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 Ghraila, 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 6-12 months, while the lower infection rate (23.85%) was recorded in the age group 6-12 months, with a significant (P ≤ 0.01) difference. Males had a close infection rate (27.27%) with females (27.72%) with significant difference (P ≤ 0.01). A higher infection rate in February (40%), while the low infection rate in December (20.00%) with a significant difference (P ≤ 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 ≤ 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 ≤ 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 Peng 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. mumps, G. microti, G. agilis and G. psicati (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- Ghraila 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, lugol’s iodine stain, Giemsa stain and flotation –NaCl) was 27.50% (55/200). (Table, 1)
Detection of *Giardia duodenalis* in sheep

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

<table>
<thead>
<tr>
<th>Number of samples examined</th>
<th>Positive</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>200</td>
<td>55</td>
<td>27.50</td>
</tr>
</tbody>
</table>

**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

<table>
<thead>
<tr>
<th>Age (Months)</th>
<th>Number of samples examined</th>
<th>Positive*</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤6</td>
<td>26</td>
<td>9</td>
<td>34.61</td>
</tr>
<tr>
<td>6-12</td>
<td>109</td>
<td>26</td>
<td>23.85</td>
</tr>
<tr>
<td>12-24</td>
<td>65</td>
<td>20</td>
<td>30.76</td>
</tr>
<tr>
<td>Total</td>
<td>200</td>
<td>55</td>
<td>27.50</td>
</tr>
</tbody>
</table>

* 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

<table>
<thead>
<tr>
<th>Sex</th>
<th>Number of samples examined</th>
<th>Positive*</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males</td>
<td>99</td>
<td>27</td>
<td>27.27</td>
</tr>
<tr>
<td>Females</td>
<td>101</td>
<td>28</td>
<td>27.72</td>
</tr>
<tr>
<td>Total</td>
<td>200</td>
<td>55</td>
<td>27.50</td>
</tr>
</tbody>
</table>

* 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

<table>
<thead>
<tr>
<th>Months</th>
<th>Number of Samples examined</th>
<th>Positive*</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>November</td>
<td>35</td>
<td>8</td>
<td>22.85</td>
</tr>
<tr>
<td>December</td>
<td>35</td>
<td>7</td>
<td>20</td>
</tr>
<tr>
<td>January</td>
<td>45</td>
<td>10</td>
<td>22.22</td>
</tr>
<tr>
<td>February</td>
<td>45</td>
<td>18</td>
<td>40</td>
</tr>
<tr>
<td>March</td>
<td>40</td>
<td>12</td>
<td>30</td>
</tr>
<tr>
<td>Total</td>
<td>200</td>
<td>55</td>
<td>27.50</td>
</tr>
</tbody>
</table>

* 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

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

* 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).
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; Mahid 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 higher than the infection rate (6.26%) of *Giardia* found in sheep in Southern Spain (Diaz et al., 1996) and Al-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-Hytel 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 (Hammes 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 duodenalis* 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., 2005) 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 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 excretion 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 Nakhadeh District (Saebi, 2005). Although, there is direct evidence of transmission of *G. duodenalis* 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 Lugol’s iodine staining and 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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Detection of *Giardia duodenalis* in sheep


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