INVESTIGATION OF THE PREVALENCE OF *CRYPTOSPORIDIUM SPP.* IN SHEEPS AND GOATS IN BAGHDAD PROVINCE OF IRAQ

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THESIS APPROVAL

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PREFACE AND ACKNOWLEDGEMENTS

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LIST OF ACRONYMS

PBS	phosphate buffered saline
FEA	Formalin-ethyl acetate
AF	Acid flocculation
PCR	Polymerase Chain Reaction
mZN	Modified Ziehl-Neelsen
"1	microlitre(s)
g	gram(s)
mm	millimetre
/im	micrometre(s)
<	less than or equal to
>	greater than

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ABSTRACT

Investigation of the Prevalence of *Cryptosporidium Spp.* in Sheeps and Goats in Baghdad Province of Iraq

In this study was aimed to microscopically identify and determine the prevalence of *Cryptosporidium oocysts* in sheep and goats in the city of Baghdad during October-November 2020. For this purpose, one hundred and twenty stool samples were collected from 60 sheep and 60 goats. Samples were collected from animals of different ages and sexes. It was examined microscopically using modified Ziehl-Neelsen stain for the detection of parasite species. In this study, two species of *Cryptosporidium* were detected in sheep and goats; these species are *Cryptosporidium parvum* and C. *andersoni*, according to its size measured by ocular lens. The total infection rate of *Cryptosporidium spp* in sheep and goats was determined as 40% and 33.33%, respectively. In sheep, according to age groups, the highest infection rate was recorded in lambs <6 months of age (65%), and the lowest infection rate (20%) was recorded in the 12-24 months age group. According to gender, *Cryptosporidium spp.* was identified in 56.66% of the teeth and 23.33% of the men. According to gender, this difference was statistically significant (p<0.01.In goats, the highest infection rate was found at >6 months of age, 50%, and the lowest rate at >12-24 months of age. According to gender, *Cryptosporidium spp* was identified in 16.66% of the teeth and 50% of the men. According to gender, this difference was statistically significant (p<0.05).

Key Words: *Cryptosporidium Spp.* Prevalence, Sheep and Goats, Iraq.
2021, 46 pages

ÖZET

Irakta, Bağdat İlindeki Koyunlarda ve Keçilerde Cryptosporidium Türlerinin Yaygmlığının Araş tınması

Bu çalışmada, Bağdat kentindeki koyun ve keçilerde, Ekim-Kasım 2020 döneminde *Cryptosporidium* ookistlerinin mikroskobik olarak tanımlanması ve prevalansını belirlenmesi amaçlandı. Bu amaçla 60 koyun ve 60 keçiden toplam yüz yirmi dışkı örneği toplandı Farklı yaş ve cinsiyetteki hayvanlardan örnekler toplandı. Parazit türlerinin tespiti için modifiye Ziehl-Neelsen boyası kullanılarak mikroskobik olarak incelendi. Bu çalışmada koyun ve keçilerde *Cryptosporidium'un* iki türü tespit edildi; bu türler, oküler lens ile ölçülen büyüklüklerine göre *Cryptosporidium parvum* ve C. *andersoni'dir. Cryptosporidium spp.* toplam enfeksiyon oranı koyunlarda ve keçilerde sırasıyla %40 ve. %33.33 olarak belirlendi. Koyunlarda, yaş gruplarına göre en yüksek enfeksiyon oran %65'in <6 aylık olan kuzularda, en düşük enfeksiyon oranının (20%) ise 12-24 ay yaş grubunda kaydedildi. Cinsiyete göre dişlerin %56.66 ve erkeklerin de %23.33 de *Cryptosporidium spp.* etkeni tanımlandı. Ciniyetlere göre bu fark istatistsel olarak anlamlı bulundu (p<0.01). Keçilerde de en yüksek enfeksiyon oranı >6 aylıkken %50 ve en düşük oran >12-24 aylıklarda tespit edildi. Cinsiyete göre dişlerin %16.66 ve erkeklerin de %50'inde *Cryptosporidium spp.* etkeni tanımlandı. Ciniyetlere göre bu fark istatistsel olarak anlamlı bulundu (p<0.05).

ANAHTAR KELİMELER: *Cryptosporidium Spp.* Prevalans, Koyun ve Keçi, Irak
2021, 46 sayfa

1. INTRODUCTION

Cryptosporidium species are apicomplexan, obligate intracellular (extracytoplasmic), single-celled parasites, which infect the gastrointestinal and/or respiratory tracts of animals and humans. It is an important parasite, causes mild to severe profuse watery diarrhea in a wide variety of animals and humans (Fayer *et al.,* 2010).

Important reservoirs of *Cryptosporidium spp.* are small ruminants and cattle, as a large number of oocysts; they shed in the environment (Xiao, 2010). In the environment under appropriate conditions oocysts can survive to more than three months and resistant to normal water treatment disinfection practices (Smith *et al.,* 1998). An important role of oocysts transmission played by insects, exoskeletons and digestive tracts of the beetles, cockroaches, house flies and synanthropic flies can spread the oocysts (Yen *et al.,* 2007). Clinical or subclinical signs, including diarrhea, vomiting, fever, abdominal pain, anorexia, anemia, dermatitis, and loss of weight may induce by *Cryptosporidium spp.* when infects the epithelial cells of the gastrointestinal tracts (primarily colon and small intestine); yet, occasionally, no symptoms may present in some infected hosts (Rossie and Latif, 2013). *Cryptosporidium* at present is considered one of an important enteric pathogen in sheep and goats (Delafosse *et al.*, 2006; Xiao 2010). The three dominant species identified in sheep and goats are *Cryptosporidium parvum, C. hominis* and C. *xiaio* (Robertson, 2009; Diaz *et al.,* 2010; Hamadand and Alkhaled, 2016). Other species also described in sheep and goats are C. *andersoni* and C. *ubiquitum* (Xiao 2010; Koinari *et al.*, 2014).

Diagnosis of *Crypotsporidium spp.* generally occurs by different methods, the common methods used for the identification and detection of oocysts are direct, concentration and staining methods, these ways gave a good laboratory practice. (Koinari *et al.,* 2014). In this study, it was aimed to microscopically identify and determine the prevalence of *Cryptosporidium spp.* in sheep and goats in Iraq-Baghdad province during October-November 2020.

2. LITERATURES REVIEW

2.1. Historical background

Cryptosporidium is a Greek name meaning "hidden spores" (Mehlhorn, 2008). This term refers to abnormal morphology, that sporocysts absence within oocysts, characteristic of another coccidian (Weller and Tyzzer, 1978), and the species naming according to host origin and redirected the zoonotic nature of this parasite (Tzipori, 1983). Cryptosporidium was first recognized and named by Tyzzer, who, in 1907, describing the sexual, asexual and oocyst stages of parasite, and found it in the gastric glands and fecal of laboratory mice (Tyzzer, 2015).

In 1910 Tyzzer offered the murine gastric isolate *Cryptosporidium muris* as the type strain and in 1912, he descripts of a new, smaller species that found in the small intestine of mouse and rabbits, which he called C. *parvum* (Tyzzer, 1912). This was confirmed with additional observation of the extracellular developmental stages, merozoites and microgametes by electron microscopy (Current and Reese, 1986). C. meleagridis causing illness and death in young turkeys was reported by (Salvin, 1955), and in 1971 conceder that as parasite a cause of bovine diarrhea (Panciera *et al.*, 1971). Barker and Carbonen discovered the first case in sheep in 1974. Cryptosporidium is considered more important due to its cause's gastritis and intestinalis associate with diarrhea in human and all animal (Pettollo-mantovani *et al.*, 1995).

In Australia, the first case of cryptosporidiosis was reported in goats, where two- week-old Angora goat kids died after a short clinical case of diarrhea (Mason *et al.*, 1981). In European countries, two major species have been reported in goat kids from two kids suffering minimum than 21 days old from diarrhea which were caused by C. *parvum* (Quilez *et al.*, 2008) and currently C. *xiaio* (Diaz *et al.*, 2010). In 1976, the first case of human cryptosporidiosis was identified when two reports were printed, first in a 3-year- old and the other in a 39-year-old girls who lived on cattle farms infected with symptoms of abdominal pain, vomiting and watery diarrhea, until the early 1980 only seven more cases were described when it was recognized in patients with acquired immune deficiency syndrome (AIDS) (Nime *et al.*, 1976).

2.2. Classification of Cryptosporidium (Finch and Belosevic, 2002)

Accordording to Finch and Belosevic (2002);

Phylum: Apicomplexa

Class: Sporozoasida

Sub-class: Coccidiasina

Order: Eucoccidiorida

Sub-order: Eimeriorina

Family: Cryptosporidiidae

Genus: Cryptosporidium

2.3. Developmental stages of *Cryptosporidium spp.*

2.3.1. Oocysts

Oocysts are sub spherical, ovoid to ellipsoidal in shape, fully sporulated containing four sporozoites and a residuum. The residuum contains a large lipid body and a crystalline protein body alongside amylopectin granules (Harris *et al.,* 2004). The oocyst wall is smooth, measures approximately 50 nm in thickness, which composed of two electron dense layers separated by an electron-lucent space and it is extremely resist chemical and mechanical disruption. Oocysts of Cryptosporidium lack sporocysts and other morphological structures such as a micropyle and polar cup and granules, which are often observed in other coccidian oocysts (Arrowood, 2002). The dimensions of the oocysts slightly vary among species of Cryptosporidium, but in general, the length ranges from 4.5 to 7.5 "m and the width ranges from 4.2 to 5.7 "m (Thigeel, 2016).

2.3.2. Sporozoites

Sporozoites have been characterized as crescent shape, with a slightly pointed anterior end and a rounded posterior end containing a prominent nucleus (Hijjawi, 2003). Sporozoites lie parallel to each other within the oocyst and upon excystation escape through a slit-likeopening created upon dissolution of the unique suture in the oocyst wall. Excysted sporozoites move by flexing and gliding, and eventually penetrate the host cells (Borowski *et al.,* 2010). The dimensions of sporozoites are 4.5 to 7.5 /im long and 1.2 to 1.8 *p.m* wide (Thigeel, 2016).

2.3.3. Trophozoites

It’s have circular to oval shape, uni-nuclear stages, epicellular, smooth surface, electron dense band, feeder-organelle, cytoplasmic granulation, hood like shape varied in size depending upon their developmental stage measured <1 /im to 2.5 /im in diameter, which represent the transitional stage between sporozoites and merozoites (Borowski *et al.,* 2010). Traditional belief is that individual trophozoites undergo multiple mitotic divisions, resulting in the formation of type I meronts (Hijjawi *et al.,* 2004).

2.3.4. Meronts

Its epicellular, smooth surface measured approximately 1.5 /im in diameter. Two different types of meronts have been described (type I and type II), the development of type I meronts occurs as a result of mitotic division of trophozoites, as well as aggregates of ’recycled’ merozoites released from type I meronts variable in size (Hijjawi *et al.,* 2004; Borowski *et al.,* 2010). Initial descriptions of type I meronts state that each can develop six or eight merozoites, and four merozoites from type II meronts (Thompson *et* a/., 2005).

2.3.5. Merozoites

It have central nucleus and are motile, circular to oval in shape, displaying gliding and flexing movements. Type I merozoites are small in size and are very active, measured (0.4 x 1 /im) with both a rounded and a pointed end; however, type II merozoites are round and measured between 0.5 /im to 1 /im in diameter (Borowski *et al.*, 2010). Type

I merozoites are either recycled, clustering together to produce more type I meronts and hence amplifying the number of type I merozoites; or give rise to type II meronts. Type

II merozoites differentiate into micro- and macrogamonts (Thompson *et al.*, 2005).

2.3.6. Macrogamonts

Typically large in size (4 x 4/im - 5 x 4/im), ovular shape, rough surface and possess a large peripheral nucleus, it have been observed adhering to the surface of macrogamonts and have also been reported being visualized within a microgamont, representing the fertilization process (Borowski *et al.*, 2010). Once fertilization is complete, a zygote (or unsporulated oocyst) is formed, which then matures into a sporulated oocyst containing four sporozoites (Thompson *et al.*, 2005).

2.3.7. Microgamonts

The size of microgamonts is (2 /im x 2 /im) which become multi-nucleate and each nucleus are incorporated into a microgamete. Microgametes appear to bud from the surface of the microgamont and appear as bullet or spherical shape with rough surface and display a jerky, gliding movement, measured 0.1 |im in diameter (Caccio. and Chalmers, 2016).

2.4. Life cycle

The life cycle of Cryptosporidium is complex, and has asexual and sexual reproductive stage (Fayer and Xiao, 2008). life cycle its completes in a single host (monoxenous), direct life cycle and completed within the gastrointestinal tract or respiratory tract of the host (Fayer *et al.*, 2000). The infection occurs, when, sporulated oocyst (infective stage) ingested by the host; it is transmitted by the fecal-oral route, indirect (contamination food and water) and direct (host-to-host) (Fayer, 2004).

Oocysts are 4-6 /im containing four sporozoites, with a thick wall in an oval or spherical shape (Fayer and Xiao 2008). The important feature of oocyst is resistant to prolonged environmental exposure in various water sources, and disinfectant such as chloride, used in treated water, in all type of animal the water represents the most important transmission route (Fayer, 2004). The life cycle takes 24-72 hour to complete, the prepatent period from 3-10 days depending on many factors some rely on a host, sex, age, strain,individual resistant and immunity or rely on the parasite like virulent of stain and site of infection (Hornok *et al.*, 1999).

The major features of the life cycle start with ingestion of sporulated oocysts by the host, by excystation processes sporozoites are released and penetrated the epithelial cells, they are extra-cytoplasmic but they have paracellular location being intracellular, parasitophorus vacuole form around the parasite, when the sporozoites penetrat the mucus layer and attach nearenterocytes. The asexual stage (merogony or schizogony) of life cycle consists of discrimination to trophozoites that promote to type I meronts (grape like aggregates), 6-8 merozoites produced from type I meronts, merozoites resemble sporozoites in structure, merozoites released from type I are small in size 1 x 1.2 |im, circular to oval in shape and actively motile (Leitcha *et al.*, 2011).

The cycling of type I meronts made the ability of Cryptosporidium spp. to continue in the host, type II meronts produce four merozoites, the main characteristic of merozoites that release from type II meronts are rounded to pleomorphic measuring 1.5 x 1.6 pm in size or broadly spindle shape with pointed ends measuring 2 x 3.5 pm in size, that promote to micro-or-macrogamete (male or female gamonts) to form a diploid zygote. (Fig 2.1). The zygote promotes into oocysts with four sporozoites by process like to meiosis division, l.ead to promote two types of oocysts, the first type with a thick wall that is shed with the feces ofthe host, second type with a thin wall that may re-infection the original host without a shed or excreted with feces (Hijjawi *et al.*, 2009; Leitcha *et al.*, 2011).

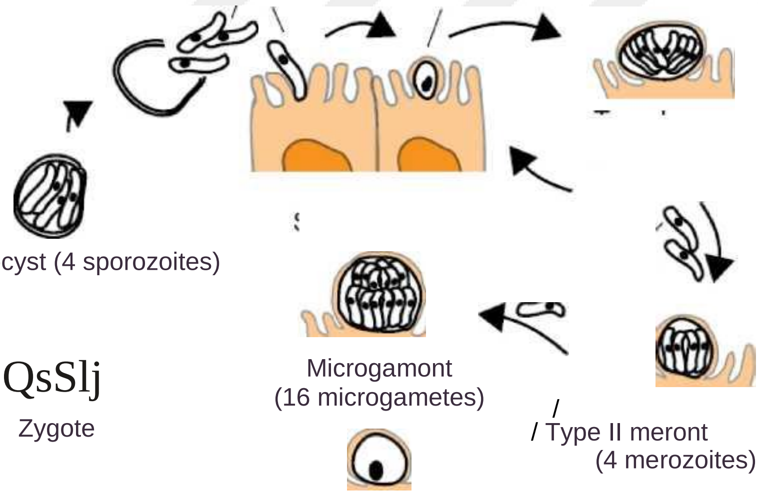

Figure 2.1. Life cycle *Cryptosporidium* spp. (Heo, *et al,*, 2018).

2.5. Transmission

Transmission of Ciyptosporidium parasite may occur in several ways, direct, indirect (contact from animal-to-human, human-to-human, ingestion of contaminated food or water), or, by a vectors like arthropods, rodent and even birds, that, play an important role in mechanical transmission of the parasite (Thompson *et al,*, 2005).

2.5.1. Animal-to-human

Zoonotic transmission of Cryptosporidium species is well known. Human can be infected by direct or indirect contact with infected animals, such as occupational exposure in the case of farmers, veterinarians, livestock owners, slaughterhouse workers, resulting in the ingestion of oocysts (Fayer, 2008; Caccio and Chalmers, 2016). C. *parvum* was an important type of *Cryptosporidium spp.* which can infected human that was reported in school children or students after exposure from infected animals (Casemore, 1999). Other species were recorded in human such as C. *hominis, C. canis, C. melegridis, C. andersoni, C. muris,* and C. *felis,* from different type of animals after exposure of infected oocysts (Guyot *et al.,* 2001; Xiao *et al.,* 2000; Akiyoshi *et al.,* 2003; Gatei *et al.,* 2003).

2.5.2. Animal-to-animal

The protozoal parasite transmission between the animals by several methods, the main important method is a direct transmission from infected mother to a newborn during the birth or through lick the anus) or by contaminated udder during sucking (Sischo *et al.*, 2000). Also, a large number of animals in a small area higher infection than the small number in the same area, and the infection can transmit from contamination stockyard to another, and a good management lead to reduce shedding of oocyst and then recovery (Mohammed *et al.,* 1999; Fresan *et al.,* 2004).

2.5.3. Human-to-human

The infection occurs by the fecal-oral route, in a day-care center, households and from patient-to-patient or patient- to health care staff in hospitals (Casemore *et al.,* 1999; Mahdi and Ali, 2002). Also, sexual practices play an important role in transmission the infection, by oro-anal contact, this way considered a major factor for the prevalence of cryptosporidiosis, studies showed that a major risk factor occurred in household contact with people special in children suffering from diarrhea (Robertson *et al.,* 2002; Hunter, 2003).

2.5.4. Water

Contaminated water is high-risk factors for increase infection with cryptosporidiosis (Castro-Hermida *et al.*, 2009). The infection may occur when the contaminated water is used for drinking, recreation, food and swimming pool; the water became contaminated through treated and untreated sewage, overflow of manure, and when the treated and untreated domestic wastewater are pouring in a large river and lakes (Rose *et al.*, 1991). Cryptosporidium oocysts have a widespread that found in surface water, oocysts can survive for up one year at 4°C in artificial seawater, even, with managed treatment plans can cause illness, when it contains high numbers of infected oocysts (Rose *et al.,* 1991; Tamburrini and Pozio, 1999).

2.5.5. Food

Infection occurs through consumption of contaminated food such as raw meat, salads, raw vegetables and fermented milk (Casmore *et al.,* 1997). The food can be contaminated by food handlers (Quiroz *et al.,* 2000). The contamination of vegetables occurs by a solid hand of farmworker, food worker, and from the contaminated surface where vegetables are packed, prepared and storage (Yoshida *et al.,* 2007 Abougrain *et al.,* 2010). Oocysts found on the surface of raw vegetables, and washing does not completely remove its (Ortega *et al.,* 1997). Cryptosporidium species have been found in oysters (Downey and Graczyk, 2007), mussels and clams (Miller *et al.,* 2005), special C. *parvum,* so, a good cooking of shellfish will reduce the risk of infection (Fayer *et al.,* 2000).

2.5.6. Arthropods

Cryptosporidium oocysts can be transmitted by flies, through unhygienic sources such as toilets, trash, sewage, carcasses and abattoirs (Szostakowska *et al.,* 2005). Also, synanthropic flies are considered as a major epidemiologic factor for spreading cryptosporidiosis (Nmrosi *et al.,* 2006). Cockroaches play a role in spreading of Cryptosporidium species. The oocysts found in the intestinal tract of cockroaches (Tatfeng *et al.,* 2005). Also, beetles play an important role in the transmission of Cryptosporidium species oocysts, when the external surface of the beetle's body is covered with oocysts (Al-Gelany, 2003).

2.6. Pathogenesis

The pathogenesis of Cryptosporidium-induced diarrhea is thought to contribute by many mechanisms (Guarino *et al.,* 1995). Throughout the clinical course and incubation period of the infection, Cryptosporidium mainly proliferated within the brush border of enterocyte that the ilium and small intestine (Noordeen *et al.,* 2000).

Main histopathological changes in the animal and human differ from mild to severe villous atrophy; the number of the pathogen is associated with the severity of the morphological changes (Genta *et al.,* 1993). The small intestine has severe damage in the mucosal layer, especially in the distal ileum and Jejunum, villi often are shorter, blunt, and wider than normal and sometimes diffused to other villi, and the crypts are hyperplastic and elongated, The inflammatory infiltrated cells found underlying of the lamina propria may be accompanied with these changes of the crypts and villi's structure, including lymphoid cells, neutrophils, and macrophages, in the paracellular region between the epithelial cells the intraepithelial neutrophils can be found during an infected with a large number of the pathogen (Godwin, 1991).

Cryptosporidium spp. attaches closely to the microvillous membrane and causes damage of microvilli and effacement, which lead to malabsorption. The organism stimulates second-signal pathways, such as the nuclear factor-kB (NF-kB) and c-srcsystems, the activation of NF-kB stimuli the production of cytokines and chemokines, such as IL-8, to stimulate an inflammatory reaction and stimulate anti-apoptotic survival signals in directly infected cells activation of c-src is connected with host-cell cy to skeletal reorganization and maybe dysfunction of tight joints. *Cryptosporidium spp.* prompts secretion of 5-hydroxytryptamine (5-HT) and prostaglandin E2 (PGE2) into the lumen, this parasite stimulates death in the epithelial cells, which lead to destruction to the epithelial partition. C. *parvum* produces many changing of the villous atrophy by an unknown mechanism lead to malabsorption (Chen *et al.*, 1998).

2.7. Epidemiology

Several factors are critical to the epidemiology of Cryptosporidium; they facilitate spread of Cryptosporidium, make the control and eradication is difficult (Dillingham *et al.,* 2002). The parasite has a direct monoxenic life cycle which is lasted 48 — 72 hours or little more (Fayer and Xiao, 2008 and Caccio and Putignani, 2014). Muli-host specificity for some species of Cryptosporidium (Smith *et al.,* 2006). The thick-walled oocysts are verystable and extremely resist to the environment, survive e.g. freezing at -10°C for one week and up to 4 days in drying feces, and remain viable in water for over than 140 days (Fayer, 2008). Thin-walled oocyst can excyst endogenously, and undergo autoinfection, which helps to explain the mechanism of persistent infections in immunocompromised individuals (Fayer, 2004). Oocysts withstand most disinfectants and the conventional water chlorination treatment, in which they don't destroy oocysts furthermore, the small oocyst size make them difficult to filter from contaminated water (Keidel and Daugschies, 2013). The infective dose is low, 50 oocysts have been shown to cause infection (Moore *et al.,* 2003). In contrast, one infected host can shed as many as (104 - 106 o.p.g.) of feces, and adult cattle, are more prone to infection, and shedding may persist for more than three months contributing to a huge infection pressure (Mahmoudi *et al.,* 2017).

Oocysts are sporulated and infective at shedding, which means that new hosts can immediately be infected (Helmy, 2014). Zoonotic transmission can take place easily through direct contact or contamination of water, food, tools and the role of rodents and insects for transmission of infection to human (Ryan *et al.,* 2017).

Most studies have focused on sheep and cattle more than goats, this made the information on the prevalence of cryptosporidiosis in goats is limited. Goat is consideredmore resistant than sheep to the infection but in some countries were reported a high infection in goats (Munoz *et al.,* 1996; Johnson *et al.,* 1999; Sevinc *et al.,* 2005).

Similarly high percentage 83.3% was recorded in both goat and sheep that examined in some herds in Cyprus (Smith and Nichols, 2010), also in Sri Lanka, a large prevalence study was conducted (33.6%) in 291 goats from 1050 animals (Noordeen *et al.,* 2000). Cryptosporidium infection was higher in goat kids under six months and in 7-12 months compared with goats that were above 12 months, the lower prevalence was detected in an older animal may be associated with gaining of active immunity (Zu *et al.,* 1992; Sreter *et al.,* 1995). Many stress factors can affect on immunity of older animal such as overcrowding, concurrent infections that causes lowering of immunity and poor nutrition, so, the cryptosporidiosis may identify in the advancing age of animal (Johnson *et al.,* J999). In AJ-Qadisiyah province, Hamed (2016) recorded the rate of infection in goats ranging in age from 7-12 months 31.57% and in age from 3-5 years 21.73%. Also increase herd size can affect the prevalence of *Cryptosporidium spp.* that has been already shown in sheep (Causape *et al.,* 2002; Sari *et al.,* 2009; Giadinis *et al.,* 2012) and cattle (Nydam and Mohammed, 2005), so, the large herd size is considered a most important risk factor for increasing the Cryptosporidium infection. As well as the 75-100% of lambs and calve become infected in the first weeks of life (De Graaf *et al.,* 1999). In Iraq, many studies have detected the prevalence of the Cryptosporidium infection in lambs. In (1998) Hasso record36%in Baghdad, as well as Halil (2000) in Mosel recorded 36.43% in lambs in age 10-30 days. 27.5% the prevalence of the infection with diarrhea in lambs in Al-Qadisiyah province (Alkaby, 2005).

Cryptosporidium parvum was a high epidemiological aspect than C. *hominis,* the first time was recorded in cow, sheep, and pig (Villacorta *et al.,* 1991; Hunter *et al.,* 2003). Al-Zubaidi (1994) found a high infection in C. *parvum* 40.2% in calve in Baghdad province. As well as C. *hominis* was few reported until (2005), after that it was recorded twice in farm. Animals (Xiao *et al.,* 1999). C. *parvum, C. hominis* and C. *xiaio* were considered the important species identified in goats (Giles *et al.,* 2009; Robertson, 2009; Diaz *et al.,* 2010). In Al-Qadisiyah province, Hamed (2016) detected C. *parvum* (55%), C. *hominis* (20%), C. *andersoni* (5%), C. *ubiquitum* (8.3%), C. *suis* (5%) and C. *xiaio* (5%) in sheep and goats by molecular analysis.

2.8. Clinical signs

Cryptosporidium is the most important parasite that causes diarrhea in many type of small animal and mammalian (Tzipori, 1983). Symptom varieties from patient to another depend on severity of infection and immune state of host (Davies and chalmers, 2009). Pre- patent period is around four days of Cryptosporidium infection. The Pre-patent period effected by dose of infection and the age of host (Noordeen *et al.,* 2000). The clinical manifestation of disease included excretion a large number of oocysts in feces. After few days of the patent period, there is a product a high percentage of oocystsexcretion, and reach to a peak at (5-8) days after infection, followed by a sharp falling in oocysts excretion, and remaining for (10-15) days post infection (Noordeen *et* a/., 2002).

The clinical manifestations of cryptosporidiosis in small ruminant are diarrhea (Watery or profuse diarrhea), anorexia, weight loss, and abdominal pain, in affected animals the color of the faeces varies from white to yellow and the consistency may differ from being soft pasty to watery with an unpleasant odor, the diarrhea may occur for 7-15 days in severe cases of the disease where as in mild form may occur for 3-5 days (Tzipori *et al.,* 1983; Noordeen *et al.,* 2002).

2.9. Diagnosis

The diagnosis of cryptosporidiosis in cattle and humans is traditionally performed by microscopically examination of a fecal smear, also immunological assays and PCR are increasingly applied in veterinary medicine (Petry, 2000).

2.9.1. Conventional methods

2.9.2.1. Clinical signs

The main clinical sign is watery diarrhea which will be green to yellow with amount of mucous but there is a lot of causative agent which is case diarrhea so diarrhea not considers the final diagnosis (Radostitis *et al.,* 2007).

2.9.1.2. Macroscopic examination

The feces examined macroscopically and record its color, consistency and smell (Olson *et al.*, 2004).

2.9.1.3. Microscopic examination

It is most widely used method for the diagnosis of Cryptosporidium by detection of oocysts in fecal smears in Light Microscopy (LM), Electron microscopy (EM) and Laser scanning confocal microscopy (LSCM) (Fayer and Xiao, 2008).

2.9.1.3.1. Direct wet smear method

The detection of the parasite oocysts is used by adding lugol’s iodine stain to the direct smears and examines under the oil immersion lenses, but this method is inadequate due to the smaller size of the oocysts and its general similarity with yeasts. Therefore, several methods were used for diagnosis as staining techniques and flotation (Crawford and Vermund, 1988).

2.9.1.3.2. Concentration methods

Concentration methods are commonly used to improve the detection limit of diagnostic techniques. This is important when low numbers of oocysts were excreted by an infected host (Pacheco *et al.,* 2013).

2.9.I.3.2.I. Flotation methods

a) Sheather's Sugar Solution is one of the most important methods used for flotation (Current and Garcia, 1991).
b) NaCl flotation solution prepared by dissolving 360g of sodium chloride in 640 ml of distilled water (Kuczynska and Shelton, 1999).
c) Zinc-sulfate flotation solution prepared bydissolving 703g of zinc sulfate in 297 ml of distilled water (Ma and soave,1983).
d) Formalin-ethyl acetate (FEA) is one of the most important methods used for flotation (Shaista *et al.,* 2016).
e) Acid flocculation (AF) concentration method separates oocysts from fibrous fecal material (Wells *et al.,* 2016).

2.9.I.4. Immunological methods

Immunoassay techniques offer both increased sensitivity and specificity when compared with the conventional staining methods, (Ahmed and Karanis, 2018).

2.9.1.4.1. Immunofluorescence techniques

The immunofluorescent antibody (IFA) is a sensitive and specific method of diagnosis in fecal samples from both human and animal sources (Rossie and Latif, 2013; Ryan *et al.,* 2016). Its high sensitivity in the detection of oocysts appeared when compared with other conventional staining methods; DMSO-MAF, MAF and auramine (Rossie and Latif, 2013). IFA allow visualization of the intact oocysts, providing a definitive diagnosis, it has achieved high sensitivity (96-100%), specificity (98.5-100%) and reliability in the diagnosis of cryptosporidiosis (Mirhashemi *et al.,* 2015).

2.9.1.4.2. Enzyme linked immunosorbent assay (ELISA)

Coproantigen detection techniques: Enzyme linked immunosorbent assay (ELISA), enzyme immunoassay (EIA) and immunochromatographic dipstick assay (ICT) are antigen detection tools that depend on the detection of Cryptosporidium antigen in fecal samples, it is provide quick and easy diagnostic tools (Ahmed and Karanis, 2018). The variability of sensitivity and specificity of coproantigen techniques are affected by the quantity of

Cryptosporidium oocysts, per gram of stool. ICT assays provide results within 10-15 min. These advantage lead to its expanded use for the routine detection and reporting of cryptosporidiosis in the United States (Roellig *et al.*, 2017) .

Enzyme immunoassays (ELISA and EIA) offer higher sensitivity and specificity than conventional microscopy as diagnostic methods for Cryptosporidium coproantigen with a detection limit of 103-104 oocysts/ ml. The spectrophotometric reading will eliminate subjectivity of microscopical interpretations. A large numbers of stool samples can be easily and quickly examined with increased reliability of results (Ghoshal *et al.*, 2018) . Longer processing (l-2h.) should be considered with enzyme assay. Variable sensitivity (59-100%) and specificity (93-100%) with this technique are also reported (Ryan *et al.*, 2016).

2.9.1.5. Histopathological examination

Examination of gastrointestinal biopsy specimens from infected individuals with Cryptosporidium species shows mild to moderate acute inflammation of the lamina propria and surface epithelial disorder. Staining of tissue sections by the Warthin-Starry staining method is an effective diagnostic pathway for the microscopic detection of microsporidia and preferable symptomatic abilities over than the haematoxylin and eosin stain (De, 2013).

2.9.2. Molecular diagnosis

Molecular techniques are being increasingly used for detection of Cryptosporidium species during the last two decades mainly because of the inability to differentiate Cryptosporidium species by conventional microscopy. These tools helped to detect and differentiate Cryptosporidium species/genotype and subtype if present (Xiao and Ryan, 2004; Caccio *et al.*, 2005). The use of molecular techniques has made significant contributions to our understanding of the prevalence and epidemiology of Cryptosporidium species (Xiao and Ryan, 2008).

2.9.2.1 Polymerase Chain Reaction

The Polymerase Chain Reaction (PCR) technique is a computerized, recycling technique and it includes large quantity processing (Ryan *et al.*, 2017; Adeyemo *et al.*, 2018). The advantages of PCR for the detection of Cryptosporidium in clinical samples are: sensitivity, specificity, ease of use, ability to analyze large numbers of samples in the same time, ability to speciate (Eliminating false positives encountered with cross reactions of antibodies to non-pathogenic protozoan species) and strain typing potential (Checkley *et al.*, 2015; Ignatius *et al.*, 2016). A variety of approaches using a range of primers have been described for clinical samples, and in all comparative trials PCR has been shown to be more sensitive and accurate than other diagnostic techniques for the detection of Cryptosporidium in different samples origins (Yu *et al.*, 2009) also the importance of PCR should be considered in terms of the practicality and cost effectiveness of using such a techniques for routine diagnosis (Arbabi and Hooshyar, 2009).

2.10. Treatment and control

Cryptosporidium parasite has high resistance to disinfection and treatment, so, there is no antimicrobial chemotherapeutic agent that reliably destroyed the parasite (Farthing, 2000), but the treatment of the animals can reduce the prevalence of the infection, and otherfactors such as appropriate hygienic measure, management the environment resistant of shedding oocysts and asymptomatic carrier, and cleaning with disinfection (Castro- Hermida *et al.,* 2002). The disinfected can be effected against Cryptosporidium oocysts are ethylene oxide, ozone, methyl bromide, and ammonia (Fayer, 2004).

Controlling of cryptosporidiosis generally depend on reduction of oocysts from the environment, occur by reducing oocysts from soil, water and contaminated food, as well as the transmission of oocysts to animals and humans, and for veterinary, laboratory and medical personnel must be avoiding of contact with contaminated material and equipment, and this equipment should be sterilized by autoclave. Also for patient care used hygienic programs including handwashing, use gloves and gowns, and cleaning the patient's room and toilet by using commercial disinfectants for killing and prevent transmission of parasites' oocysts (Fayer and Ungar, 1986; Sears and Kirkpatrick, 2001).

2.10.1. Vaccines

No vaccines are available to prevent cryptosporidiosis in both farm livestock and humans (Thompson, 2016). Calves that were immunized with killed (gamma-irradiated) Cryptosporidium oocysts showed a reduction in oocyst shedding and severity of diarrhea compared to non-immunized calves (Jenkins *et al.,* 2004). Some attempts to immunizebovine for produce antibodies against Cryptosporidium which can be passed to their calves in the colostrum have been made, calves receiving colostrum from mother vaccinated with recombinant Cryptosporidium oocysts were protected against diarrhea, also a reduction in oocyst shedding occur compared to the calves, which did not receive colostrum from vaccinated mother (Thompson, 2016; Arsenopoulos *et al.*, 2017; Guo *et al.*, 2018).

2.10.2. Disinfectants

Some disinfectants amine-based, peroxygen compounds are effective against Cryptosporidium oocysts at least in vitro (Naciri *et al.*, 2011). Few field trials testing these disinfectants found that even though these might be powerful in vitro, they are not sufficiently compelling to depend on for the successful elimination Cryptosporidium oocysts on the farms (Keidel and Daugschies, 2013).

2.10.3. Farm management

Good management and hygiene practices are recommended for controlling cryptosporidiosis because oocysts are very difficult to eliminate, so it is better to try and reduce environmental contamination in the first instance (Thompson, 2016).

Frequent messing out of calving zones joined with steam-cleaning and sterilization can decrease ecological development such as exhaustive cleaning with heated water pursued bydrying as the oocysts are susceptible to extremes of temperature and desiccation (Harp and Goff, 1998; Castro-Hermida *et al.*, 2002). Ultraviolet light introduction in these zones additionally restricts feasible Cryptosporidium oocysts determination (Story, 2016).

3. MATERIALS AND METHODS

3.1. Materials

Materials and instraments were shown in Table 3.1.

No.	Equipments (Devices)	Company
1	Autoclave	Sturdy (Taiwan)
2	Centrifuge	Hettich (Gennany)
3	Digital camera	Samsung / China
4	Distiller	GFL (Germany)
5	Exispin centrifuge	Bioneer/ Korea
6	Disposable gloves China	
7	Light microscope	Olympus (Japan)
8	Plastic containers	China
9	Slides and cover slides	China
10	Ocular micrometer	Japan
11	Refrigerator	Concord /Lebanon
12	Disposable gloves	China
13	Disposable syringe	China

Table 3.1 Materials and instruments

3.1.1. Solutions

3.1.1.1 Sheather's sugar solution

Sheather'ssugar solution was prepared by dissolved 500 gram sugar in 320 ml distilled water, with 6.5 gram phenol as preservative (Chennette and Boufassa,1988).

3.1.1.2 Phosphate buffer saline

Phosphate Buffer Saline was prepared by dissolved: 0.2 gram *KH^PO^*, 0.9 gram *NciiHPO^* 8 gram *NaCl* and 0.2 gram *KCl* in 1 liter of distilled water (Coles, 1986).

3.1.1.3 Potassium Dichromate 2.5%:

Potassium Dichromate 2.5% was prepared by dissolved 25 gram of potassium dichromate in 1 liter of distilled water (Ma and Soave, 1983).

3.1.1.4 Add Alcohol:

Acid Alcohol was prepared by adding 3 ml of concentrated HCL to 97 ml of ethanol 95% (Coles, 1986).

3.1.2. Stains

3.1.2.1. Modified Ziehl-Neelsen Stain

a) Components: Basic carbol fuchsin (4 grams); Phenol (8 grams); Ethanol 95% (20 ml); Distilled water (100 ml).

b) Preparation:

Solution A: It was prepared by dissolved basic carbol fuchsine stain (4 gram) in 20 ml of ethanol 95%.

Solution B: Phenol (8 gram) was added to 100 ml distilled water with continuous mixing until completely dissolved, then solution B was added to solution A and mixed well (Beaver and Jung, 1985).

3.1.2.2. Methylene blue stain (1%):

It was prepared by dissolving 1 gram methylene blue in 100 ml distilled water (Levine, 1961).

3.2. Methods

3.2.1. Samples collection

One hundred and twenty fecal samples from(60 sheep and 60 goats) were collected randomly in Baghdad city during the period from beginning of September 2020 to the end of November 2020. These samples were collected from different age (from less than 6 month to 24 months) and both sex. Fecal samples were collected directly from the rectum, also stool samples were collected in a clean plastic container and were tightly closed, given sequential numbers, age, sex, date of sampling also included protective measure was taken such as wearing disposable gloves. The samples were transported incool box to parasitology laboratory for traditional examination, in College of Veterinary MedicineUniversity of Baghdad.

3.2.2. Study design

The study design was summarized in Fig 3.1.

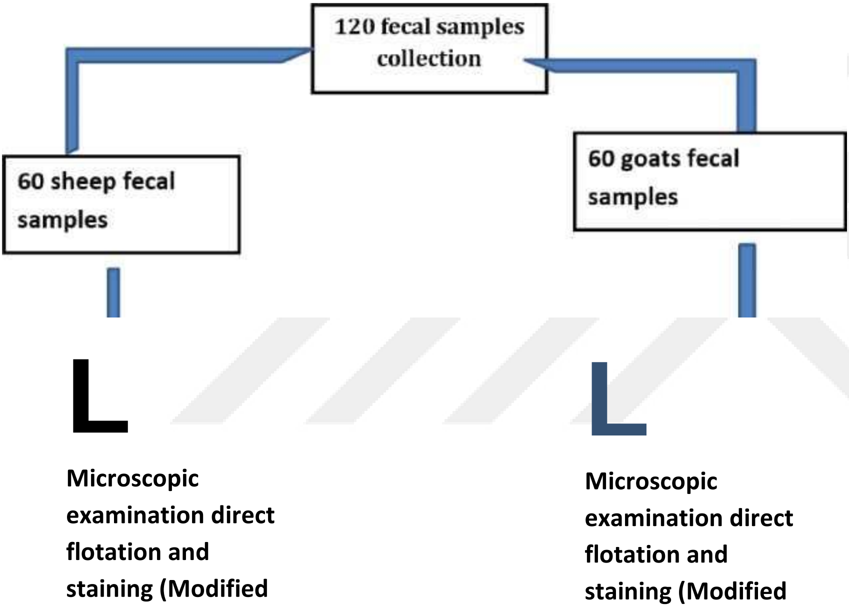

Figure 3.1. Study design

3.2.3. Laboratory tests of fecal samples

3.2.3.1. Macroscopic examination

The feces were examined macroscopically and recorded the abnormal color, smell and consistency.

3.2.3.2. Microscopic examination

3.2.3.2.I. Staining methods

Smears were prepared and stained by Modified Ziehl-Neelsen (mZN) stains to investigate Cryptosporidium oocysts according to (Beaver and Jung, 1985).

3.23.2.2. Flotation methods

Sheather's solutions were used to investigate Cryptosporidium oocysts according to (Chermette and Boufassa, 1988).

3.2.4. Calibration by using ocular micrometer

Calibration by using ocular micrometer for oocyst measurements (Zeibig, 1997):

a) Ocular eyepiece was replaced with one containing an ocular micrometer.

b) Stage micrometer was placed on the stage microscope.

c) Using the lOx objective, it was focused in on the stage micrometer and was arranged so that the left edge of the stage micrometer lines up with the left edge of the ocular micrometer. Successful completion of the zero points of each calibration device, that is, the numbers are superimpose.

d) A point farthest to the right of the zero was located where both devices again super imposed. The number of microns equal to each unit on the ocular micrometer was calculated by using the following.

e) Number of ocular micrometer units

The process was repeated for each objective and the microns equivalent to each ocular unit was calculated. Number of stage micrometer units x 1000

3.3. Statistical analysis

The Statistical Analysis System-SAS (2012) program was used for analyzing the effect of different factors in this study such as age, sex, region of study, and during the months of the year on prevalence, by used Chi-square test which was used to compare significant difference between percentages in this study ($p > 0.01$) (SAS, 2012).

4. RESULTS

4.1. Macroscopic examination of samples

Macroscopic examination of the positive fecal samples collected from (sheep and goats) were revealed that the consistency may differ from being softy-to-wateiy diarrhea, and while the color ranging from yellow-to-dark green, with an unpleasant odor.

4.2. Microscopic examination of samples

Cryptosporidium spp. oocysts appeared microscopically in flotation method oval- to-spherical shape and were examined under (X40and X100) of the microscopic (Fig 4.1) lens as in Fig 4.2, was two species determination of Cryptosoridium in both sheep and goats by using ocular lens measurement (length x width), the oocysts of C. *parvum* (Fig 4.3), C. *andersoni* (Fig 4.3) and stained with modified Ziehl Neelsen stain appeared as spherical/ rounded in shape stained red bodies with a clear halo around the oocyst, against a dark blue background of the methylene blue stain, and the measurement were 4.2 x 4.8 pm and 7.0 x 5.5pm respectively.

Figure 4.1. Examination of anmals and microscopic examination of *Cryptosporidium spp.* in sheep and goats fecal samples.

4.3. Infection rate of *Cryptosporidium spp.* in sheep according to age.

The total infection rates of Ciyptosporidium spp. in sheep were 40% stained with Ziehl-Neelsen stain and examined microscopically. The significant ($p < 0.01$) difference

Figure 4.2. *Cryptosporidium spp* oocysts, floatation with Sheather's solution (X100).

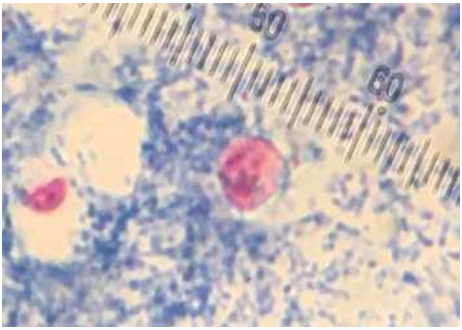

Figure 4.3. *Cryptosporidiumparvum* oocysts, stained with modified Ziehl Neelsen, measured 7.0 x 5.7/im /im (X100).

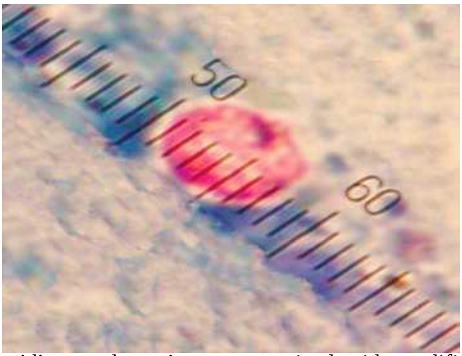

Figure 4.4. *Cryptosporidium andersoni* oocysts, stained with modified Ziehl Neelsen, measured 7.0 x 5.5pm (X100).

among the different age groups, and the results showed the highest infection rate 65% was recorded in lambs at the age group <6 months, while the lowest infection rate 20% was recorded at the age group >12-24 months as in Table 4.1 and Fig 4.5.

Age groups	No. of samples examined	Posit No.	ive samples %
< 6 months	20	13	65.00
>6- 12 months	20	7	35.00
>12 - 24 months	20	4	20.00
Total	60	24	40%
Chi-Square —X^2 (P-value)			26.527 ** (0.0001)
** (P<0.01)-Highly Significant.			

Table 4.1 Infection rate of *Cryptosporidium spp.* in sheep according to age by Ziehl-Neelsen stain.

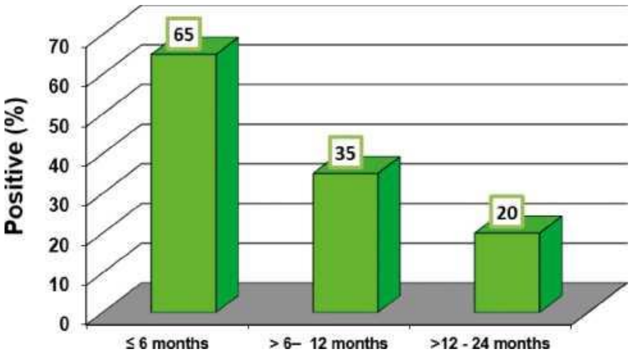

Figure 4.5. Infection rate of Ciyptosporidium spp. in sheep according to age.

4.4. Infection rate of *Cryptosporidium spp.* in sheep according to sex

The results showed that male and female recorded 23.33 % and 56.66 % respectively, with significant differences (p <0.01) as in Table 4.2 and Fig 4.6.

4.5. Infection rate of *Cryptosporidium spp.* in goats according to age

Cryptosporidium spp. infection rate was 33.33% in goat samples in Baghdad. The goat's age groups were ranged between (>6months to- <24months) which were infected with Cryptosporidium at variable rates. Highest infection rate showed in age >6 months

Sex	No. of samples examined	Positive samples	
		No.	Percentage (%)
Male	30	7	23.33
Female	30	17	56.66
Total	60	24	40%
Chi-Square —X^2 (P-value)			13.887 ** (0.0002)
** (P<0.01)-Highly Significant.			

Table 4.2 Infection rate of *Cryptosporidium spp.* in sheep according to sex.

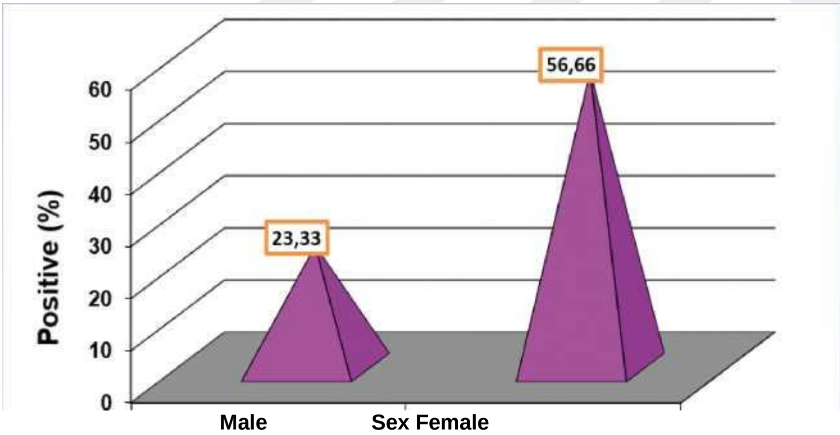

Figure 4.6. Infection rate of *Cryptosporidium spp.* in sheep according to sex.

(50%) and the lowest rate at age >12- 24 months was (20%) as in Table 4.3 and Fig 4.7.

Age groups	No. of samples examined	Posit No.	ive samples %
< 6 months	20	10	50.00
>6- 12 months	20	6	30.00
>12 - 24 months	20	4	20.00
Total	60	20	33.33%
Chi-Square —X^1 (P-value)			14.151 ** (0.0008)
** (P<0.05)-Significant.			

Table 4.3 Infection rate of *Cryptosporidium spp.* in goats according to age.

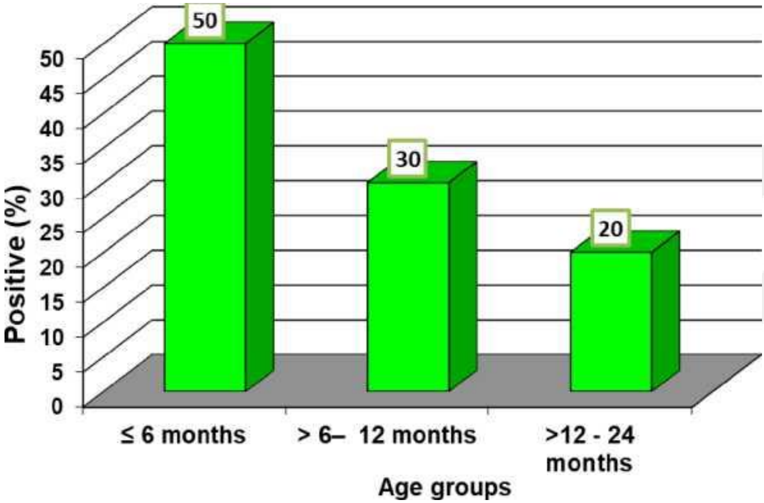

Figure 4.7. Infection rate of *Cryptosporidium spp.* in goats according to age.

4.6. Infection rate of *Cryptosporidium spp.* in goats according to age

The infection rate of *Cryptosporidium spp* in females was high at 50% while in the males was 16.66% with significant difference between male and female at level of p<0.05, as in Table 4.4 and Fig 4.8.

Sex	No. of samples examined	Positive samples	
		No.	Percentage (%)
Male	30	5	16.66
Female	30	15	50.00
Total	60	20	33.33
Chi-Square —X^1 (P-value)			16.675 ** (0.0001)
** (P<0.05)-Significant.			

Table 4.4 Infection rate of Cryptosporidium spp. in goat according to sex

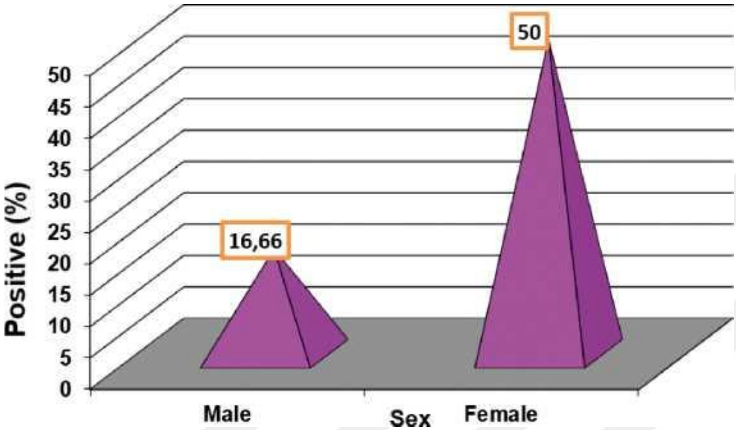

Figure 4.8. Infection rate of *Cryptosporidium spp.* in goat according to sex.

5. DISCUSSION

By microscopic examination, Cryptosporidium species oocysts isolated from the feces of the naturally infected animal were spherical, ovoid-shaped to ellipsoidal with a thin greenish membrane. It contains four sporozoites that resemble black bodies in oocysts on a wet smear and ovoid-shaped bodies with a transparent halo around the oocyst on a dark blue background of methylene blue in (Modified Ziehl Neelsen stain), but with slightly different dimensions. Four species of Cryptosporidium parasite were detected in this study by examine fecal smears with ocular lens measurement (length x width). Two species of Cryptosporidium parasite were detected in this study by examine fecal smears with ocular lens measurement (length x width).

The most frequently or dominant species that reported in goats is C. *parvum,* round, small oocysts size measured of 4.3 to 5.0 X 4.5 to 5.5 |im (mean: 4.6 x 5.0 |im), which agreed with Upton and Current (1985), who gave measurements of 5.0 x 4.5 (4.5 to 5.4 x 4.2 to 5.0) |im, and Tilley et al. (1990) reported thatthe oocysts measured 5.2 x 4.6 (4.8 to 5.6 x 4.2 x 4.8) |im. C. *andersoni* is more oval and larger oocysts than other species which were detected in this study, its measured 6.0 to 7.5 x 5.0 to 6.5 |im (mean: 7.0 x 5.7|im), this agreed with Anderson (1991), the original finder of the parasite, which reported the oocysts passed fully sporulated, lacked sporocysts, and its measured 7.4 x 5.5|im (6.0 to 8.1 x 5.0 to 6.5) (Lindsay *et al.,* 2000). Results are announced that the total infection rate of *Cryptosporidium* in sheep is 40%. The studies other recorded different results by using microscopic examination in Baghdad it found 5.85% (AL-Zubaidi, 2009), 15.8% in sheepin Al-Diwanyah (Dawood and Abdullah, 2007), 17.7% in Al-Muthana (Mohammed, 2013), 18% in Basrah (Ali, 1998), 10.1% (Khalil, 2000) in Mosul. In the world, the rate of the infection was 3.8% in Turkey (Erman *et al.,* 2000), in Nigeria by (Faleke *et al.,* 2006) which is 22.7% in sheep, in Bangladesh 11.3% (Siddiki *et al.,* 2015).

The overall infection rates for *Cryptosporidium spp.* in goats by microscopic examination were 20 (33.33%). This study recorded high infection rate than Al-Zubaidi (2009) who was recorded 10.77% in Baghdad city, also in Diylla province the lowest infection rate was recorded by Al-Tahie (1997) 7.2%, and by Al-Bakri (2002) in Mosul 14.7%; moreover, it was not in agreement with Mahoodi et al. (2017) when recorded the lowest infection rate in Iran 18.9% and in Bangladesh 15%. In European countries, this study agreed with Park et al. (2006) in Korea 42.9% and 35.8% in Sri Lanka (Mahoodi *et al.,* 2017). The convergent rate was recorded in Italy with Giallettil etal (1986) 25%; in other countries.

The variance of these infection rates is happens to depending on the effect of different factors such as environmental and health conditions of host, methods of animal husbandry and management, different age, sex and immune status of the animal, and the difference in the number of samples; also, the experience of the examiner and methods of examination have a significant impact on the rate of infection. In Iraq small ruminants breeding in opening farmyard with a low number, but in the world it breeds in the flock in large number in the small area, this crowded and defection of animals in opining area near water sources that considered a great source of transmission of the oocysts and increased the infection rate (Heithman *et al.*, 2002). The effect of environment factor is limited due to the oocysts will be sporulated without any special circumstance, which have resistant to high temperature and humidity (Jenkins *et al.*, 2005). Most infection was found in young lambs (65%) and lower in older sheep (20%) Similarly Khalil, (2000) from Iraq reported 80% in lambs, Fatimah et al. (1995) 36% in Malaysia, in Egypt EL-Wahed, (1999) 30% in lambs and Sari et al. (2008) 38.8% in Turkey. The study indicated that goat age groups were showed a significant difference, which the highest rate was recorded in>6 months (50%) and the lowest rate at age > 12- 24 monthswas (20%). This result agreed with Hamad (2016) who found the highest rate in the ages 7-12 months 31.57%, while the lowest rate in the ages 1-3 years 11.11% in Al-Qadisiyah province. Also Bejan et al. (2009) recorded the high prevalence rate in goat kids between one day and six weeks of age, and it was observed at the age 1-2 weeks as 39.4% and 2-3 weeks as 30.2%, as well as(Mi *et al.*, 2014).

These results could be due tothe mothers which infected in the younger age but it stops shedding oocysts in the post-weaning which return to shedding oocysts in a birth season that caused a decline in immunity level (Khalil, 2010). While the adult age is less susceptible to infection compared with young age that may be due to the adult animals are more resistant than young, this related to the physiological state of the animal, and maybe adult age have been adaptive immunity, which gets it from an old infection. Also the difference of sample number, various numbers of age group, mixed rearing, bad management; season and regions of collection sample which are an effect on infection rate.

According to sex, suggested statistical differences infection in sheep males 23.33% compared with sheep females 56.66%. Also, The females goats showed higher than goats male which recorded 50% and 16.66% respectively, this agreed with Gumar and Al-Zubaidi (2019) in Baghdad, Hamad (2016) in Al-Qadisiyah province. The infection with Cryptosporidium has no sex-specific, this study was showed both sexes were infected, but this variation in the infection rate was associated with females that it undergoes different conditions that lead to depressing immunity particularly during pregnancy and lactation, as well as a physiological state of female compared with male. In another side, the infected animal was not separated from the flock and its breeding mix together.

6. CONCLUSION AND RECOMMENDATIONS

According to the results obtained from this study, the following conclusions could be below;

a) Modified Ziehl-Neelsen stain was considered the basic stains for detection of Cryptosporidium oocysts.
b) This study recorded high prevalence of Cryptosporidium was endemic in Baghdad city in sheep and goats.
c) Different factors could be affected on the prevalence of Cryptosporidium such as age, sex, months of present study, as shown a significant difference between them
d) Conducting epidemiological studies in all provinces of Iraq to detection of Cryptosporidium infection in small ruminants.
e) Using molecular methods for detection of *Cryptosporidium spp.* and identification of genotyping in different animals in Iraq.
f) Developing modern diagnostic and immunological tests for early diagnosis of infection with this parasite and reveal infection in humans and animals Inevitability.
g) Experimental studies for the pathological effect of *Cryptosporidium spp.* in different hosts.
h) Instigation of control programs is suggested to reduce the risk of human infection and small ruminants mortality, morbidity rate and loss of production in dairy small ruminants farms.

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