DETECTION OF GIARDIA INFECTION IN DOGS OF BASRAH CITY

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ABSTRACT

The present study was conducted to determine detect the infection rate of Giardiasis in local canine breeds of Basrah, Iraq. Two hundred and twenty-five (225) fecal samples were collected from dogs of both sexes from March to October 2016. Suspected animals either asymptomatic (56.7%) or symptomatic (43.3%) the suspected dogs show different clinical signs such as diarrhea (6.66%), pasty feces (13.33%), steatorrhea (8.00%), anorexia (10.66%), emaciation (15.55%) and vomiting (0.88%). Diagnosis of Giardia spp was done on basis of microscopic examination which detects Giardia cyst and trophozoite with infection rate (40 %), moreover, it was confirmed by ELISA technique(Giardia-specific antigen) to detect (75.55%) was infected with G. lamblia with a high infection rate comparison with microscopic examination. It had been shown that high infection rate (43.75%) was detected in March than the lowest in July (34.78%). Moreover infection with Giardia was found more common at (1 day to 6 months) of age; furthermore, females show infection rate more males. It has been concluded that giardiasis affected canine of Basrah city lead to substantial effect, therefore animals must be screened periodically.

INTRODUCTION

Giardiasis is a common parasitic disease which can infect human as well as a great verity of domesticated animals such as cats, dogs, cattle, sheep, goats, and horses, numerous species of wild mammals and birds (1, 2).

Giardia was protozoan parasites that cause enteric disease in human and animals (3).

Infection with Giardia is the most common infectious disease worldwide, which Transmission of disease occurs in animals or humans via ingestion of viable cysts (4). Animals may have disease through ingestion of fecal contaminated by Giardia

cysts; moreover drinking of contaminated water was playing an active role for infection. (5).

Giardiasis is considered as a zoonotic disease (6) since domestic animals had important source of giardiasis for human's infection (7).

The Common clinical signs exhibited by diseased animals are enteritis accompanied with diarrhea, weight loss, mucoid and soft faeces, and the presence of fats in the feces. Most dogs infected with Giardia are subclinical; however, in diseased animals diarrhea is the most common clinical sign. Feces are often pale, malodorous, and steatorrheic (8).

Diagnosis of giardiasis were done by conventional methods following the direct examination of smear feces under a microscope, flotation techniques particularly Zinc Sulphate Centrifugal Flotation (9), furthermore diagnosis could be confirmed by ELISA test (10).

Little information had been provided on canine giardiasis therefore the present study were conducted to determine the disease in animals of Basrah city and to identify the infections species, however evaluation of infection rats and recognizing clinical manifestation of diseased dog were also concerned.

MATERIAL AND METHODS

Present study was conducted on 225 dogs (Domestic dogs, guarding dogs and stray dogs) of different age and of both sexes (120 females and 105 males) which examined clinically and recorded the signs which appear on dogs which suspected infected with Giardia, and fecal samples were collected from different age, sex and health status, from center of Basrah city, during period from March to October 2016.

Samples Collection

1- Fecal samples

All fecal samples were collected freshly, directly or as soon as after defecation, collected samples stored in sterilized cups were used for each animal and all these cups were labeled by information with number, date of collection, animal condition, nature of feces, and age of animal. After collecting samples, transported to laboratory of internal and preventive medicine/ College of Veterinary Medicine -

University of Basrah for confirmed diagnosis. After diagnosis, the sample is kept in formalin and kept at room temperature until the diagnosis is confirmed by the ELISA test.

2- Diagnosis

2-1 - Conventional Microscopic Methods:

2-1-1 Direct smear with normal saline: is done According to (11).

1. Fecal balls or 2-4 gm with pestle and mortar.

2. Transfer feces a using an applicator stick and a drop of normal saline were placed on glass slide and thoroughly mixing to form a uniform suspension.

3. Spread it on the slide and apply cover slip.

4. Examine the slide microscopically under low power (10X) and high power (40X).

2-1-2 Direct smear with Lugol′s iodine: After prepare the direct smear, used Lugol′s iodine to kill and staining the trophozoites and cysts (11, 12).

2-2- Concentration Methods:

2-2-1 Floatation Techniques: include**:**

❖ **Zinc Sulphate Centrifugal Flotation:** is done according to (13**)**

2-3 Staining: include:

❖ **Giemsa Stain:** The procedure of staining is done according to (14):

1. Fix the air-dried film in methanol for 2 minutes.

2. Allow the smear to dry and then immerse it in a solution of 1 part of Giemsa solution to 10 parts of water for 45min.

3. Discard the sediment stains and rinse the slides in buffered water.

4. Drain the slides thoroughly in a vertical situation and allow them to air dry.

5. Mount the stained smears in a neutral mounting medium (Canada balsam).The slide examination under oil immersion objective (100X magnification).

2-4 Immunological diagnosis: using Enzyme Linked Immunosorbent **Assays** (ELISA)

The biomerica Giardia ELISA is intended for the qualitative detection of Giardia antigen in fecal specimens. Antibodies to Giardia-specific antigen (GSA) have been immobilized on breakaway microwells. Diluted case specimens are added to the microwells along with horseradish peroxidase conjugated antibodies to GSA. If GSA is present in the sample, it will bind to the detecting antibody and the immobilized antibody to form a complex, which will remain in the microwells after washing to remove unbound enzyme. After washing, the substrate is added which develops a blue colour in the presence of the enzyme complex. The stop solution ends the reaction and turns the blue colour to yellow.

All data were subjected to Statistical analyzers, the significance of variation were statistically analyzed using spss using Chi-Square test (15).

RESULT AND DISCUSSION

Result of Clinical examination

The total numbers of clinically examined dogs are 225 of various ages and sexes. Present study revealed that the, microscopically examination of fecal samples showed that the number of positive infected animals are (90), with infection rate (40 %).

Table (1) show the clinical signs in dogs which include: diarrhea (6.66%), pasty feces (13.33%), steatorrhea (8.00%), anorexia (10.66%), emaciation (15.55%) and vomiting (0.88%).

Table (1): Clinical Signs of giardiasis in dogs

Sings	No of animal	Percentage
Diarrhea	15	6.66%
Steatorrhea	18	8.00%
Anorexia	24	10.66%
pasty feces	30	13.33%
Emaciation	35	15.55%
Vomiting	2	0.88%
Asymptomatic	130	57.77%

Diseased animal shows different clinical manifestation which mentioned also others (16; 17, 18) like diarrhoea and malabsorption, and Emaciation, fatty diarrhoea, weight loss, soft faeces, and anorexia.

The distortions caused by the parasite in the synthesis of the villi, which represented Palace of villa , especially in the duodenum the observed increase in the length of villa in the ileum with the reduction in the surface area of the membrane microvillus and an increase in the number of Cisternae cells in the small intestine (19)

Table (2) show the results of giardiasis types and percentage of infection among dogs, the percent of symptomatic type was 43.3 % , while the percentage of asymptomatic giardiasis was 56.7 % , with no significance differences**.**

Table (2): Giardiasis types and percentage of infection in dogs.

Giardiasis types	No. of infected animals	Percentage of infection
Symptomatic cases	39	43.3%
Asymptomatic cases	51	56.7%
Total	90	100%
X^2=1.97, df= 1, P= ns* *Non-significant		

(20) Showed that there are two types of giardiasis asymptomatic and symptomatic infections. The clinical picture of giardiasis varies ranging from asymptomatic infection or acute self-limiting to severe, chronic one (21).

Result of Microscopic examination

The microscopic examination was done by light microscope at different power (10X, 40X and 100X). Through laboratory examination of 225 fecal samples which using

direct smear for diagnosis Giardia trophozoite and flotation methods for diagnosis Giardia cyst.

Giardia trophozoite were appear re-sampled pear cut half or tear-drop in shape or fun-face shape, binucleate, four pairs of flagella, ventral disc and two median bodies, Fig. : 1

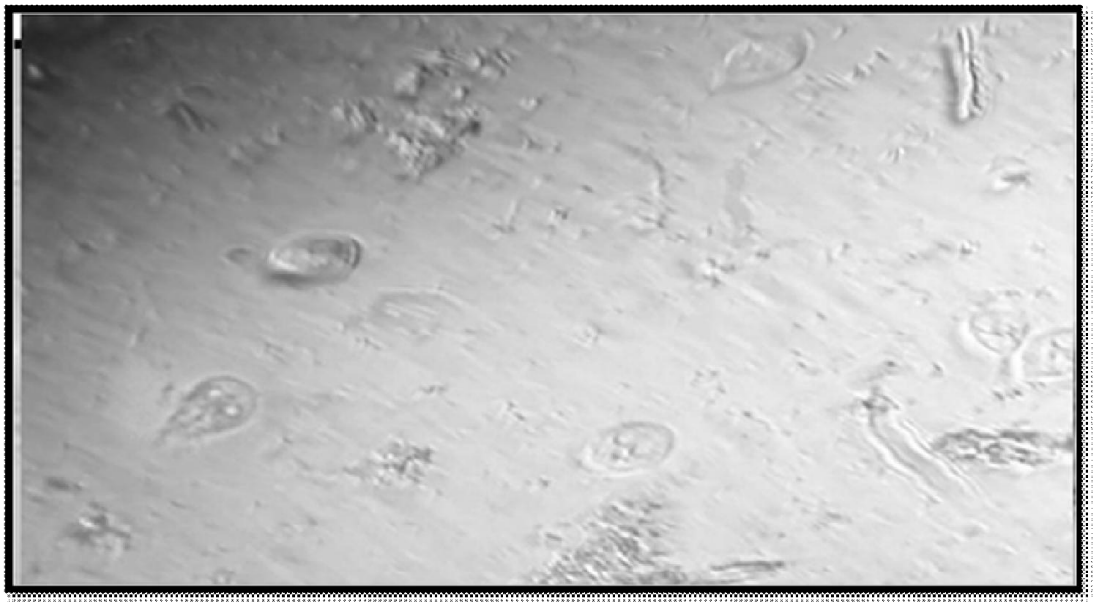

Fig. (1): *Giardia* trophozoite, (40X) direct smear with normal saline.

Giardia cyst on microscopically examination was appeared oval in shape, colorless, have 4 nuclei, median bodies and axostyle *Fig. 2:*

Fig. (2): f *Giardia* cysts: flotation methods with normal saline (40X)

Results of ELISA test

Table (3) show the results revealed that 90 samples out of a total 225 samples were recorded as positive for *G. lamblia* when using microscopically examination method with prevalence rate (40%), and From ELISA method, 68 samples positive out of a total 90 samples which give positive result when using microscopically examination with prevalence rate (75.55 %) with significant differences (P<0.05).

Table (3): Prevalence of *G. lamblia* according to type of detection test.

Test	No. of examined samples	No. of Positive samples	Percentage of infection
Microscopic examination	225	90	40%
ELISA	90	68	75.55%
X^2= 5.72, df=1, P<0.05			

(18) In Nineveh province show that the prevalence rate of Giardia 26% and; (22) in Baghdad 24.1%. Showed the prevalence rate with Giardia 24.1 % and (23) The ELISA test results showed a prevalence rate of 55.2% after examined 183 samples, while results (24) showed reduction in the prevalence rate 7.0% after the examination of 102 samples.

Table (4) shows the percentage of infection with Giardia depending on the sex. The high percentages of infection with Giardia were recorded in infected females (43.3%).While the percentage of infection in the male is (36.1%).with no significance differences ($p \geq 0.05$).

Table (4): Percentage of infection according to sex.

Sex of dog	No. of samples	No. of infected samples	Percentage of infection
Male	105	38	36.19
Female	120	52	43.33
	225	**90**	**40**
X^2= 0.64, df=1, P= ns			

Table (5) shows the percentage of infection with Giardia depending on the age of animals. The microscopic examination were showed highest percentage of infection with Giardia occurs in age group between one day to six months (41.1%), followed by 7 months to 1 year (39.4%), and in age more than 1 year , (38.5%), with no significance differences ($p \geq 0.05$).

Table (5): Prevalence of Giardiasis according to Ages.

Ages	Examined Samples	Positive samples	Prevalence Rates
1 day to 6 months	84	35	41.1
7 months to 1 year	71	28	39.4
More than 1 year	70	27	38.5
Total	225	90	40
X^2=0.09, df=2, P= NS			

The result of present study agree with the results of (18) in Nineveh province (27.77%) higher percentage rate appeared in animals aged less than one year, whereas lower prevalence rate (25%) were indicated in animals aged more than one year .

Animal age might play an important role, young animal were highly susceptible to giardiasis and this might relate to the development of immunity, age-related resistances and movement of animals to less contaminated environmental (Ayaz, 2007).

Table (6) shows the percentage of infection depending on months of years. The highest percentage of infection which occurs in March (43.75%), and the lower percentage of infection with Giardia occurred in July (34.78%) and August (37.93%). with no significance differences ($p \geq 0.05$).

Table (6): Percentage of infection according to months

Months of year	No. of samples	No. of infected Samples	Percentage of infection
March	32	14	43.75
April	35	13	37.14
May	30	12	40
June	21	9	42.85
July	23	8	34.78
August	29	11	37.93
September	30	13	43.33
October	25	10	40
Total	225	90	40
X^2=1.825774 , df = 8, P= NS			

These results agree with result of (22) in Baghdad recorded higher rate (40%) were seen in March and lower prevalence rate (10%) recorded in July.

The highly significant differences in the prevalence of giardiasis among different months was related to environmental condition which inconstancies around the year, warm temperature, relative humidity and rainfall will increase availability of cysts (25)

Table (7) shows highly significant differences ($p<0.01$) were detected in the prevalence of giardiasis according to the type of faces and results showed high percentage rate (100%) in cases of steatorrhea whereas low percentage of giardiasis (20%) was detected in cases of diarrhea.

Table (7): prevalence rate of giardiasis according to the type of feces

Type of Feces	Examined animals	Positive Cases	Prevalence Rates (%)
Normal	**162**	**54**	**33.33%**
Diarrhea	**15**	**3**	**20%**
pasty feces	**30**	**15**	**50%**
Steatorrhea	**18**	**18**	**100%**
Total	**225**	**90**	**40%**
*** X^2=43.5 → p<0.01**			

(27) Explained that the rate of infection is high in cases of diarrhea (40.3%) compared to normal cases (20.9%). (29) Explained that Veterinarians often consider Giardia in a list of differential diagnoses of diarrhea in clinically ill animals. 64 of 82 (78.0%) dogs with giardiasis did not have diarrhea. however (26) and (28) explain mention relationship between Giardia infection and diarrhea due to attachment of trophozoite with intestinal brush border which result in mechanical irritation or mucosal injury, moreover villous atrophy, crypts cells hypertrophy, crypt death were result, on the other hand epithelial permeability were change.

الكشف عن الاصابه بالجارديا في الكلاب في مدينه البصره

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الخلاصة

أجريت الدراسة الحالية لتحديد معدل الإصابة بالجياردیات في سلالات الكلاب المحلية في البصرة، العراق. تم جمع مائتين وخمسة وعشرين (٢٢٥) عينة برازية من الكلاب من الجنسين من مارس إلى أكتوبر ٢٠١٦. الحيوانات المشتبه بها إما بدون أعراض (٥٦.٧٪) أو أعراض (٤٣.٣٪) الكلاب المشتبه فيها تظهر علامات سريرية مختلفة مثل الإسهال(٦.٦٦٪)، البراز العجيني(١٣.٣٣٪)، اسهال دهني(٨.٠٠٪)، فقدان الشهية(١٠.٦٦٪)، الهزال(١٥.٥٥٪) و التقيؤ(٠.٨٨٪). وقد تم تشخيص الجياردیا على أساس الفحص المجهري الذي يكشف كيس الجياردیا و الطور الخضري مع معدل اصابة (٤٠٪)، علاوة على ذلك، تم تأكيده من قبل تقنية الاليزا (مستضد الجارديا) للكشف عن (٧٥.٥٥٪) كان مصابا بالجارديا لامبليا مع نسبة عالية من

العدوى مقارنة مع الفحص المجهري . وقد تبين أن معدل الإصابة العالي (٤٣.٧٥٪) تم اكتشافه في مارس مع أدنى مستوى له في يوليو (٣٤.٧٨٪) . علاوة على ذلك عدوى الجيارديا أكثر شيوعا في (١ يوم إلى ٦ أشهر) من العمر، وعلاوة على ذلك، الإناث تظهر معدل الإصابة أكثر من الذكور. وقد خلص إلى أن الجيارديا يؤثر على الكلاب في مدينة البصرة يؤدي إلى تأثير كبير، وبالتالي يجب فحص الحيوانات بشكل دوري .

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