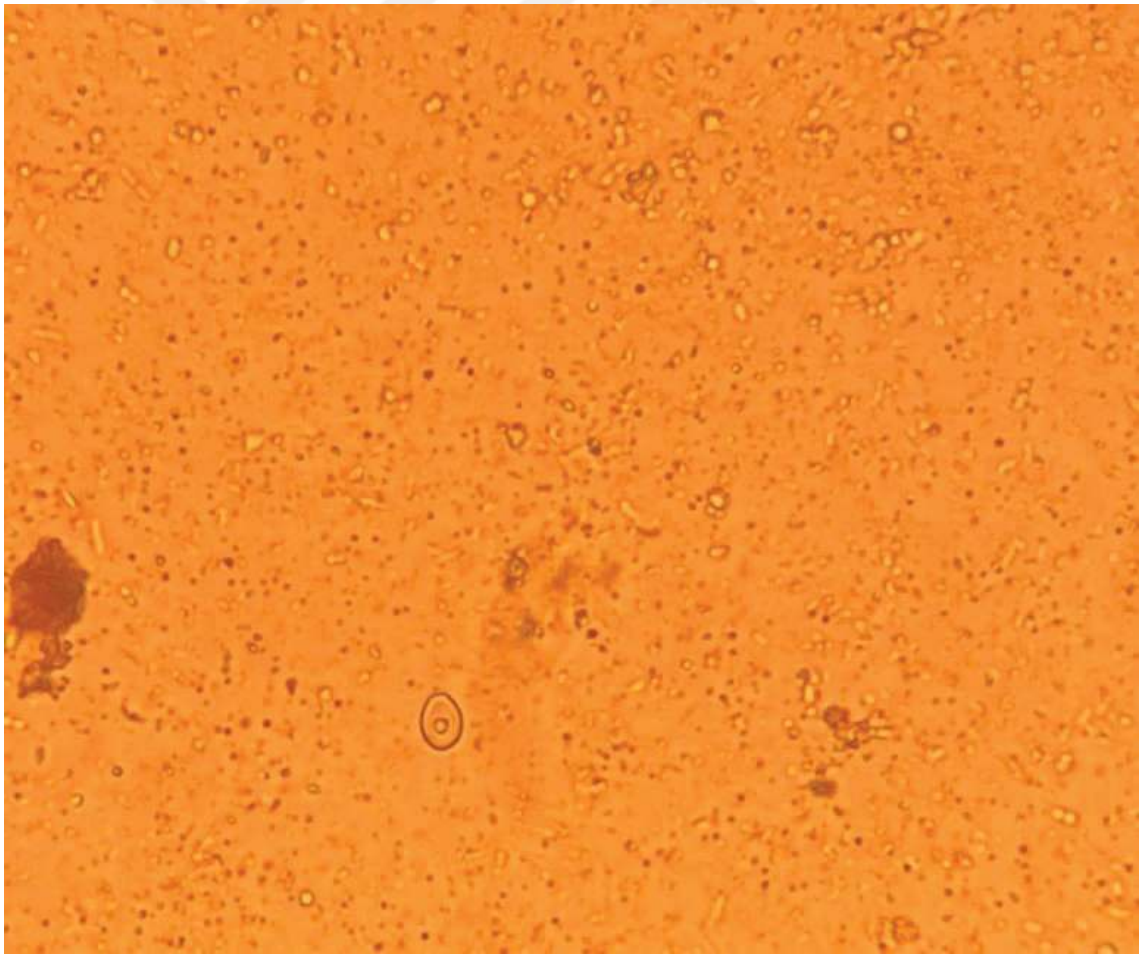

Figure 3.2. Dilute lugol's iodine method.

3.4.3. Paracon parasite concentration

If *Giardia* trophozoites are not detected in direct smears, cysts should be examine by Parkon parasite concentration. Preparing the sample by take 2 or 5 grams of feces and put it in the paracon tube and left the sample for 24 h under room codition 25 C.

Centerifuge turns the parakon upside down and places it in acentrifuge machine centrifuge it for 2 minutes in 1000 cycles (Zajac et al,. 2002). Then take the sample from the precipitate and place it along with lugol's iodine . this will allow you to see possible protozoa . Examine 200 to 300 fields using 40× or higher objectives.

Figure 3.3. Parkon parasite concentration tubes.

3.4.4. Giemsa staining

Prepare a smear with 1 to 2 drops of the specimen on the slide and dry on a slide warmer at 60°C until dry. Fix with absolute methanol for 30 seconds. Stain with Kinyoun's carbol fuchsin for one minute. Rinse briefly with distilled water and drain. Destain with acid alcohol for 2 minutes. Rinse with distilled water and drain. Counterstain with Malachite green for 2 minutes. Rinse briefly with distilled water and drain. Dry on a slide warmer at 60°C for about 5 min. Mount with a coverslip using desired mounting media. Examine 200 to 300 fields using 40X or higher objectives. To confirm internal morphology, use 100X oil immersion objective (Anne *et al.* 2019).

3.4.5. Trichrome stain for a stool sample

Procedure : Preparations were procced using deparaffinized method and made ready for staining. Mix equal amounts of solutions **A** and **B** drop onto the preparations and leave for 10 min and then, quickly pass preparations through distilled water. Drop the solution **C**

on the preparations and leave for 4 min, quickly pass preparations through distilled water, Drop solution **D** and leave for 15 min, quickly pass preparations through distilled water. Drop from **E** solution and leave for 5 min. Pour the solution over the preparations and drop **F** sol without washing and leave for 7 or 8 min, quickly pass preparations through distilled water. And later, pass preparations alternately in 96% and 99.9% grades alcohols. Spend preparations with xylene every 2 min, 2 times apart. Cover slides with mounting medium (Masson, 2017).

3.5. Statisticals analysis

The data were presented as percentages and frequencies and analyzed by using of nonparametric test-frequencies and crosstabs analysis and finally by Chi-square test to find the differences between groups. The analysis done using SPSS V.23 software (IBM, Chicago, USA).

4. RESULTS

A study aimed at investigating the prevalence of *Giardia* infections in dogs in Cankiri municipality stray animal treatments and rehabilitation centre was conducted. Various laboratory tests were done; wet mount smear, flotation test, dilute lugols iodine. Parkon parasite concentration, and Giemsa stain. There was also a test done to investigate the relationship between the gender and age of the dogs. Statistical methods and techniques were applied to test and describe the relationship between age, gender and disease prevalence. The following are the descriptive statistics of the variables used.

On the gender basis, it was observed that 43% of the dogs investigated were female and 57% were male. On the other hand, the variable age was categorised into three categories; less than 1 year accounting for 52%, 1 to 3 years accounting for 38% and more than 3 years accounting for 10%.

The chi square test was conducted to assess if there existed a statistically association between age and gender. The test describes the distribution of the age groups based on gender. The results were statistically significant implying that there was association between gender and age. The p-value of 0.025 less than 0.05. the Pearson chi square test score was 7.339.

The laboratory tests were done on the samples and had various results. The wet mount smear test revealed that 27% of the samples were positive and 73% of the samples were negative (Table 4.1). To access the association in wet mount smear test and gender, Chi square test was conducted. The aim was to access a statistically significant association between the two groups. The test indicated that there was a statistically significant between gender and wet mount smear with a Pearson chi square test score of 63.145 and a p-value of 0.00. The test indicated that the males had high-recorded positive cases than female and lower negative cases.

The flotation test was also done and revealed that 4% of the samples were positive and 96% were negative (Table 4.2). On dilute lugol's iodine test, there were 12% positive and 88% negative samples (Table 4.3).

The Parkon parasite concentration test revealed that 12% were positive and 88% were negative (Table 4.4). The Trichrome stain test was done and the results were 12% positive and 88% negative (Table 4.5). Finally, the Giemsa stain test revealed that all the samples were negative (Table 4.6).

Table 4.1 Positive and negative wet mount smear test.

Wet mount smear	Positive				Negative			
	Gender		Age		Gender		Age	
	M(%)	F(%)	M(%)	F(%)	M(%)	F(%)	M(%)	F(%)
Less than 1 year	82.6	25	95	5	44.1	43.6	46.9	53.1
1-3 Years	8.7	50	50	50	47.1	46.2	47.1	52.9
More than 3 years	8.7	25	66.7	33.3	8.8	10.3	42.9	57.1
TOTAL	**27**				**73**			
Significant at P≤0.05	0.013*				0.979 NS			
M: Male; F: Female * (P≤0.05), ** (P≤0.01), NS: Non-Significant.								

Table 4.2 Flotation test

Flotation test	Positive				Negative			
	Gender		Age		Gender		Age	
	M(%)	F(%)	M(%)	F(%)	M(%)	F(%)	M(%)	F(%)
Less than 1 year	50	0	100	0	60	43.9	64.7	35.3
1-3 Years	50	100	33.3	66.7	30.9	43.9	48.6	51.4
More than 3 years	0	0	0	0	9.1	12.2	50	50
TOTAL	**4**				**96**			
Significant at P≤0.05	0.248 NS				0.130 NS			
M: Male; F: Female * (P≤0.05), ** (P≤0.01), NS: Non-Significant.								

Table 4.3 Dilute lugols iodine.

Dilute Lugol's Iodine	Positive				Negative			
	Gender		Age		Gender		Age	
	M(%)	F(%)	M(%)	F(%)	M(%)	F(%)	M(%)	F(%)
Less than 1 year	87.5	50	77.8	22.2	55.1	41	62.8	37.2
1-3 Years	12.5	50	33.3	66.7	34.7	46.2	48.6	51.4
More than 3 years	0	0	0	0	10.2	12.8	50	50
TOTAL	**27**				**73**			
Significant at P≤0.05	0.157 NS				0.204 NS			
M: Male; F: Female * (P≤0.05), ** (P≤0.01), NS: Non-Significant.								

Table 4.4 Parkon parasite concentration.

Parkon parasite concentration	Positive				Negative			
	Gender		Age		Gender		Age	
	M(%)	F(%)	M(%)	F(%)	M(%)	F(%)	M(%)	F(%)
Less than 1 year	87.5	50	77.8	22.2	55.1	41	62.8	37.2
1-3 Years	12.5	50	12.5	66.7	34.7	46.2	48.6	51.4
More than 3 years	0	0	0	0	10.2	12.8	50	50
TOTAL	**12**				**88**			
Significant at P≤0.05	0.157 NS				0.204 NS			
M: Male; F: Female * (P≤0.05), ** (P≤0.01), NS: Non-Significant.								

Table 4.5 Giemsa stain.

Giemsa stain	Positive				Negative			
	Gender		Age		Gender		Age	
	M(%)	F(%)	M(%)	F(%)	M(%)	F(%)	M(%)	F(%)
Less than 1 year	0	0	0	0	59.6	41.9	65.4	34.6
1-3 Years	0	0	0	0	38	31.6	46.5	52.6
More than 3 years	0	0	0	0	8.8	11.6	50	50
TOTAL	**-**				**88**			
Significant at P≤0.05	0.084 NS							
M: Male; F: Female * (P≤0.05), ** (P≤0.01), NS: Non-Significant.								

Table 4.6 Trichrome stain.

Trichrome Stain	Positive				Negative			
	Gender		Age		Gender		Age	
	M(%)	F(%)	M(%)	F(%)	M(%)	F(%)	M(%)	F(%)
Less than 1 year	87.5	50	77.8	22.2	55.1	41	62.8	37.2
1-3 Years	12.5	50	33.3	66.7	34.7	46.2	48.6	51.4
More than 3 years	0	0	0	0	10.2	12.8	50	50.0
TOTAL	**12**				**88**			
Significant at P≤0.05	0.157				0.240			
M: Male; F: Female								

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5. DISCUSSION

Intestinal protozoa of the genus *Giardia* are parasites of worldwide distribution, being considered one of the ten most common parasites in man and one of the main causes of non-viral diarrhoea in humans and animals (Ballweber et al,. 2010). Approximately 200 million people in Asia, Africa and Latin America have symptomatic giardiasis, and approximately 500,000 new cases are described each year. Its prevalence has variable rates, depending on the geographic location, the population studied and the method used for diagnosis. The possibility that bivalve molluscs can accumulate cysts has also been demonstrated, indicating that their consumption without prior heat treatment can lead to cases of disease. In developed countries the prevalence of *Giardia* in patients with diarrhoea varies from 3% to 7%, and in developing countries around 20%. (Ballweber et al,. 2010) reported that giardiasis is a notifiable disease in different industrialized countries, such as Canada, Japan, the United States and New Zealand. Its greatest clinical impact on humans is linked to children in their first years of life. Children and young animals are particularly susceptible to protozoan infections due to an incompletely developed immune system. Some studies indicate that there is greater sensitivity in animals less than one year old than in adults, suggesting the development of a certain degree of resistance with increasing age. Animals that have greater contact with the street are more exposed, due to greater contact with water, food and feces from contaminated animals or people (Bouzid *et al.* 2015) found values of 15.2% for dogs and 12% for cats in an analysis of the prevalence of *Giardia* worldwide. Being in cats a greater number in young animals. The industrial regions of northern Europe and North America had a lower prevalence than the rest of the world. The protozoan has been reported in feces of dogs and cats in different countries in Europe. (Dawson, 2005) in a study carried out in 377 clinics in 7 countries of the European Union, showed that *Giardia* is a common enteric agent in dogs and cats. Studies carried out in Belgium and Portugal to assess the prevalence of *Giardia* spp. in municipal kennels and shelters, they revealed prevalences of 43.9% and 61.1% respectively, being higher than the European global value. In Portugal, there was a concern with the parasite in humans, and some studies were found regarding *G. duodenalis*. Infection in asymptomatic children in the district of Lisbon was 6.8% and the author showed a positive association between the prevalence of the parasite and contact with companion animals, especially dogs. (Pitães

et al. 2015) , when studying two kennels in the region of Viseu, based on three diagnostic methods: Speed *Giardia*, coprology using the zinc sulfate flotation method and fecal smears stained by the Ziehl-Neelsen technique, observed the following prevalences: 21 .6%, 19.6% and 17.6%. Parameters such as sex, age and race were also evaluated, and no association between them was described. Results described by (Epe *et al.* 2010) in European countries are in line with the value described by (Pitães *et al.* 2015). However, these values are relatively higher than those of other studies carried out in various regions and dog populations in Portugal, with a prevalence of 23% in Évora and 61.1% in Lisbon. The value of 9% of positive animals was obtained in a study carried out in cats in the Lisbon region, from the detection of *G. duodenalis* by the immunochromatography method. This value is below the results obtained in studies carried out in Portugal with dogs, where the prevalences were 15.5% and 17.5%. (Braga, 2017), observed a prevalence of 33% for *Giardia* spp. in canids from the Beja region, noting that kennel environments combine ideal characteristics for continuous infection by parasites with a direct life cycle, some of which have zoonotic potential in a screening of gastrointestinal and pulmonary parasites in catteries cats in the districts of Lisbon and Setubal, found a value of 3.9% for *Giardia* spp. (Carvalho, 2017). The prevalence of infections in stray cats in the Évora region ranged from 5% for cats in the wild and 50% for animals in showed a prevalence of 62.5% for the gastrointestinal parasite *Giardia duodenalis*, through questionnaires directed to cat owners and veterinarians in Portugal. This value is in agreement with studies published by several authors that highlight this protozoan as one of the most common etiological agents of diarrhoea of parasitic origin globally. In dairy cattle, in the region of Entre Douro and Minho, *G.duodenalis* cysts were found in 18% of the fecal samples analyzed (Hooshyar *et al.* 2019).

EK 2. ÖZGEÇMİŞ

Adı – Soyadı : ALHAN M SHEET

Doğum yeri ve tarihi :

İletişim adresi ve telefonu :

Öğrenim Durumu :
Lisans : Mezuniyet yılı, Üniversite, Fakülte, Bölüm
Yüksek lisans : Mezuniyet yılı, Üniversite, Anabilim Dalı

Mesleki Deneyimi

Üye Olduğu Bilimsel Kuruluşlar

Bilimsel Çalışma Alanları

Yayınları: (Ulusal ya da Uluslararası makale, bildiri, poster, kitap ya da kitap bölümü vb.)

Bilimsel Etkinlikleri

Aldığı Burslar, Ödüller, Projeleri

Diğer Bilgiler

Eğitim programı haricinde aldığı kurslar ve

Katıldığı eğitim seminerleri

Organizasyonunda katkıda bulunduğu bilimsel toplantılar

Diğer üyelikleri