



Detection of *Giardia duodenalis* in Cattle in Mosul City, Iraq

Nadia S. Alhayali, Mostafa S. Alneema and Eman G. Suleiman

Department of Microbiology, College of Veterinary Medicine, University of Mosul, Mosul, Iraq.



THE TOTAL percentage of infection with *Giardia duodenalis* in (50) ingesta of small intestine and (50) gallbladders which were collected from cattle with different ages and both sexes from abattoir and some butcher shops in Mosul city were 28% ,12% respectively. Staining with Giemsa technique showed a higher percentage of Giardia infection rates for ingesta , gallbladder fluid , intestinal mucosal scraping and gallbladder mucosal scraping were 22%, 16%, 6% and 10%, respectively. There were no significant differences appeared between male and female cattle. The highest rate of infection with Giardia was 50% in animals less than one year old while the lowest rate was 17.64% in 3 years and more with no significant differences between those two ages. There was a significant difference between cattle suffering from diarrhea (60%) than without diarrhea (14.28%). The histopathological technique of the gallbladder sections revealed presence of trophozoites of *Giardia duodenalis* in the lumen of the gallbladder with chronic inflammatory response.

Keywords: Giardia, Cattle, Gallbladder, Fecal Sample.

Introduction

Giardia is an intestinal flagellate protozoan parasite of the small intestine. It was newly considered in the World Health Organization's Neglected Disease Initiative because the parasite is environmentally ubiquitous which has the ability to continue for prolonged periods in the environment since the cysts are capable of propagating through anthroponotic route [1]. Infection with *Giardia* occurs by the ingestion of contaminated water or food with cysts which result in giardiasis [2].

Giardiasis exhibited different clinical signs ranged from asymptomatic to acute or chronic diarrheal manifestation accompanied by abdominal pain and nausea; most of the infections are self-limiting although re-infection and chronic infection can occur [3]. This disease occurs in a wide variety of hosts including human, monkeys (*G. intestinalis* syn *G. lamblia*), dogs (*G. canis*), cats (*G. cati*), cattle (*G. bovis*), goats (*G. capare*), horses (*G. equi*), rabbits (*G. duodenalis*), mice and rats (*G. muris*) and guinea-pigs (*G. caviae*) [4].

The favored sites of infection of *Giardia* in human and animals are the duodenum and proximal jejunum [5]. Several previous researches have implied that *Giardia* was either a parasite of gallbladder and bile ducts. Others did not believe that *Giardia* was either a parasite of the hepatic system or it caused any disease related to the liver or its ducts [6]. The biliary phospholipid in the duodenum and proximal jejunum is a significant source for preformed membrane phospholipid of the parasite [7, 8]. In general *Giardia* parasite was detected in cow and calf in the fecal samples in different countries all over the world with different percentages of infection such as Italy (19%), Romania 5%, India 52%, America 10-89% [9] while Degeri and Ozcelik (2003), [10] has been reported the infection with *Giardia* in the gallbladder of adult cows in Turkey the percentage of infection 1.7%. Since there are few studies in Iraq and other countries in the world about the isolation of *Giardia* from gallbladder and the study was conducted due to the importance of this parasite in human and animals.

Materials and Methods

Sample collection

A total of (50) small intestine ingesta and (50) gallbladders were collected from cattle with different ages and of both sexes from abattoir and some butcher shops in Mosul city, Iraq to detect infection with *Giardia duodenalis*, these samples were collected from animals with and without diarrhea during the period from March 2019 to July 2019. All samples were labeled and transmitted by ice bag to the parasitology lab., College of Veterinary Medicine, University of Mosul, Iraq.

Parasitological study

- Intestinal ingesta was examined by direct concentration methods using flotation with sheather's sugar solution and staining the ingesta and intestinal scraping with iodine and Giemsa stain 5% [11].
- The gallbladders were washed with tap water and then distilled water and the gross pathological changes were recorded. The gallbladders were opened and the fluid was collected to determine the nature of the fluid. The collected fluids were examined directly under light microscope using the concentration sedimentation methods by centrifuging with speed 1500 rpm for 5-15 min. The sediment examined by using sheathers sugar solution [11], also mucosal scrapings were obtained from gallbladders and examined under light microscope. Each gallbladder fluid mucosal scrapings were stained with iodine and Giemsa stain 5% for the detection of the parasite.

Histopathological Study

The gallbladder which gave a positive result was placed in 10% neutral buffer formalin for 48 hours for the preparation of the histopathological examination. Slides at thickness 4-6µm and

then stained with hematoxylin and eosin stain to detect the *Giardia* and exam the pathological changes [12]. The sizes of parasite were measured by using ocular micrometer.

Statistical analysis

The obtained data of this research were analyzed statistically using Chi-square [13].

Results

The total percentages of infection with *Giardia duodenalis* in each ingesta of small intestine and gallbladder were 28%, 12% respectively (Table 1).

Giardia duodenalis was diagnosed in the ingesta of small intestine and gallbladder by using different techniques. High ratio of infection with *Giardia* was clear with staining the specimens (ingesta of small intestine, Intestinal mucosal scraping, gallbladder fluid. Gallbladder mucosal scrapings) with Giemsa stain include 22%, 16%, 6%, 10% respectively (Table 2).

High ratio of infection with *Giardia* appeared in ingesta of small intestine of male 30% with no significant differences between male and female of cattle (Table3).

Cattle less than one year old show the highest rate of infection with *Giardia* 50% while the lowest rate 17.64% recorded in age 3years and more. Statistical analysis showed significant differences between animals aged less than one year and animals aged 3years and more. While there were no significant differences between animals aged less than a year and animals aged 1-2 year and between animals aged 1-2 years and animals aged 3years and more (Fig1).

Animals suffering from diarrhea showed higher percentage of infection (60%) than animals without diarrhea (14.28%). However statistical analysis revealed significant differences between both groups (Table 4).

TABLE 1. The total percentage of infection with *Giardia duodenalis* in fecal material of small intestine and gallbladder of cattle.

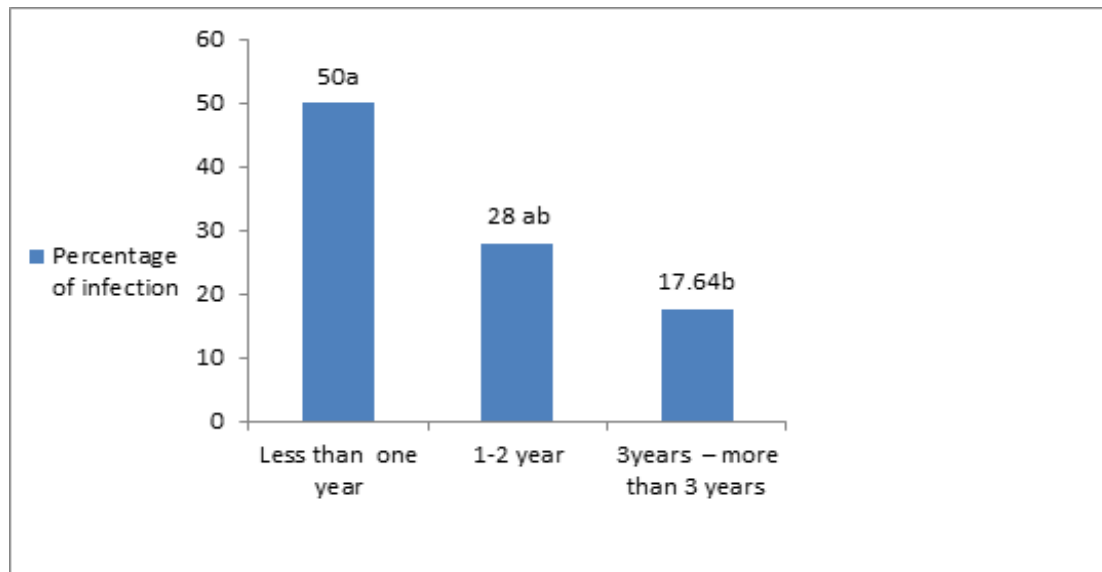
The samples	Number of examined samples	Number of infected samples	Percentage of infection
Ingesta of small intestine	50	14	28%
Gallbladder	50	6	12%

TABLE 2. The percentage of infection with *Giardia* in (50 fecal samples and 50 gallbladder) by using different technique.

The sample	Technique			
	Direct method	Flotation method	Staining with iodine	Staining with Giemsa
Ingesta of small intestine	4(8%)	7(14%)	7(14%)	11(22%)
Gallbladder fluid	3(6%)	7(14%)	4(8%)	8(16%)
Intestinal mucosal scraping	2(4%)	2(4%)	2(4%)	3(6%)
Gallbladder mucosal scrapings	1(2%)	3(6%)	3(6%)	5(10%)

TABLE 3. The relationship between percentage of infection with *Giardia* in ingesta sample and sex of animals.

Sex of animals	Number of examined samples	Number of infected samples	Percentage of infection
Male	30	9a	30%
Female	20	5a	25%
Total	50	14	28%

**Fig. 1.** The relationship between percentage of the infection with *Giardia* in fecal samples and age of animals.**TABLE 4.** The percentage of infection with *Giardia duodenalis* in ingesta of animals with and without diarrhea.

Animals	Number of examined animals	Number of infected animals	Percentage of infection
Cattle with diarrhea	15	9a	60%
Cattle without diarrhea	35	5b	14.28%
Total	50	14	28%

Trophozoites of *Giardia* appeared as pear shaped body and looked inactive when the specimens of ingesta, gallbladder fluid and mucosal scrapings examined by direct methods. However, when stained with iodine and Giemsa appeared in typical form (pyriform with broad anterior end, pointed posterior end), have two nuclei, and flagella, the trophozoite measuring $10.5-21 \times 5-13 \mu\text{m}$ with mean $15.723.36 \pm 8.22.58 \pm \mu\text{m}$ (Fig.2). Also these parasite aggregates in colonies (Fig 3). The cysts of *Giardia* were diagnosed in the ingesta and appeared ovoid in shape with two or four nuclei when stained with iodine and Giemsa and measuring $11.635 \pm 2.450 \times 7.0833 \pm 2.213 \mu\text{m}$ with mean $9-15.5 \times 4-10 \mu\text{m}$ (Fig. 4&5).

The gross examination of the infected gallbladder with *Giardia* showed thickened gallbladder (6), congested mucosa layer and the gallbladder fluid was thickened with dark brown color Fig.7.

As for histopathological examination of the gallbladder sections showed presence the trophozoites of *Giardia duodenalis* in the lumen of the gallbladder attached to the mucosal epithelium (Fig.8,9). Histopathological changes have been noticed accompanying presence of the parasites manifested by chronic inflammatory response including congestion blood vessels, lymphocytes, plasma cells and macrophages infiltration in mucosal and submucosal layer, Fig.10,11,12,13 with degeneration in the epithelial layer.

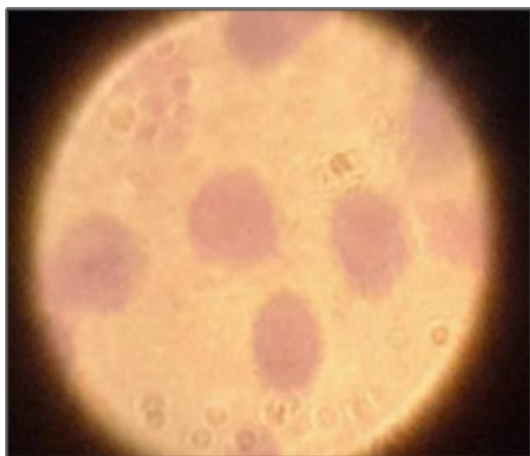


Fig. 2. Trophozoites of *Giardia duodenalis* stained with Giemsa 100X .

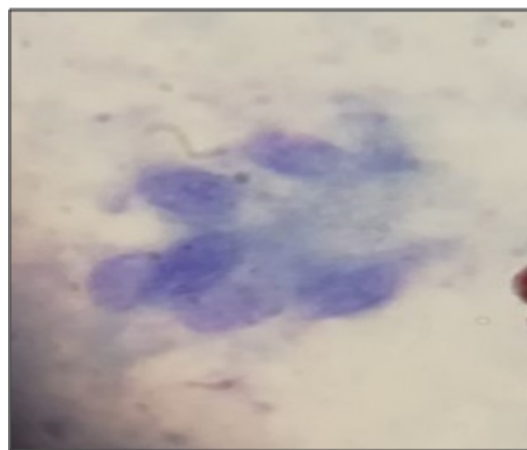


Fig. 3. Trophozoites of *Giardia duodenalis* stained with Giemsa appeared as colonies 100X .

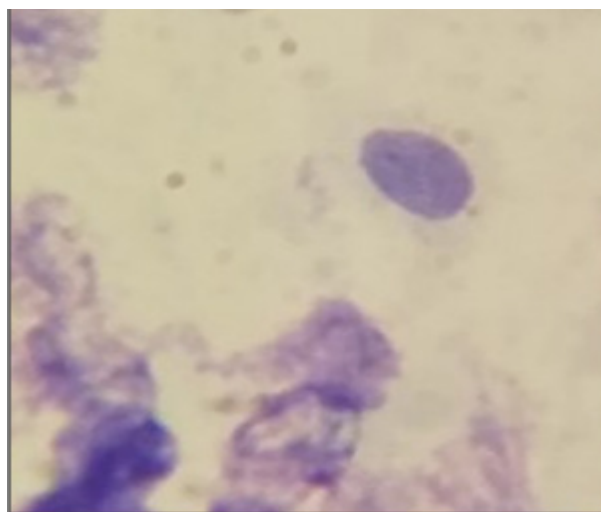


Fig. 4. Cyst of *Giardia duodenalis* stained with Giemsa 100X .



Fig. 5. Cysts of *Giardia spp.* stained with iodine 100X by using digital camera.



Fig. 6. Thickened wall of the infected gallbladder by *Giardia duodenalis* 10X.

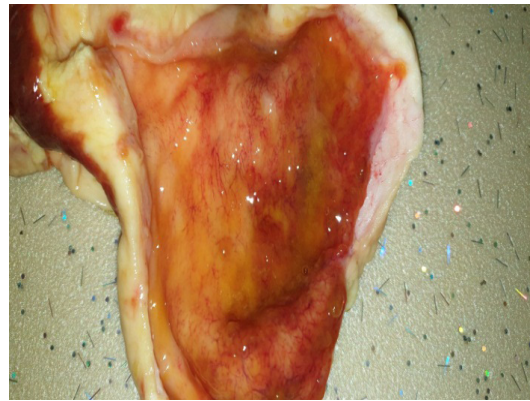


Fig. 7. Congested mucosal layer and thickened gallbladder fluid 10X.

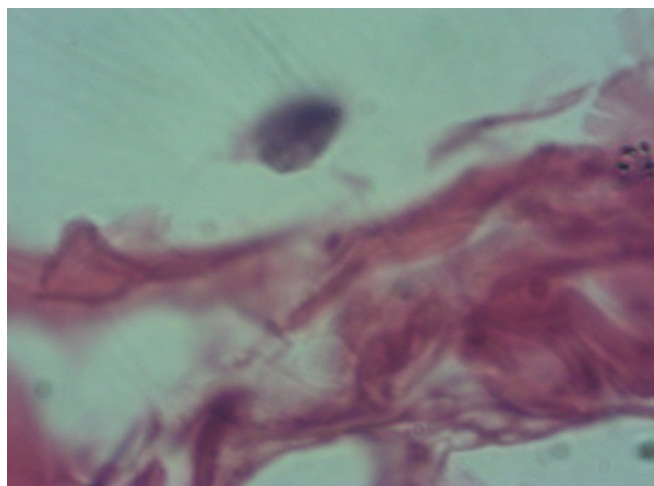


Fig. 8. Trophozoite of *Giardia duodenalis* in the lumen of the gallbladder and attached to the mucosal epithelium 100X .

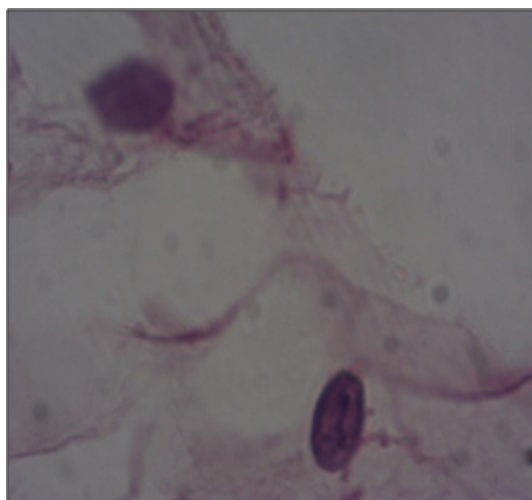


Fig. 9. Cyst and Trophozoites of *Giardia duodenalis* in the lumen of the gallbladder 100X .

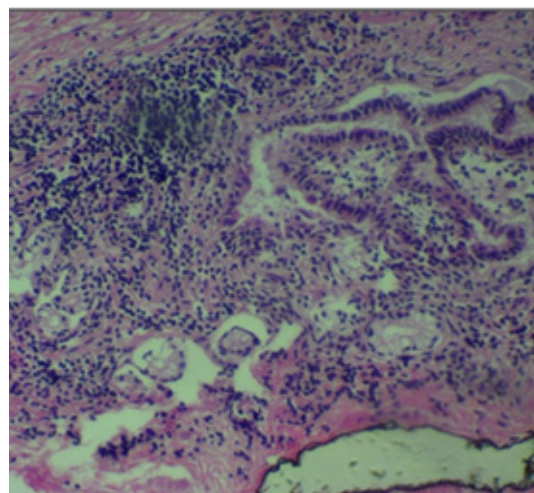


Fig. 10. Chronic inflammatory cells (lymphocyte, plasma cells, surrounding submucosal glands of gallbladder) H&E stain 10X.

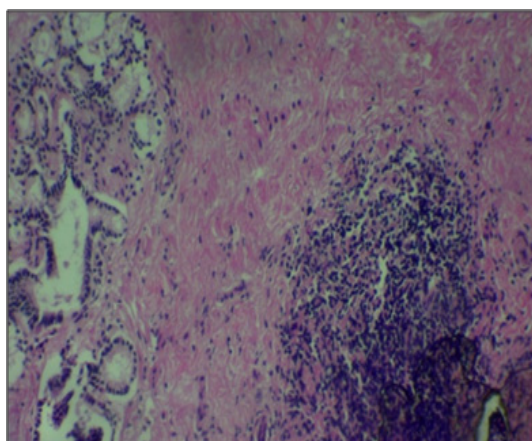


Fig. 11. Submucosal inflammatory foci (multifocal chronic cholecystitis) 10X .

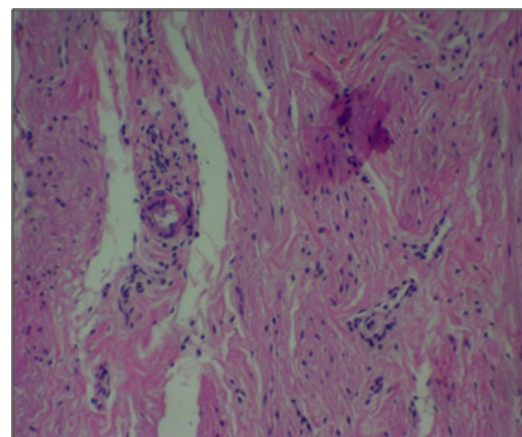


Fig. 12. Mild to moderate inflammation characterized by infiltration of chronic inflammatory cells (lymphocyte, plasma cells) 10X

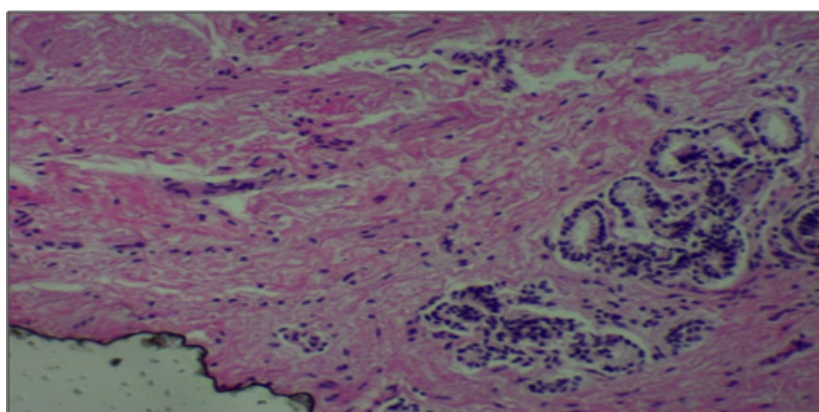


Fig. 13. Submucosal subacute to chronic inflammation characterized by infiltration of lymphocyte, plasma cells and macrophages , slight degenerative changes in epithelial mucosa 10X.

Discussion

This study, total percentages of infection with *Giardia spp.* in fecal material of small intestine was 28%. These results were in agreement with that occurred in Babylon province's cows 24.5% and calves 35.5% [14] and with study of the Al-Saad and Al-Emarah, [15] in the northern Basrah on fecal samples of cattle 30.6%. Other studies which were conducted in the different parts of the world recorded varied percentages of infection with *Giardia* such as 10.4% in domestic ruminants [16], 7.6% in the fecal samples from asymptomatic calves and cows in Denmark farms [17], 44.79% in dairy cattle of Chitwan, Nepal [18], 31.11% in cattle in Lahore [19]. Diagnosis of *Giardia* with different percentages of infection may be related to the number of samples, methods of examination and degree of contamination of the farms with cyst of the *Giardia* especially in water. In this study trophozoites of *Giardia* diagnosed in the gallbladder of cattle the percentage was 12%. This is the first survey in Mosul, Iraq. This study agrees with Degeril and Ozcelik [10] they found *Giardia* trophozoites in the gallbladder of an adult cow in Turkey. Beaver et al., [20] showed that gallbladder may become infected by *G. Intestinalis* and associated with gallbladder colic and jaundice due to obstruction of the bile passages or irritation and edema of Vater's ampulla. Biliary giardiasis should be taken into consideration when intend to diagnose acute a calculus cholecystitis,

Giardia duodenalis was diagnosed in the ingesta of small intestine and gallbladder by using different techniques. High percentage of infection with *Giardia* appeared when staining the specimens (ingesta of small intestine, gallbladder fluid, Intestinal mucosal scraping and Gallbladder mucosal scrapings) with Giemsa stain 22%, 16%, 6%, 10% respectively. These results were in agreement with Wotfe [21] who referred that examination of stool permits trophozoites to be seen in direct wet saline methods and on direct wet saline. The parasites may appear clearly but not all internal structures are visible because the cysts are three dimensional. Therefore, the internal structures may be seen adequately if iodine solution is added to the preparations. The addition of Lugol's iodine can be helpful. Examination of fluid from the duodeno-jejunal junction may reveal *Giardia* trophozoites, also the mucus and fluid from the bile may be fixed and stained for a permanent mount.

High percentage of infection with *Giardia* seemed in ingesta of small intestine of male was 30% with no significant differences between male and female cattle. Male and female animals may be exposed to infection with equal affection to predisposing factors of infection with this parasite. These results were not in agreement with Ayaz et al., [19] in Lahore who recorded high prevalence in cows 31.71% as compared to bulls 26.25% and referred that there were significant differences in *Giardia* prevalence's among the sampled farms in relation to age, sex of the cattle and season of the year.

Statistical analysis showed significant differences between animals aged less than one year and animals aged 3 years and more while there were no significant differences appeared between animals aged less than a year and animals aged 1-2 year and between cattle aged 1-2 years and cattle aged 3 years and more. These results were in agreement with Hussian et al., [14] in Babylon province who recorded percentage of infection with *Giardia* in calves and cattle were 35.5%, 24.5% respectively and Mahato et al [18] who found the prevalence of *Giardia duodenalis* in dairy cattle in Chitwan, Nepal significantly < 0.05 higher in 1-6 months age group compared to more than 3 years. Kakandelwa et al [22] showed the prevalence of *Giardia* was highest in calves less than three months. According to numerous studies carried out in different parts of Turkey giardiasis has been found in 11.2% of the adult and 13.8% of children population. Ayaz et al. [19] referred that the overall cattle 2-3 years observed to have higher *Giardia* prevalence compared to cattle of 3-7 years while Ozcelik and Degeril [23] showed high prevalence of giardiasis was dependent on the age group examined as well as environmental hygiene and climatic conditions [22].

High percentage of infection appeared in animals suffering from diarrheas with percentage 60% while the percentage of infection in animals without diarrhea was 14.28%. Statistically, there were significant differences recorded between animals with and without diarrhea. *Giardia* has been recorded to cause diarrhea among humans, dogs, cats, calves and horses (Centers for epidemiology and animal health [24] Infected hosts may present with a broad spectrum of clinical signs ranging from asymptomatic carriage to acute or chronic diarrhea [25]. The result of this study was in agreement with Al-Saad and Al-Emarah [15] who

showed that the presence of symptomatic type was higher than the percentage of asymptomatic giardiasis in cattle while Kakandelwa *et al.* 2016[22] showed the prevalence of *Giardia* was not associated with occurrence of diarrhea in the calves. Mahato *et al.* [18] referred that the higher occurrence of *Giardia* was found in diarrheic animals compared to Quilez, [25] who showed that the infection rates of *Giardia* were higher in non-diarrheic than diarrheic calves. The morphology and measurements of trophozoites and cysts of *Gairdia duodenalis* were in agreement with Soulsby [4] and Tayler *et al.* [26].

The histopathological examination of the gallbladder sections showed presence the trophozoites of *Giardia spp.* in the lumen of the gallbladder and attach to the mucosal epithelium. The presence of trophozoites of *Giardia* in the lumen of the gallbladder and attach to the epithelial cells without invading was in agreement with Quilez *et al.* [26] who mentioned that the biopsies of various site of the duodenal and jejunal mucosa may display patch distribution of *Giardia* on villi and intervillous spaces. Also Buret and Cotton [27] showed that the trophozoites colonize the lumen of the small intestine without invading host tissue or entering the blood stream. The result was in agreement with Howard *et al.* [28] who referred that *Giardia* invades the biliary system and entrance to the gallbladder. Histopathological changes have been noticed accompanying presence of the parasites manifested by chronic inflammatory response including slightly hyperemic blood vessels, lymphocytes, plasma cells and macrophages in filtration in mucosal and submucosal layer with degeneration in the epithelial layer. These results were in agreement with Scott *et al.* [26] who showed that in Giardiasis, the acute pathophysiology happens without invasion of the small intestine tissue by the trophozoites and in the absence of distinct inflammatory cell infiltration with the exception of a modest increase in intraepithelial lymphocytes. Increased lymphocytic infiltrates have been described in some children with inflammation has been recorded in some adults with either acute [29, 30].

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Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this manuscript.

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تشخيص الجيارديا المعوية (*Giardia duodenalis*) في الابقار في مدينة الموصل، العراق

نادية سلطان الحيالي ومصطفى سالم النعمة وايمان غانم سليمان
فرع الاحياء المجهرية - كلية الطب البيطري - جامعة الموصل - الموصل - العراق.

بلغت نسبة الاصابة الكلية بطفيلي الجيارديا في كل من (٥٠) عينة من محتوى الامعاء و (٥٠) كيس مرارة جمعت من الابقار المذبوحة من مختلف الاعمار ومن كلا الجنسين في المجزرة وبعض محلات القصابين في مدينة الموصل ٢٨٪ و ١٢٪ على التوالي. ظهرت اعلى نسبة اصابة بطفيلي الجيارديا بتقنية التصبغ بصبغة الكيمزا لمحتوى الامعاء وسائل المرارة وقشطات الطبقة المخاطية للامعاء والطبقة المخاطية لكيس المرارة ٢٢٪ و ١٦٪ و ٦٪ و ١٠٪ على التوالي. اظهرت النتائج عدم وجود فرق معنوي بين نسبة اصابة كل من ذكور واناث الابقار بطفيلي الجيارديا. كما اظهرت النتائج ان اعلى نسبة اصابة بلغت ٥٠٪ ظهرت في الحيوانات التي عمرها اقل من سنة و اقلها ١٧,٦٤٪ ظهرت في الحيوانات التي عمرها ٣ سنوات فاكثر مع عدم وجود فرق معنوي بينهما . كما لوحظ وجود فرق معنوي في نسبة الاصابة ٦٠٪ في الحيوانات التي تعاني من اسهال مقارنة ١٤,٢٨٪ للحيوانات التي لا تعاني من اسهال. اظهرت نتائج التقنية النسجية تشخيص طفيلي الجيارديا في تجويف كيس المرارة مع وجود استجابة التهابية مزمنة.