Microbes, Infection and Chemotherapy

ORIGINAL RESEARCH

https://doi.org/10.54034/mic.e1268

Prevalence and Molecular Characterization of *Giardia duodenalis* Assemblage D of Dogs in Egypt, and Its Zoonotic Implication

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Abstract

Background, Methods: To elucidate the prevalence and molecular characterization of *Giardia* infection in dogs, a cross-sectional study was performed on stray and housed dogs from different localities of Egypt. **Results**: A total of 986 fecal specimens were collected from dogs. The overall infection rate was 8.5%. The diarrheic dogs revealed higher prevalence of 14.43% than asymptomatic animals. The infection was more prevalent in younger dogs (⩽ 6 months) (9.5%) compared to older animals. Higher prevalence of infection was observed during the cold winter months (11.24%). The community owned dogs (stray dogs in the street) showed (11.75%) higher prevalence rate than pet dogs in the household environment (5.59%). Moreover, it was found that dogs fed on undercooked meat, and offal's were showing higher prevalence of giardiasis than dogs fed on canned meat. On the other hand, dogs subjected to regular grooming and good hygienic practices had lesser prevalence rate of the infection compared to unclean neglected dogs. **Conclusions**: The sequencing and phylogenetic analysis of the amplicons of 18SrRNA gene of *G. duodenalis* revealed that; they were closer to assemblage D necessitating urgent attention due to their zoonotic importance.

Key word: *giardia*, epidemiology, 18S rRNA, phylogeny.

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Submitted: october 15, 2021
Reviewed : october 27, 2021
Approved : december 11, 2021

How to cite: Abdelaziz AR, Sorour SSG. Prevalence and Molecular Characterization of Giardia duodenalis Assemblage D of Dogs in Egypt, and Its Zoonotic Implication. Microbes Infect Chemother. 2021; 1: e1268

Introduction

Giardia spp, is a flagellated protozoan which colonizes the upper small bowel, binds to the epithelium's apical surface, and found in the trophozoite form inside the intestine, and reproduces in this form. Shedding occurs with feces to change to cyst stage under challenging environmental conditions (1-3). Giardiasis annually accounts for about 200 million gastrointestinal disorders and 171 million disability-adjusted life years (DALYs). The disorder is frequently detected in the developed countries of Latin America, Africa, and Asia (4).

Nausea, loss of appetite, flatulence, greasy stool, diarrhea, weight loss, and abdominal cramps are the clinical manifestations of the symptomatic giardiasis. Nevertheless, several *Giardia* infections are entirely asymptomatic (5). *Giardia duodenalis* is transmitted through the faeco-oral route, either indirectly or directly. Even though it is a rare reason for food-borne diarrhoea, transmission via ingestion of cysts in polluted water or feed is a significant factor for spreading of the infection (6,7).

Giardia spp., is one of the parasites of dogs with zoonotic potential and is the common cause of diarrhoea in various domestic, pet animals, and humans. Previous reports revealed a higher prevalence of *Giardia* spp., in animals in Egypt with changes in the relative distribution of the different genotypes, (4,5, 8-10). The organism was detected from various environmental resources including water supply, soil, and field collected crops (11,12), The purpose of the present investigation is to find out the role of different epidemiological factors that may trigger the rate of infection among dogs. The molecular characterization based on small subunit ribosomal RNA gene of isolates of *Giardia* spp., and their zoonotic implications were also discussed.

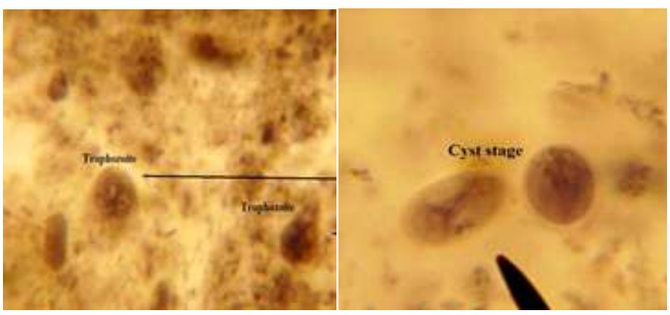
Material and Methods

The study region and ethical considerations

The study protocol was evaluated and accepted by

the ethics committee of the Faculty of Veterinary Medicine, Sohag University, Egypt. The fecal samples were collected from household dogs that were brought to private pet clinics in the different governorates (13). Dogs owners were notified about the purpose of work.

Figure1. Trophozoite and cyst of *Giardia* spp., stained by lugol solution.x100



The cross-sectional study was performed in five governorates from January 2020 till January 2021 [Fig. 2], including Alexandria, Kafrelshiekh, Giza, Assiut, and Sohag. Alexandrina and Kafrelshiekh governorates are located in the northern part of Egypt (latitude 31°12'56.3''N, and longitude 29° 57' 18.97''E), on the Mediterranean Sea, with an average annual rain fall of 169 mm. Giza governorate represents a city in the middle Egypt (latitude 30°36' 15.37''N, and longitude 32°16'20.1''E), with a civil population of 1366,669. It has a hot desert temperature and is in the northern portion of the country in the Nile Delta (longitude 31°013082''E and latitude 30° 565,576''N), with a population of 13,941,293 and a tropical desert climate. Assiut and Sohag are rural governorates in Upper Egypt (longitude 31°695,671''W and latitude 26°559,074''N). There are two different seasons in each year, viz., a cold winter from November to April and a warm summer from May to October, with an average yearly rainfall of 1 mm (14).

Figure 2. *Giardia spp*., among different governorates in Egypt

Animals and epidemiological factors

A total of 986 fecal samples were collected during the period from January 2020 to January 2021 for screening of trophozoites and cysts of Giardia spp. Fresh fecal samples were collected directly from rectum of dogs visiting pet hospitals. Samples were also collected from stray dogs of the nearby street from the floor where they had defecated (15).

Coprological examination

The fecal samples were immediately transported to the laboratory for examination using the usual flotation method (15). To summarize, a small amount of fecal sample (approximately 3–5 g) was mixed with saturated sodium chloride solution (Sp. Gr. 1.2) until a homogenous suspension was obtained. Then, the suspension was filtered through pores of a 60-micrometer mesh sieve, and the filtrate was collected. After filling the tubes to the top with the saturated sodium chloride solution, a cover glass was applied and kept the tube vertically for 15–20 minutes, the area under the coverslip was examined microscopically (x40 objective) for the presence of trophozoites and cysts (12).

Molecular characterization:

The fecal samples positive for Giardia spp., (n=4) were used for DNA extraction using a Qiagen fecal Kit (Qiagen, Germany) according to the manufacturer's instructions. The isolated DNA was spectrophotometrically quantified at 260/280 nm and stored at (-20 °C) until used for polymerase chain reaction (PCR).

The PCR for the amplification of small subunit-ribosomal RNA gene (SSU-rRNA) 590 bp was performed (16). The primers used were GiarF (5'- GAC GCT CTC CCC AAG GAC-3') and GiarR (5'- CTG CGT CAC GCT GCT CG-3') (17), and the conditions of PCR cycling were 94 °C for 5 min (initial denaturation), then 30 cycle of 94 °C for 1 min (denaturation), 50 °C for 35 s (annealing), 72 °C for 1–3 min (extension) followed by, a final extension at 72 °C for 10 min. The PCR products were purified then sequenced on an ABI 3370 DNA sequencer at the Molecular Biology Unit, Assiut University, Egypt) using a primer walking strategy (18).

The sequence was assembled using MEGA6 software as a sequence editor (18), and were aligned by pair-wise and multiple alignments by Clustal W 12.1 V. (19). They were also subjected to BLAST algorithms with the help of databases from the National Center for Biotechnology Information NCBI (http://www.ncbi.nlm.nih.gov/html), like tBLASTn. Phylogram was constructed using the neighbor-joining method, using various published sequences (20).

Statistical analysis

A comparative analysis by binary logistic regression analysis (negative binomial regression) and odds ratio (OR) assays were performed to verify the mean differences

between probable relationships and local groups with proper prevalence. The proper level of logical significance was chosen (P<.05), with an odds ratio >1.0).

Results

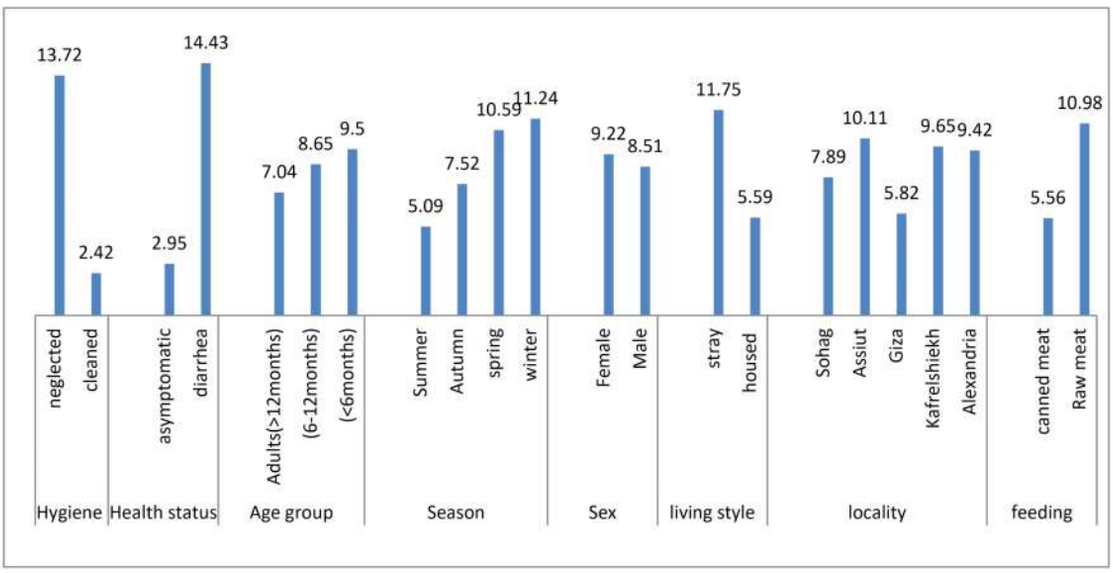
Prevalence and associated risk factors

The outcomes of this study indicated that the total prevalence of *Giardia* infection among the dogs was 8.5% (86/986) at a p-value (≤0.05) with odd ratio (95% CI) 0.29 (0.17-.38) [Table1], and [Fig. 2, 3]. The highest infection rate observed in Assiut was 10.11% (18/178), followed by Kafrelshiekh 9.65 % (11/115), Alexandria 9.42% (28/297), and Sohag 7.89% (15/190). The lowest rate of infection observed in Giza was 5.82% (12/206). The seasonal variation showed a significant impact on the infection rate, [Table 1, Fig. 3]. The cold winter season revealed the highest rate (11.24%) of infection, followed by spring (10.59%); and the lowest in Summer (5.09%). The animals at pre-weaned ages showed more infection with giardiasis, (Table 1, Fig. 3). The highest percent of infection (9.5%), was seen in puppies under six

Table 1: Prevalence and risk factors of Giardia ssp., in dogs from Egypt

locality	Exam.	Inf.	%	Odds Ratio (95% C.I.),	*P-value* (≤ 0.05)
Alexandria	297	28	9.42		
Kafrelshiekh	115	11	9.65	0.29 (0.17-.38)	0.0911
Giza	206	12	5.82		
Assiut	178	18	10.11		
Sohag	190	15	7.89		
Total	986	84	8.5		
Season	**Exam.**	**Inf.**	**%**	**Odds Ratio (95% C.I.),**	***P-value* (≤0.05)**
winter	249	28	11.24		
spring	236	25	10.59	2.89 (2.69-3.06)	0.021*
Autumn	226	17	7.52		
Summer	275	14	5.09		
Total	986	84	8.5		
Age	**Exam.**	**Inf.**	**%**	**Odds Ratio (95% C.I.),**	***P-value* (≤0.05)**
≤ 6 months	399	38	9.5		
6 – 12 months	289	25	8.65	1.76 (1.69-1.86)	0.0262*
≥ 12 months	298	21	7.04		
Total	986	84	8.5		
Sex	**Exam.**	**Inf.**	**%**	**Odds Ratio (95% C.I.),**	***P-value* (≤0.05)**
Male	458	39	8.51		
Female	488	45	9.22	0.92 (0.877-0.98)	0.0762
Total	986	84	8.5		
Health status	**Exam.**	**Inf.**	**%**	**Odds Ratio (95% C.I.),**	***P-value* (≤0.05)**
Diarrheal	478	69	14.43		
Asymptomatic	508	15	2.95	1.23 (1.19-1.46)	0.0162*
Total	986	84	8.5		
Living style	**Exam.**	**Inf.**	**%**	**Odds Ratio (95% C.I.),**	***P-value* (≤0.05)**
household	518	29	5.59		
Stray	468	55	11.75	1.34 (1.06-1.76)	0.003**
Total	986	84	8.5		
Feeding type	**Exam.**	**Inf.**	**%**	**Odds Ratio (95% C.I.),**	***P-value* (≤0.05)**
Raw meat	537	59	10.98		
Canned meat	449	25	5.56	1.099 (0.929-1.26)	0.025*
Total	986	84	8.5		
Hygiene	**Exam.**	**Inf.**	**%**	**Odds Ratio (95% C.I.),**	***P-value* (≤0.05)**
Regular clean	454	11	2.42		
Neglected	532	73	13.72	0.59 (0.39-0.76)	0.0462*
Total	986	84	8.5		

Figure 3: Prevalence of Giardia spp., infection of dogs in Egypt



months, but the lowest was seen in adults above one year (7.04%). The gender of dogs did not reveal any significant effects on the infection rate. The prevalence of infection was higher in dogs with diarrhoea (14.43%) than apparently healthy dogs (2.95%). The street dogs revealed more infection (11.75%) than house dogs (5.59%). Dogs that were fed with uncooked or raw meat showed more infection (10.89%) compared to those fed on canned meat (5.56%). In addition, those dogs subjected to regular cleaning and grooming showed a significantly lower infection rate (2.42%) with Giardia than neglected dirty dogs, either housed or stray dogs.

Sequence analysis and phylogeny of *Giardia duodenalis*

The amplified DNA fragment (18S rRNA gene) of *Giardia* spp., was subjected to gene sequence analysis, (Figure 4), the pairwise and multiple nucleotide comparison with the other previous sequences from GeneBank (Figure 5,6) showed that; the isolates in this study was closely similar (93%) to G. duodenalis Assemblage D (Accession No: (EF507636, U60986, JX448631, EF507619), and it was homologous to the human isolate (94%) of Giardia spp., (LN875383, U47632).

Figure 4: PCR product on agarose gel electrophoresis of target (18SSU-rRNA) gene of *Giardia* spp., show bands at 590 bp. of 4 samples

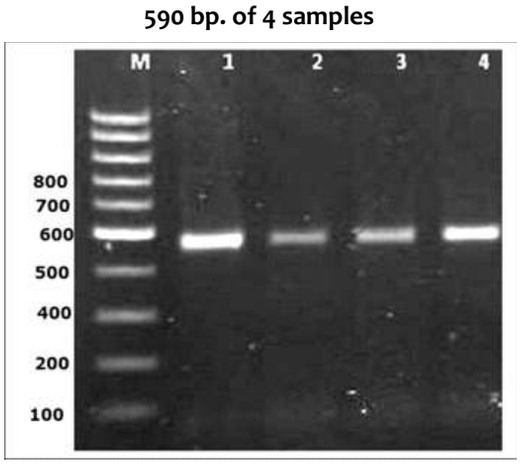


Figure 5. Sequence alignment of giardia duedenalis isolates based on the 18 SSU-rRNA gene locus amplification

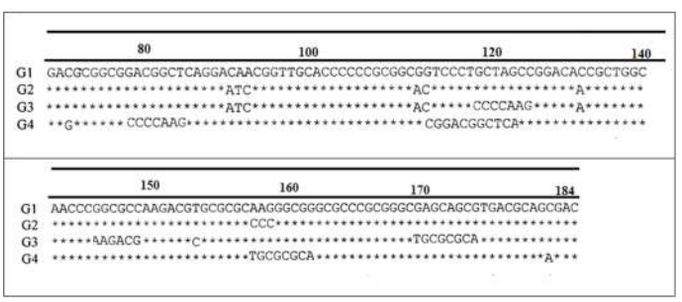
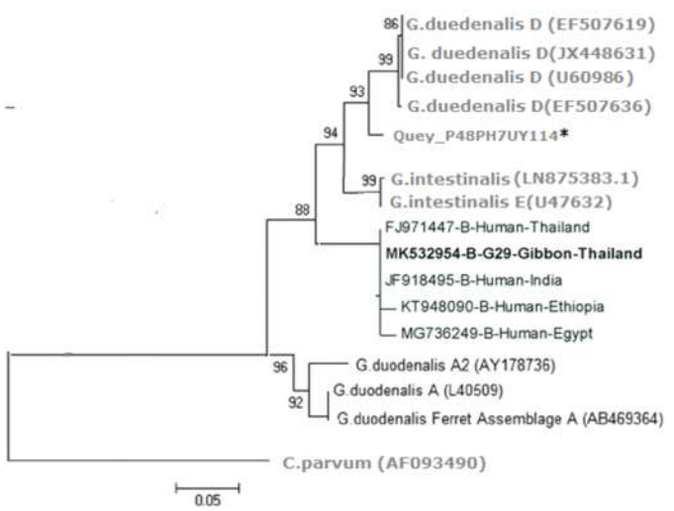


Figure 6. Genetic and phylogenetic analysis of *Giardia duedenalis* sequences of amplicons of 18S r RNA genes from fecal samples of dogs from Egypt *: The isolates in this study that was closely similar (93%) to *G. duedenalis* Assemblage D (Accession No: (EF507636), (U60986), (JX448631), (EF507619), and it was genetically closer to human isolate of *Giardia* spp., 94% (LN875383), (U47632)



Discussion

The importance of this study comes from the zoonotic aspect of giardiasis infection from dogs to humans. *Giardia* spp., which has a diverse host range, is a prevalent cause of diarrhea in animals and man (5). The companion animals can transfer various zoonotic infections, like giardiasis, to their owners, but the magnitude of this risk is unknown (6).

There are several studies on the evidence for zoonosis due to giardiasis, the disease was identified as a prevalent protozoan infection in the children, with an infection rate of 24% (21) in Egypt. The low frequency of helminth parasites in Egypt is due to the availability of deworming drugs and campaigns (22).

The present study revealed that the overall prevalence rate of giardiasis among dogs in Egypt was 8.5%. This is comparatively low infection rate compared to the previous reports from Egypt or worldwide. The geographical area selected in this study covered a large area, including northern (Alexandria, Kafrelshiekh) and middle (Giza), and southern Egypt (Assiut, Sohag). It was clear that; animal gender has no significant effect on the prevalence rate.

On the other hand, seasonal variation, age of dogs, feeding type, hygienic measures, and living location either housed or free-living stray dogs, diarrhoeic or apparently healthy dogs etc were identified as potential risk factors as previously described (23, 24). The performance of detection method used in the present study, the microscopy, always depends on the skill and experience of the technician. In addition, the concentration technique used (floatation technique) can substantially improve the sensitivity of microscopy.

Low temperature in winter season can provoke more stress, thereby; lowering the immune status of the hosts that can trigger the infection especially in preweaned young puppies. The free-living dogs are more exposed to infectious organisms than housed dogs reared under good managemental practices like, provision of clean bedding, regular grooming (25) as previously observed in northwest England.

The results of phylogenetic analysis revealed that there is a significant intraspecies diversity in *G. duodenalis*. The canine isolate (D) was different from feline (F) and bovine isolates (A) in several polymorphic regions of the 18S rRNA gene. The evolutionary distance between *G. duodenalis* assemblage D and the human G. intestinalis (E) genotypes is much smaller than the evolutionary distances between other assemblage of *G. duodenalis*. This suggested a significant danger of zoonotic disease, as several strains discovered in dogs are part of assemblies that represent a special risk of human giardiasis (25). The phylogeny of sequences from positive samples revealed the genetic relatedness of the isolate of dogs with that of humans in Egypt. Previously, the genotyping of *G. duodenalis* isolate (26) from dogs in Guangdong (China) based on multi-locus sequence such as 18SrRNA gene locus, glutamate dehydrogenase (gdh), triose phosphate isomerase (tpi), ββ-giardin (bg), identified assemblage A (7 samples), C (2 samples), and D (1 sample). They also found that 11 samples showed mixed infections with assemblage A which is potentially zoonotic.

Conclusion

We concluded that *Giardia* infection in dogs is of a low prevalence rate; the most associated risk factors for infection were seasonal variation, younger age of puppies, unhygienic managemental measures, free-living conditions of stray dogs and feeding of raw or undercooked meat. The Assemblage D of *G. duodenalis* identified in the present study was genetically closer to human isolate and it may be a source for human infection.

Conflict of interest

None.

Acknowledgements

The researchers are very grateful to all owners of cattle farms, veterinarians and staff, personnel of the molecular biology unit, Assiut University, for their support and generous assistance.

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