GLOBAL JOURNAL OF BIO-SCIENCE AND BIOTECHNOLOGY

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DETECTION OF *GIARDIA DUODENALIS* IN SHEEP BY DIFFERENT LABORATORY METHODS

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ABSTRACT
The study was conducted to estimate the prevalence of *Giardia duodenalis* in sheep in Baghdad city (Abu Ghraib, Al-Bayaa, Al-Mahmudiah and field of Veterinary Medicine College -Baghdad University), and the effects of age and sex of the animal in the infection rate by using 200 fecal samples during the period from the beginning of November to the beginning of March, 2017. All fecal samples were examined by conventional microscopic examination {Direct wet smear, lugol's iodine smear, Giemsa stain smear and flotation technique (NaCl)**}**, The total infection rate of microscopic examination was 27.50% and the higher infection rate (34.61%) was recorded in the age group ≤6 months, while the lower infection rate (23.85%) was recorded in the age group 6-12 months, with a significant ($P \leq 0.01$) difference. Males had a close infection rate (27.27%) with females (27.72%) with significant difference ($P \leq 0.01$). A higher infection rate in February (40%), while the low infection rate in December (20.00%) with a significant difference ($P \leq 0.01$). The results of the present study showed a higher infection rate (80.00%) of *Giardia* in the field Vet. Med. College and the low infection rate (16.66%) in Al-Mahmudiah region with significant differences ($P \leq 0.01$). A different infection rates of *Giardia* in sheep were recorded by different conventional diagnostic methods. A higher infection rate (9%) was recorded by lugol's iodine smear , while a lower infection rate (5%), was recorded by the flotation methods (NaCl) with significant ($P \leq 0.01$) difference.

KEY WORDS: *Giardia*, sheep, conventional methods, trophozoites, cyst

INTRODUCTION

Giardia is a flagellate protozoan parasite causes a disease called Giardiasis (Geurden *et al*., 2010 and Feng and Xiao, 2011). It is infected numerous hosts that range from different mammals to amphibians and birds (Thompson and Caccio, 2004; Caccio *et al*., 2005; Monis *et al*., 2009). Different animal species have been reported worldwide infected by the parasite and considerable economic losses in livestock animals that are associated with the morbidity and mortality (Aloisio *et al*. 2006 ; Sweeny *et al*. 2011). Transmission of *Giardia*, particularly may occur through either direct contact (farmers, veterinarians, and petting zoos) or through indirect routes such as contaminated water or foods (Dixon, 2009).This parasite have many species such as *G. duodenalis* (syn. *G. lamblia*, *G. intestinalis*), *G. muns*, *G. microti*, *G. agillis* and *G. psicatti* (Olson *et al*., 2004). The organism exist in two forms, vegetative form (trophozoite) capable of causing illness in the host which lives principally in the upper part of small intestine and cyst form that considered as an infective stage of the parasite (Peter and Lisa, 2010). Infected animal revealed symptoms range from asymptomatic to acute or chronic disease (Gardner and Hill, 2001) and the predominant clinical signs in ruminants are diarrhea and reduction in growth rate (Lalle *et al*., 2005**).** Diagnosis of the parasite is based upon the demonstration of cysts or motile trophozoites in the feces, duodenal aspirates or serologically (Smith and Mank, 2011).

MATERIALS & METHODS

Two hundred direct fresh sheep fecal samples were collected from different areas (Al- Bayaa , AL-Mahmudiah , Abu- Ghraib and Field of Veterinary Medicine College, Baghdad University) in Baghdad city during the period from 1/ November/2016 till 31/ March /2017. The samples were conveys to the parasitic laboratory at Veterinary Medicine College/ Baghdad University. Each fecal sample was examined by direct wet smear (Griffiths, 1978), Lugol's iodine stains (Levine, 1961), Giemsa stain (Coles, 1986) and by concentration method -NaCl flotation (Soulsby, 1982).The information about the age and sex of the animals were recorded.

RESULTS

Total infection rate of *Giardia sp.*

The total infection rate of sheep *Giardiasis* by different conventional methods
(Direct fecal wet smear, logul's iodine stain, Giemsa stain and flotation –Nacl) was 27.50% (55/200). (Table, 1)

TABLE 1: The total Infection rate of *Giardia* in sheep by different conventional methods

Number of samples examined	Positive	Percentage (%)
200	55	27.50

Infection rate of *Giardia* sp. according to age of the animal

The study revealed that all age groups of sheep were infected by *Giardia* sp. with different infection rates, but with a significant (P≤ 0.01) difference among groups .The higher infection rate was recorded in animals aged more ≤ 6 month 34.61 while the lowest infection rate in 6-12 month 23.85% (Table, 2).

TABLE 2: Infection rate of *Giardia* sp. in sheep according to the age of the animals

Age(Months)	Number of samples examined	Positive*	Percentage (%)
≤6	26	9	34.61
6-12	109	26	23.85
12-24	65	20	30.76
Total	200	55	27.50

*P≤ 0.01

Infection rate of *Giardia* sp. in sheep according to sex of the animals

Table (3) was showed with significant (P≤ 0.01) difference between both sexes with an infection rate 27.72% in females, and 27.27 % in males.

TABLE 3: Infection rate of *Giardia* sp.in sheep according to the sex of the animals

Sex	Number of samples examined	Positive*	Percentage (%)
Males	99	27	27.27
Females	101	28	27.72
Total	200	55	27.50

* P≤ 0.01

Infection rate of *Giardia* sp. in sheep according to Months of the study

The infection rate along the months of the study was variable with significant (P≤ 0.01) difference**.** The higher *Giardia* sp. infection rate (40 %) was estimated in February, while the lower infection rate (20%) was recorded in December (Table, 4).

TABLE 4: Infection rates of *Giardia* sp.in sheep according to the months of the study

Months	Number of Samples examined	Positive*	Percentage (%)
November	35	8	22.85
December	35	7	20
January	45	10	22.22
February	45	18	40
March	40	12	30
Total	200	55	27.50

*P≤ 0.01

Infection rate of *Giardia* sp. in sheep according to the areas of the study

A different infection rate of *Giardia* sp.in sheep was recorded in different areas of the study but with significant (P≤ 0.01) difference. A higher infection rate (80%) was found in the field of Veterinary Medicine College and the lowest infection rate (16.66 %) was recorded in Al-Mahmudiah area (Table, 5).

TABLE 5: Infection rates of *Giardia sp.* in sheep according to the areas of the study

Areas	Number of samples examined	Positive*	Percentage (%)
Abu Ghraib	33	13	39.39
Al-Biae	85	22	25.88
Al-Mahmudiah	72	12	16.66
Field of Vet. Med. College	10	8	80.00
Total	200	55	27.50

*P≤ 0.01

Infection rate of *Giardia* sp.in sheep according to the methods of diagnosis

There was a different infection rate of *Giardia* sp. in sheep recorded by different conventional diagnostic methods with significant (P≤ 0.01) difference. A higher infection rate (9%) was recorded by the Lugol′s iodine smear, while the lower infection rate (5%) was found by the flotation - Na Cl) method (Table, 6).

TABLE 6: Infection rate of *Giardia sp.* in sheep by using the different conventional diagnostic methods

Methods	No. of Samples *Examined	Positive	Percentage (%)
Direct wet smear		15	7.5
Lugol′s iodine smear	200	18	9
Giemsa stain smear		12	6
Flotation -Na Cl		10	5

*P≤ 0.01

DISCUSSION

Giardia is the most commonly intestinal protozoal diagnosed parasite in the world and a numerous methodologies are available to identify the parasite (Olson, 2002), such as the traditional methods for fecal analysis (direct fecal wet smear or fecal concentration techniques by sedimentation or flotation techniques (Cheesbrough, 2005) or by the serological tests (El-Nahas *et al*., 2013) that were used in the present study. The total infection rate of *Giardia* sp. in the present was recorded 27.50% from 200 sheep fecal samples that examined by using conventional methods (Direct wet fecal smear, lugol′s, Giemsa stain and flotation) that was agree or different from reports before in many countries of the world, and the species *G. duodenalis* has a worldwide distribution (Wade *et al*., 2000; Degerli and Ozcelik, 2003; Bomfim *et al*., 2005; Thompson *et al.*, 2008; Winkworth *et al*., 2008; Muhid *et al*., 2011; Geurden *et al*., 2012; Di-Cristanziano *et al*., 2013 and Wang *et al*., 2014). Also, it has been reported in sheep in many countries including Australia, Canada, England and USA (Buret *et al*., 1990; Taylor *et al*., 1993; Olson *et al*., 1997; Ryan *et al*., 2005 and Santín *et al*., 2007), and in China (Zhang *et al*., 2012). On the other hand, the result was high than the infection rate (6.26%) of *Giardia* found in sheep in Southern Spain (Diaz *et al.*, 1996) and AI-Fetly *et al*. (2010) was estimate an infection rate 13.5% in AL-Diwaniya Province and was less than that identified in sheep from different Canadian farms 38% (Olson *et al.*, 1997). Also a wide range of an infection rates were estimated (6–82%) in North America (Xiao, 1994), while cumulative incidence of *Giardia* in sheep has been reported in the previous literatures to be nearly 100%. Also, a different *Giardia* sp. infection rate was estimated in different animal species in different countries of the world; in goats a different incidence of the *Giardia* infection was recorded that may reach 100% (Castro-Hermida *et al*., 2005, 2006) and among dairy goats in Brazil (14.3%) by microscopic examination and 42.2% in Spain and 20% among asymptomatic adult goats in France by immunofluorescence technique (Bomfim *et al*., 2005; Castro-Hermida *et al*., 2005 and Ruiz *et al*., 2008), and it has been established in beef and dairy cattle with prevalence reach to 100% (Xiao and Herd, 1994; O'Handley *et al.*, 1999; O'Handley, 2002 and Ralston *et al.*, 2003). The prevalence of infection in cats and dogs was different from <1 to 45% in sheltered dogs (Upjohn *et al.*, 2010*)* and 4 to 11% in cats (Olson *et al.*, 2010). The difference in the infection rates between the results of present study and other studies may be due to many factors that may be associated with risk of infection that can be narrowed down to the demographic and management factors (Xiao, 1994 and O'Handley *et al*., 1999). Demographic factors may include age distribution of animals sampled, size of the farm, geographic location, herd size, and other species of animals present on the farm (Gow and Waldner, 2006). Management factors include general management (type of flooring, calf housing, and frequency and method of cleaning) (Maddox-Hyttel *et al*., 2006). Also separation of the dam from the calf and administration of colostrum, and don′t direct contact with infected animals. Generally, intensive management has been found to favor transmission of *Giardia* cysts (Hamnes *et al*., 2006). Previous studies revealed that animals reared indoors especially under group housing were more likely to be infected with the parasite than those housed outside (Quigley *et al*., 1994 and Reust *et al*., 1998). Some management practices that reduce direct contact between animals such as separation of new born from the dam immediately after birth may aid in reducing the transmission of the cysts (Wade *et al*., 2000), because adult animals are a potential source of the parasite especially for neonates as a per parturient rise in cyst excretion has been reported in cattle, sheep, goats and pigs (Xiao and Herd, 1994; Xiao *et al*., 1994; Wade *et al*., 2000 and Castro-Hermida *et al*., 2005). Different studies showed that synanthropic flies are the most important mode of transmission of *Giardia duodenal* which mechanical transmission of pathogens by flies is intensive and it is achieved through defecation regurgitation or mechanical dislodgment .They carry the viable parasite from unhygienic sites (Graczyk *et al*., 2001 and Graczyk *et al*., 2003) and nonbiting flies can deposition of this pathogen on the visited surfaces (Bean *et al*., 1996 and Wallace *et al*., 2000). The role of animal infections remains controversial particularly that of livestock and wildlife because of their potential role as zoonotic reservoirs of infection (Cifuentes *et al*., 2002). The great potential for zoonotic transmission of *Giardia* is with genotype A and domestic animals, wildlife, and possibly pets act as reservoirs (Guy *et al*., 2004), and a several studies referred to a variety of birds suggest they may be zoonotic reservoirs (Franssen *et al*., 2000). Also, the transmission of parasite in humans and animals are restricted largely to the presence of genetic recombination (Ashford and Snowden, 2001, Coope *et al.*, 2007; Teodorovic *et al*., 2007; Lasek-Nesselquist *et al*., 2009 and Sprong *et al*., 2009). There is an effects of age in infection rates of *Giardia* and cysts of the parasite were found in all age categories, but the high infection rate in this study was recorded in the age group between 1-6 months 9 of 26 (34.61%) and there were an apparent declining infection rate with an increasing age of the animals that was agreement with the previous studies (epidemiological studies) which referred that young

animals were more susceptible to opportunistic parasites than adults in Giardiasis of sheep and goats and the majority focused on the occurrence in lambs and kids. In a longitudinal study of lambs, overall prevalence of *G. duodenalis* was 23.0% in the first samples and 31.0% in the second samples (Robertson *et al*., 2010) also, in Belgium, the prevalence was 25.5% in lambs and 35.8% in kids (Geurden *et al*., 2008), but these results were differ and disagreement with Taylor *et al*. (1993) who found 68.6 % of lambs excreted *Giardia* cysts. On the same hands; a higher prevalence of *Giardia* infections in neonatal lambs than in adult sheep has been reported in Spain (Castro-Hermida *et al*., 2011). Another study in Australia also revealed that infection with the parasite was higher in lambs aged below twelve months than in adult sheep (Ryan *et al*., 2005). In the Brazilian study, infections were more in kids from one to three months of age than they were in adult goats (Bomfim *et al*., 2005).Also, calves aged over nine days were found to be more likely to be infected with *Giardia* (Gow and Waldner, 2006). On the same way, a study in North America revealed that dairy calves as young as two days of age were harboring the parasite (Mark-Carew *et al*., 2010) and the burden of infection in dairy cattle has been reported to be low above six months of age (Buret *et al*., 1990 and Becher *et al*., 2004). High infection rates in the younger animals may be due to many reasons; It has been suggested through experimental studies that lambs do not rapidly develop high antibody titers against *Giardia* (Yanke *et al*., 1998), that develop a specific immunity by the host against the parasite (O'Handley *et al*., 2003).Wolf (1992) emphasized that the antibodies type IgM and IgA play a major role in the extermination of parasites, and noted that the chronic Giardiasis link with low immune globulin IgG, and acquired immunity after initial infection may emerge as an important protection toward the parasite (Hanevik *et al.*, 2011). As a result, young animals can be considered to be a source of infection for susceptible hosts and high levels of infection with the parasite have been recorded on farms located in areas with poorly drained soils (Tiranti *et al*., 2011). Poorly drained soils may increase the retention of moisture, which in turn prolongs survival of the cysts in the environment (Barwick *et al*., 2003). Sex of the animals doesn't affect the infection rate in sheep in the present study that results agreement with Diaz *et al*. (1996) and Xiao (1994). The infection rates of *Giardia* were variable with significant difference in the different months of the year. A high infection rate was 40 % in February, while a low infection rate (20%) was found in December that agreement with Al-Dulaimi (2016) who refers variation in the infection rates during different months of the year. Furthermore, different infection rates of *Giardia* were recorded in different areas of the study with significant difference. The infection is diversely dispersed throughout all over Iran, such as East Azerbaijan Province and the incidence in this Province were variable from 15.2% in Tabriz city to 43.8% in Naghadeh District (Saebi, 2005). Although, there is direct evidence of transmission of *G. duodenal* from small ruminants to the other sheep via contaminated water and it is considered a threat. Also, the prevalence of *Giardia* in water was significantly higher in the inland area, with higher concentration of livestock and fewer water treatment plants (Castro-Hermida *et al*., 2011).On the same hand , the pastures surrounding the drinking water basins are all grazed by small ruminants lead to a substantial public health threat (Tzanidakis *et al*., 2014).

There were a different infection rates of *Giardia* in sheep recorded by different conventional diagnostic methods .A high infection rate (9%) was recorded by Lugol′s iodine smear and a low infection rate (5%) was estimated by flotation methods that was agreement with Zhang *et al*. (2012) who referred that a different results of each sheep fecal specimen was directly used to smear three slides for iodine wet mount staining and wet smears were examined for the presence of *G. duodenalis* cysts by light field microscopy (40x magnification) .The average prevalence of *G. duodenalis* infection was 5.0% (34/678) by microscopy after Lugol's iodine staining 5.6% (30/539). Furthermore, the prevalence of *G. duodenalis* in goats varies (<10% to >40%), depend on the age of animal, geographical locations and diagnostic techniques used in the examination (Robertson, 2009).

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