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Detection Of Non Zoonotic Giardia Duodenalis Assemblage C And D In Dogs In Baghdad Province.

Hisham A Swadi[1]*, and Mohammad M Zenad[2].

[1]Department of Animal Production Technical, Al-Musaib Technical Institute, Al-Furat Al-Awset technical University, Iraq.
[2] Department of Internal and Preventive Medicine, Veterinary Medicine Collage, Baghdad University, Iraq.

ABSTRACT

The aims of current study were detection and identification of Giardia duodenalis in fecal samples collected from household, stray and police dogs in Baghdad, Iraq, from March till October 2017. The total infection rate of was 9% (PCR). By direct smear examination, the rate of infection was 5%, same rate of infection (5%) was found by utilized the Immnuo chromatography assay whereas by using Zinc sulphate flotation method, rate of infection was increased to 7%, the higher rate of infection (9%) was obtained by using polymerase chain reaction. A significant higher infection rate was recorded among males (10.86%); furthermore the rate of infection was significantly increased among younger dog. Also rate of infection of Giardia duodenalis showed variant values according to different raring methods: A significant higher infection rate (25%) was observed in stray dogs as compared with household (7.14%) and police dogs (0%). It was worthy to refer for a Giardia duodenalis found in diarrheic stool more than non diarrheic stool 33.33% and 4.70% respectively. This study successful to identify the Giardia duodenalis assemblage of nine isolates from dogs by sequencing of the PCR amplified Giardia glutamate dehydrogenase (GDH) gene. Non zoonotic Giardia duodenalis assemblage C and D was detected in the nine isolates.

Keywords: Giardia, Dogs, Iraq, PCR.

Corresponding author

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INTRODUCTION

Giardia duodenalis is a protozoan infects millions peoples and domestic animals yearly through the world causing moderate to severe diarrhea (1). The main route of transmission is contamination of the water by cysts of Giardia (2) . Animals play important reservoirs of Giardia for human infection, which it's may transmitted from infected pet animals to human especially children (3). Giardia duodenalis has several assemblages defined from A to H by DNA sequencing analysis (4,5), the assemblage A have I and II sub-assemblages while assemblage B divided into III and IV sub-assemblages (6). The sub-assemblage A-II is restricted to human, while A-I identified in human, dogs and other animals except cattle (7, 8), the assemblages C and D are specific for dogs (7), whereas assemblage E is specific for livestock (9), assemblage F isolated from cats and assemblage G found in rats (10) and assemblage H found in grey seal and gull (5).

In Iraq few studies were performed on assemblages of G. duodenalis in human. Some studies in Baghdad, Najaf and AL-Qadisiya, found assemblages A, B and A+B (11, 12, 13, 14), but in Al-Muthanna province the assemblages A, B, E and F found in human (15). Only few studies had been carried out to find the different Giardia duodenalis assemblages in animals in Iraq, assemblage A (sub-assemblage in AI) was found in dogs (11), while assemblages A, B and A+B had been detected in cattle (14). Another study in Baghdad revealed 38 stool samples from human were positive for giardiasis by using conventional PCR and different assemblages were detected (A, B and A+B) (13). Furthermore 17 stool samples (out of 30) from Baghdad were positive for genotyping of Giardia duodenalis by RFLP-PCR and gdh gene was successfully amplified, all were belong to A and B assemblages (11). Amplification of tpi gene had been done in Al-Muthanna government (south of Iraq) to detect Giardia duodenalis and, it was found these belonged to A, B, E and F assemblages (15).

MATERIAL AND METHODS

Specimen Collection:

Faecal specimens from housed, stray and police dogs with and without diarrhea and are with different ages and from both gender in many regions in Baghdad. Fresh faecal specimens from 100 dogs were collected from March to October 2017 and were labeled and transferred to laboratory of Collage of Veterinary Medicine / University of Baghdad. Microscopical detection was done directly by examination of fecal smear and flotation method using concentrated zinc sulphate solution. Chromatographic immunoassay was done also for further qualitative diagnosis of Giardia duodenalis in the fecal sample by (CerTest Giardia one step card test, CERTEST BIOTEC S.L., Zaragoza, Spain), then all samples were kept frozen (-20 ºC) for DNA extraction and amplification of specific gene glutamate dehydrogenase (gdh) by polymerase chain reaction in ASCo. Learing Center, Baghdad/ Iraq.

Microscopical examination:

Direct smear was done as descriptive in (16) and concentrated method by zinc sulphate solution done as descriptive in (17).

Chromatographic Immunoassay Test:

According to the company instruction CerTest BIOTEC ® One Step test to detect Giardia in card format (Pol. Industrial, Zaragoza, Spain) the test had been done.

DNA extraction and PCR:

DNA extracted from faecal sample according to instruction of Manufacture Company of QIAamp® Fast DNA Stool Mini Kit (QIAGEN® GmbH, Hilden, Germany). DNA of all samples was amplified by semi-nested PCR. Positive faecal samples were sequenced to identify the Giardia assemblages found in the infected dogs. A fragment of the gdh gene of 432 bp in length was amplified using primers External-GDHeF: 5'-CAACGTYAAYCGYGGYTTCCGT-3'; Internal-GDHiF: 5'-CAGTACAACTCYGCTGG-3' and Reverse-GDHiR: 5'-GTTRTCCTTGCACATCTCC-3'. Two replicates were done for each samples in PCR analysis (the thermal cycler: BioRad, USA) was used under the following amplification condition: the first run, 1 cycle at 95ºC for 5 minutes, followed by 55 cycled of 94 ºC for 2 minutes, 56 ºC for 1 minute and 72 ºC for 2 minutes. In the second run,

addition 55 cycles the temperature and time were 94 ºC for 30 seconds, 56 ºC for 20 seconds and 72 ºC for 45 seconds, the final extension at 72 ºC for 7 minutes. The PCR products were separated on a 1 % agarose gel, after that stained with ethidium bromide and visualized on a UV transilluminator (18).

PCR product purification and sequencing

Purification and sequencing of the nine PCR products were done commercially (PCR product were send for Sanger sequencing using ABI3730XL, automated DNA sequencer, by Macrogen Corporation – Korea). Results were analyzed using genious software. Sequences were compared with GenBank references.

Statistical Analysis

The Statistical Analysis System- SAS (2012) program was used to effect of difference factors in study parameters . Chi-square test was used to significant compare between percentage in this study (19).

RESULTS

Comparison polymerase chain reaction with other methods for detection of Giardia in faecal samples showed no significant detection than both microscopical methods (direct and concentration) and immune chromatography assay. In comparison of all 100 faecal samples collected, G. duodenalis was detected in 9% by PCR versus 5% by direct smear with logal´s iodine, 7% by concentration method by used zinc sulphate flotation test and 5% by immune chromatography assay (Table 1).

Table 1: Prevalence of infection according to methods of detection of G. duodenalis in faeces of the dogs.

Method	Infected/Total	(%)
Direct smear	5/100	5.00
Zinc sulphate flotation	7/100	7.00
Immnuochromatography assay	5/100	5.00
PCR	9/100	9.00
Chi-Square	---	1.834 NS

NS: Non-Significant.

No significant differences between male (10.80%) and female dogs (7.01%) in the rate of infection (Table 2).

Table 2: Prevalence of infection by G. duodenalis according to dogs genders (by PCR method)

Gender	Infected/Total	(%)
Male	5/46	10.86
Female	4/54	7.01
Chi-Square	---	0.961 NS

NS: Non-Significant.

A significant ($P<0.01$) highest infection was recorded in stray (25%) dogs as compared with household (7.01%) dogs, whilst no infection with G. duodenalis recorded in police dogs (Table 3).

Table 3: Prevalence of infection by G. duodenalis according to dog breeds (by PCR method)

Breed	Infected/Total	(%)
Household	3/42	7.14
Stray	6/24	25.00

Police	0/34	0.00
Chi-Square	---	8.362 **

** (P<0.01)

A significant increase in G. duodenalis infection was found in young aged dogs (less than one year), whilst the rate of infection was low above 2 years (Table 4).

Table 4: Prevalence of infection by G. duodenalis according to age of the dogs (by PCR method)

Ages (years)	Infected/Total	(%)
<1	2/13	15.38
1-2	4/30	13.33
>2	2/57	3.50
Chi-Square	---	5.378 *

* (P<0.05)

The environmental temperature influence the rate of infection, the rate was increased significantly (P< 0.01) in hot months (May 28.57% and July 23.07% respectively), whereas in temperate months (April, September and October) no G. duodenalis infection was recorded (Table 5).

Table 5: Prevalence of infection by G. duodenalis in dogs according to months (by PCR method)

Months	Infected/Total	(%)
March	2/15	13.33
April	0/15	0
May	2/7	28.57
June	1/12	8.33
July	3/13	23.07
August	1/18	5.55
September	0/9	0
October	0/11	0
Chi-Square	---	9.503 **

** (P<0.01)

Moreover the diarrheic dogs were showed higher rate (33.33%) of infection than those non diarrheic (4.70%) with high significant differences (P<0.01) table 6.

Table 6: Prevalence of infection by G. duodenalis in dogs according to present of diarrhea (by PCR method)

Status health	Infected/Total	(%)
Diarrheic	5/15	33.33
Non diarrheic	4/85	4.70
Chi-Square	---	8.466 **

** (P<0.01)

Genotyping of Giardia duodenalis

The detection and identification of G. duodenalis genotyping in faecal samples were achieved by PCR and the sequencing. All faecal samples of dogs were tested by semi-nested PCR, and the specific gene (gdh) of Giardia duodenalis was successfully amplified at 432 bp (Fig. 1).

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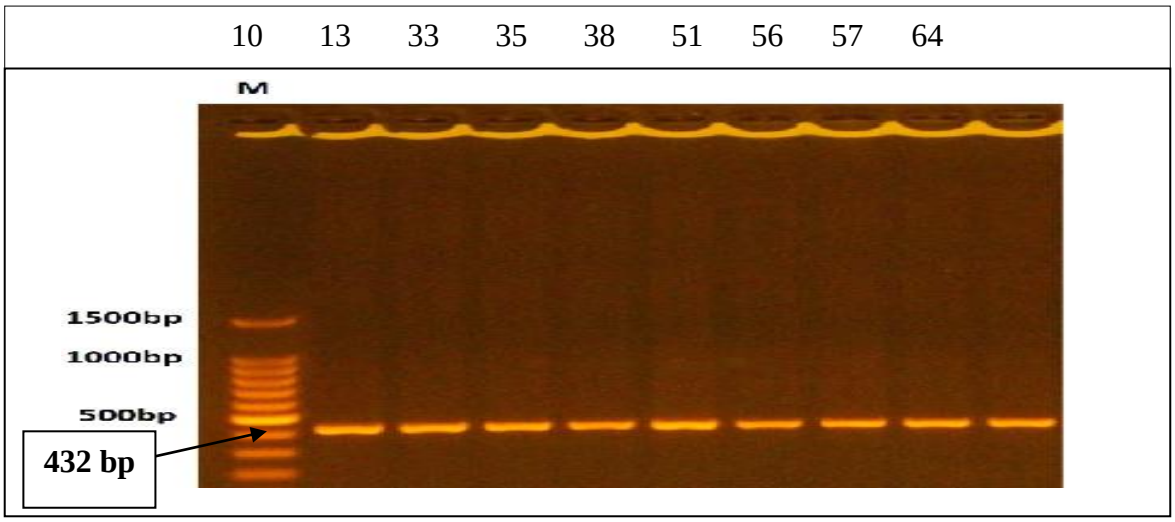

Fig 1: A semi-nested PCR at the gdh locus for detection of Giardia duodenalis (line 1 is 100bp molecular ladder and lines 2-10 are positive samples).

Phylogenetic analysis of Giardia duodenalis, sequence was conducted using BLAST search tool. A dataset was assembled using sequences retrieved from this study and GenBank at the National Centre for Biotechnology Information representing the diversity of gdh sequences. The nine isolates from the present study had 100% similarity to sequences deposited in GenBank. The nine isolates detected in present study were Giardia duodenalis assemblage C and D depended on cluster of others in GenBank.

DISCUSSION

Giardiasis a common enteric protozoan among human and animals in developing countries including Iraq, The prevalence of stool positivity may range from 1% to 40% depending on the geographic area and age group surveyed, it is higher in poor sanitations area (20). In developed countries, infection rates varied from 2-5 % (21). Depending on the previous many surveys studies performed in different regions in Iraq , the prevalence of infection range from 1.77% to 38.5%. In many different surveys performed in animals, the highest infection rate was 76.4% in asymptomatic cattle (22) and the lowest was 2.5% in cats (23). In 2000, the first detection of Giardia sp. in dogs in Baghdad with infection rate7.05% (24). The total infection rate of dogs with Giardia duodenalis in Baghdad was 9% (by PCR) which is lower than that found previously in Baghdad (24.1%) (25) and that found in Mosul (23, 26). The high infection in young dogs compared with old was noticed by many authors (24, 27, 28); this variation explained the state of immunity as the maternal immunity lowered significantly in puppies after 6 months (29). Giardia infection appear higher in diarrheic dogs than non diarrheic, this protozoan causing intermittent diarrhea, also other enteric pathogens causing diarrhea may assist in shedding of protozoan in faeces(30). No significant difference among sex variation was observed, similar observations were reported in many surveys among human being and animals (31, 32). In contrary, the study performed in Mosul in stray dogs which it's found the infection rate with Giardia spp. higher in the female of dogs (26). In present study high prevalence infection in males of dogs may be due to differences in types and levels of hormones consider a predisposing factor to infection with giardiasis.

The low infection rate in household and police dogs was due to management and hygienic feeding, whereas a significant raised in the infection rate in stray dogs was because of freely feeding from environment particularly contaminated and infected carcass as well as contaminated water (24). The hot environment significantly influenced the rate of infection (table 5), due to increase thirst, leading to drink more water, which increase the chances of infection, also hot weather act as stress factor which contributes in suppression of immune response in dogs (30).

Polymerase chain reaction is one of important molecular tools for detection and identification of Giardia duodenalis genotypes. In this study, isolated of Giardia duodenalis detected were genotyped through nucleotide sequence analyses successfully. Giardia duodenalis assemblages C and D were showed to be only

assemblages detected in both diarrheic and non diarrheic dogs. The significance of this finding is that dogs were infection with non zoonotic Giardia duodenalis and dogs in Baghdad province are getting infection from dog to dog transmission as the assemblages C and D have been reported to be restricted to infected dogs (7).

Source of Funding

The research was performed independently, there is no funding, influence over study design, analyses, manuscript preparation, or scientific publication.

Ethical clearance

The project was approved by the local ethical committee (College of Veterinary Medicine/ Baghdad University).

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