

IDENTIFICATION AND GENOTYPES OF *GIARDIA INTESTINALIS* IN RUMINANT LIVESTOCK IN SOUTH SINAI GOVERNORATE, EGYPT

By

SAFAA M. BARGHASH^{1*}, SAMIR A. TAHA², SARA S. A. SERAG²,
AND EHAB A. RAGAB³

Parasitology Unit, Department of Animal and Poultry Health, Animal Production and Poultry Division, Desert Research Center¹, El-Naam, Postal Code, 18511 Cairo, Department of Zoology, Department, Faculty of Science, Zagazig University², and Agricultural Economics Institute³, Agriculture Research Center, Cairo, Egypt
(*Correspondence: Barghash_7@yahoo.com)

Abstract

This study may be the first to detect genotype of *Giardia* among ruminants in South Sinai Governorate. Fresh iodine-fecal stained smears were examined by microscopy, followed by a nested PCR on frozen fecal specimens. The results showed *Giardia* in 40% (78/195) & 28.72% (56/195) of the examined ruminants by microscopic and nPCR, respectively. In cattle was (52.94%, 39.2%), in goats (37.5, 25.0%), and in sheep (34.82%, 25.0%). Animals <3 months old were more *Giardia* infected than older ones. *Giardia* infection was high in El Tur City (50%, 35.1%) than in Ras Sudr City (25.9%, 19.8%), and in males (51.3%, 35.5%) than in females (32.8%, 24.4%). Symptomatic animals were more susceptible to the infection than asymptomatic ones. *Giardia* was more prevalent in diarrheic samples than those with soft and hard nature with significant differences ($P < 0.05$). Nested-PCR of *G. intestinalis* identified positive β -*giardin* gene band of 511 & 753bp. Analysis of five sharp generated fragments in both directions showed sequences with accession numbers MW925063, MW925066, & MW925067 (Assemblage A) & MW925064 & MW925065 (Assemblage B). It clustered with some Kenyan and Egyptian isolates with identity from 93.9% to 100% and genetic distance from 0.0 to 6.5 compared to other GenBank strains. Genotyping at sub-Assemblage level was essential for zoonotic transmission.

Keywords: Sinai, *Giardia intestinalis*, nested-PCR, *beta-giardin* (*bg*), Gene sequencing, Ruminants.

Introduction

Giardia duodenalis (*G. lamblia* & *G. intestinalis*) is one of the commonest protozoa parasites worldwide (Savioli *et al*, 2006) with significant health and economic problems (Escobedo *et al*, 2010). *Giardia* exists in two forms trophozoite and cyst, and transmitted by fecal-oral route either directly or indirectly by cyst ingestion (Hooshyar *et al*, 2019). Giardiasis causes asymptomatic infection or abdominal pain, diarrhea, and vomiting, seriously impact on humans and animals health (Cacciò *et al*, 2018). Also, infected animals are source of zoonotic transmission by contaminating vegetables, fruits and even water (Abd El-Latif *et al*, 2020). Both cysts and trophozoites can be seen in fecal samples, but only trophozoites in watery diarrhea (Hooshyar *et al*, 2019). Microscopic examination of the fecal samples remains the cornerstone of diagnosis (McH-

ardy *et al*, 2014). Nowadays, by molecular tools eight assemblages (A, B, C, D, E, F, G, & H) were identified (Minetti *et al*, 2016). Globally, assemblages A & B are zoonotic, particularly in Egypt (Elhadad *et al*, 2021).

The present study aimed to evaluate, and identify genotype *Giardia* species among ruminant livestock in South Sinai Governorate, Egypt.

Materials and Methods

Study area: The present study was carried out in South Sinai Governorate. Sheep and goats were settled for centuries in Sinai, and locally imported cattle were established. Two cities were selected, Ras-Sudr and El-Tur due to the increased number of established humans dwellings and animal farms.

Sampling of data: *Giardia* infections were examined in 15 randomly selected farms over a year from October 2020 to September 2021. A total of 114 diarrheic and 81 non-

diarrheic fecal samples were collected from 51 cattle, 112 sheep, and 32 goats and classified according to their ages into G1: aged 0-3 months, G2: aged from 3 months to <1 year & G3: aged > 1year. Questionnaires data were recorded for analysis. Labeled collected samples were transported in a cool box to the Parasitology Lab., Faculty of Science. Modified precipitation technique was done within 24hr and was divided into two parts; one for microscopic examination and second was stored at -20°C for molecular studies.

Macroscopic examination: Fecal samples were examined for consistency as normally (well-formed), pasty (soft, not well-formed) or diarrheic (liquid), odor, mucus or blood.

Microscopy examination: Direct smears were stained with Lugol's iodine and/or haematoxylin and Eosin (H & E) and examined under low and high powers (Eldeek *et al*, 2019).

Genomic DNA extraction and PCR amplification: Genomic DNAs were extracted using a QIAamp Fast DNA Stool Mini Kit (Qiagen, Hilden, Germany), after the manufacturer's recommendations and subsequently frozen at -20°C until use. Extracted DNA was amplified by the nested-PCR targeting *beta-giardin* (*bg*) gene, which included two sequential PCR reactions. First PCR reaction was conducted with 1µM of each outer primers, G7 & G759, in a 25µl total volume containing 12.5µl Premix Taq DNA Polymerase (TaKaRa, Tokyo), and 5µl of genomic DNA. Semi-nested-PCR reaction started with a step initial denaturation at 94°C for 5min, followed by 35 cycles of denaturation at 94°C for 30s, (50°C for 40s) annealing, and extension at 72°C for 45 s, with a final extension at 72°C for 10min. Primers 200F & 200R, respectively were used as the forward and reverse primers. The reaction mixture in the nested-PCR had the same composition, as in the first step, except template was replaced by 1µl of the first PCR product, the initial denaturation step of 94°C for 5min, annealing was 55°C for 40s, and extension cycle was 72°C for 45s. Final

extension step was at 72°C for 10min. Reactions were performed in a gradient thermal cycler after adjusting the thermal profiles. Amplified nPCR products were stained with ethidium bromide and electrophoresed on agarose gel (1.5%) in TAE buffer, and seen under a UV trans-illuminator.

Molecular characterization of *Giardia* isolates: PCR products were purified using a QIA-quick PCR product extraction kit (Qiagen Inc. Valencia CA). They were sequenced in forward and reverse directions on an Applied Bio-systems 3130 automated DNA Sequencer (ABI, 3130, USA) using a ready reaction Big-dye Terminator V3.1 cycle sequencing kit (Perkin-Elmer/Applied Biosystems, Foster City, CA, No. 4336817). Sequence identity to GenBank accessions was analyzed using the basic local alignment search tool (Altschul *et al*, 1990). Comparative analysis of sequences was carried out using the CLUSTALW multiple sequence alignment programs, version 1.83 of MegAlign module of Laser-gene DNASTar software Pairwise (Thompson *et al*, 1994). Phylogenetic analyses were done using maximum likelihood, neighbor-joining and maximum parsimony in MEGA6 (Tamura *et al*, 2013).

Results

Giardiasis were 78/105(74.29%), in cattle 27/51(52.94%), followed by goats 12/32 (37.5%), and least in sheep 39/112 (34.82%) without significant differences ($P > 0.05$). Males were more infected than females (51.3% & 32.8%) respectively ($P=0.344$). Positivity was higher ElTur than Ras Sudr (50.0% & 25.9%) respectively ($P=0.962$). Symptomatic animals were (45.1%) and asymptomatic ones (37.1%) without significant association ($P=0.867$). Stools consistency showed a difference ($P=0.05$) with infection rates among diarrheic, soft, and non-diarrheic ones (76.6%, 37.7%, & 20.2%), respectively. Infection showed a significant difference ($P < 0.05$) in animals aged < 3 months (60%) than < 1 year (34.8%) but greater >1 year (30.2%).

Molecular identified positive β -giardin gene band of 511 & 753bp, respectively in 56/195 (28.72%). Cattle, sheep and goats were 39.2%, 25%, & 25%, respectively. Infection rate was higher in ElTur (35.1%) than Ras-Sudr (19.8%), with males more infected (35.5%) than females (24.4%) without significance ($P>0.05$). Giardiasis was more prevalent in animals < 3 months old (34.0%) compared to < a year (30.4%) and > a year (20.7%) without significance ($P=0.524$). No significance ($P=0.530$) was between symptomatic (40.9%) and asymptomatic (21.8%) positive ones. A difference ($P<0.05$) was between diuretic feces (49%) and soft ones (30%) with cysts than well-formed ones (16.5%), with significant ($P<0.0001$).

Of 56 samples amplified at sizes of 511 & 753bp, respectively, gave strong DNA products with available outer and inner primers sequencing. Fragments in both directions proved the *G. intestinalis* assemblages. Phylogenetic analysis showed 5 submitted isolates in 2 main clusters of assemblages A & B. Three assemblages A were MW925063, MW925066, & MW925067, and two were MW925064 & MW925065 clustered together as Assemblage B. They were closely related to some Kenyan and Egyptian isolates. Identity ranged from 93.9% to 100% and divergence was 0.0 to 6.5, without different SNPs generated within each one.

Details were given in tables (1, 2, & 3) and figures (1, 2, 3, & 4)

Table 1: Parasitological and PCR detection of *Giardia spp.* in ruminants in South Sinai Governorate

Parameter	Symbol	No.	Giardia-Microscopy		P value	Giardia-PCR		P value
			+ve	(%)		+ve	(%)	
Site	El-Tor	114	57	50.0	.059	40	35.1	.225
	Ras-Sudr	81	21	25.9		16	19.8	
Species	Cattle	51	27	52.94	.344	20	39.2	.100
	Goats	32	12	37.5		8	25.0	
	Sheep	112	39	34.82		28	25.0	
Sex	Male	76	39	51.3	.962	27	35.5	.636
	Female	119	39	32.8		29	24.4	
Age	X<3 months	50	30	60.0	.047	17	34.0	.524
	X< 1 year	92	32	34.8		28	30.4	
	X>1 year	53	16	30.2		11	20.7	
State	Asymptomatic	124	46	37.1	.867	27	21.8	.530
	Symptomatic	71	32	45.1		29	40.9	
Status	Hard	79	16	20.2	.050	13	16.5	.043
	Soft	69	26	37.7		20	30.0	
	Diarrhea	47	36	76.6		23	49.0	
Intercept					.000			.000

Table 2: Correlation between variable factors using microscopy and molecular methods

Variable	<i>Giardia</i> - Microscopy						<i>Giardia</i> -PCR					
	Sp.	Age	Sex	Site	State	Status	Sp.	Age	Sex	Site	State	Status
Species	--	.000	.005	.000	.455	.010	--	.000	.004	.000	.393	.014
Age	.000	--	.088	.060	.412	.426	.000	--	.046	.141	.420	.470
Sex	.005	.088	--	.005	.046	.046	.004	.046	--	.002	.034	.031
Site	.000	.060	.005	--	.209	.001	.000	.141	.002	--	.258	.001
State	.455	.412	.046	.209	--	.002	.393	.420	.034	.258	--	.003
Status	.010	.426	.046	.001	.002	--	.014	.470	.031	.001	.003	--

Table 3: Gen-Bank data of present isolates compared to the closest ones

No.	Accession no.	Assemblage	Host	Country	References
1	MW925063 <i>G. intestinalis</i> SG1-DRC-Eg	A	Cattle	Egypt	Present study
2	MW925064 <i>G. intestinalis</i> SG1-DRC-Eg	B	Sheep	Egypt	Present study
3	MW925065 <i>G. intestinalis</i> SG1-DRC-Eg	B	Sheep	Egypt	Present study
4	MW925066 <i>G. intestinalis</i> SG1-DRC-Eg	A	Cattle	Egypt	Present study
5	MW925067 <i>G. intestinalis</i> SG1-DRC-Eg	A	Sheep	Egypt	Present study
6	LC508625 <i>G. intestinalis</i> K8-10	A	Homo sapiens	Kenya	Tokoro <i>et al.</i> (2020)
7	LC508641 <i>G. intestinalis</i> K9-14	A	Homo sapiens	Kenya	
8	LC508624 <i>G. intestinalis</i> K8-9	A	Homo sapiens	Kenya	
9	MG736240 <i>G. intestinalis</i> Egyh34	A	Homo sapiens	Egypt	Yu <i>et al.</i> (2019)
10	LC508594 <i>G. intestinalis</i> K1-5	B	Homo sapiens	Kenya	Tokoro <i>et al.</i> (2020)
11	MG736247 <i>G. intestinalis</i> Egyh36	B	Homo sapiens	Egypt	Yu <i>et al.</i> (2019)
12	MG736244 <i>G. intestinalis</i> Egyh52	B	Homo sapiens	Egypt	Yu <i>et al.</i> (2019)
13	LC508633 <i>G. intestinalis</i> K9-6	B	Homo sapiens	Kenya	Tokoro <i>et al.</i> (2020)

Discussion

In the present study, *Giardia* spp. infected cattle, sheep, and goats are of public health significance in the study area, resulting in diarrhoea and inefficient weight gains that lead to economic losses to owners due to morbidity, mortality, and abortion. The fecal samples of ruminant livestock (cattle, sheep & goats) showed that *Giardia* infection was higher, by microscopy, than by using nPCR targeting the *Bg* gene. No doubt, microscopy distinguished stained *Giardia* spp. with its characteristic shape and size whether trophozoite and/or cyst (Thompson *et al.*, 2016). Toledo *et al.* (2017), and Benhassine *et al.* (2020) reported the failure of PCR amplification in microscopy-positive giardiasis infections, could be due to low parasite burdens and. They attributed the negative nPCR to the presence of naturally-occurring PCR inhibitors in fecal samples, cyst quantity and quality, and samples storage. Also, the DNA extraction method, the gene targeted and the number of copies and choice of primers and cycling conditions (Wilke and Robertson, 2009). The present study focused on cysts, and applied freeze-thaw cycles before DNA extraction that might lower DNA recovery and consequently decreasing PCR sensitivity. This agreed with the fact that PCR analysis was less sensitive if the number of cysts was less or not completely break (Fayer *et al.*, 2007; Mueller-Doblies *et al.*, 2008).

In the present study, cattle were more giardiasis than sheep and goats. In sheep, *Giardia* were 25%, which agreed with Saharaoui *et al.* (2019) in Algeria who found 23/83 (28%) of sheep were infected giardiasis. But, in China higher infection rates of *G. intestinalis* were reported for sheep 4.64% in Heilongjiang (Zhang *et al.*, 2012) and 5.24% in Henan (Wang *et al.*, 2016). In contrast, by nPCR the present results were lower than in China that were 64.11%, by nPCR detection followed by sequence analysis of the small subunit ribosomal RNA (SSU rRNA) gene (Cao *et al.*, 2020).

In the present study, cattle showed higher

infection than 31.6% in Iraq (Al-Saad and Al-Emarah, 2014), 7.6% in Brazil (Toledo *et al.*, 2017), and 20.6% in China (Wang *et al.*, 2019). However, it was more or less similar to (53%) in Ismailia Governorate (Helmy *et al.*, 2014).

In goats, the prevalence of giardiasis was 37.5% & 25% by microscopy and nPCR, respectively, was nearly similar to that recorded in urban and peri-urban areas (33.8%) in northern India (Utaaker *et al.*, 2017), and 35.8% (53/148) in goat kids reported in 8/10 farms in Belgium (Geurden *et al.*, 2008). In contrast, it was higher than that reported in goat fecal samples (22.4%) from two dairy farms (A & B) located in Rio de Janeiro state, Brazil (Sudré *et al.*, 2014). These variations in the prevalence may be due to the livestock farming methods used (free-range or intensive farming), sampling size, geographical region, climate, and animals' ages, a diagnostic tool used, breed, hygiene conditions, and the poor hygienic-sanitary conditions in agro-ecological environment (Jin *et al.*, 2017; Santin, 2020). Additionally, sampling time, numbers of samples, breeding methods, animal health status, and experimental methods also have assured impacts on the infection rate (Wang *et al.*, 2020).

Concerning age, the present results showed that the prevalence of *Giardia* infection was higher in animals' age (< 3 months) than those (< 1 year), & higher than those (> 1 year) without significant difference between age groups and infectivity prevalence, which agreed with Kiani-Salmi *et al.* (2019). Wang *et al.* (2020) found the infection rates of *G. intestinalis* in dairy cattle were 7.5%, 1.8%, & 0.4% in <3-months old, >3-11 months, and 12-24 months-old, respectively, with a significant negative correlation between the infection rate and ages. Also, Lee *et al.* (2019) reported that the prevalence of *G. intestinalis* was significantly high in calves aged 31-50 days, especially in those aged between 41 & 50 days (P = 0.00). Besides, the highest prevalence rate of *G. intestinalis* infection was in feces of cattle among 2-

months old young ones (Santín *et al.*, 2009; Toledo *et al.*, 2017; Naguib *et al.*, 2018; Feng *et al.*, 2019).

Nonetheless, a higher prevalence of *G. intestinalis* was commonly reported among post weaned calves as compared to pre-weaned ones or those older than 12 months, due to the changes in management of the infected animals (Trout *et al.*, 2005; 2006; 2007; Santín *et al.*, 2009; Paz Silva *et al.*, 2012; Ouchene *et al.*, 2014). Other studies showed higher infection rates of *G. intestinalis* in pre-weaned lambs in China, Algeria, Brazil, Canada, and Greece due to the lack of acquired immunity in younger ones (Paz e Silva *et al.*, 2014; Tzanidakis *et al.*, 2014; Ye *et al.*, 2015; Chen *et al.*, 2019; Sahraoui *et al.*, 2019; Benhassine *et al.*, 2020). The prevalence in reported variations in different studies within the same age group might be due to environment, climatic or feeding behavior factors of the animals. However, closed collective environments were favorable for the development and prevalence of *Giardia* species and others gastrointestinal protozoan parasites affecting man and animals particularly in rural areas (El Shazly *et al.*, 2007). Also, the higher the animal density in animals' farms led to so many sources of protozoa contamination and thus, more prevalence and incidence of infections (Vargas *et al.*, 2014).

In the present study, there was no significant difference between male and female infection rates, however was higher in males than females. This result agreed with Kiani-Salmi *et al.* (2019) in Iran who found no significant difference between livestock species, but without difference sexes as to *Giardia* spp. infection. Benhassine *et al.* (2020) in Algeria found *Giardia* infection rate in male lambs was 6.7% and in females 7.7% ($P = 0.72$). Also, Peng *et al.* (2020) in China found *G. duodenal* prevalence was (14.47%) with slight differed among Tan female sheep than males (14.58%), but without 1 significant difference ($P > 0.05$). It may be due to the equal possibility of exposure to the con-

taminated sources (Fayer and Xiao, 2007).

In the present study, the stool samples consistency and symptomatic pictures coincided were risk factor for giardiasis, but with significant difference ($p \leq 0.05$) between the *Giardia* infection rates among the diarrheic, soft, and non-diarrheic animals by the two diagnostic methods. This agreed with Benhassine *et al.* (2020) in Algeria found that the *G. intestinalis* infection rate was higher in lambs with diarrhea (15.9%) than those without diarrhea (5.6%) with significance ($p = 0.02$). Similarly, higher *G. intestinalis* infections was in lambs with diarrhea compared to healthy animals, which caused outbreaks of diarrhea in pre-weaned lambs in Iceland (Skirnisson and Hansson, 2006), in Belgium (Geurden *et al.*, 2010) and in Spain (Carmena *et al.*, 2012). Nevertheless, Wang *et al.* (2020) in China found that infection rates of *G. intestinalis* was in <3-month-old dairy cattle with diarrhea (10.3%) and without diarrhea (4.8%) without significant difference ($P = 0.312$). But, O'Handley *et al.* (2003) in Canada reported that *Giardia* spp. infected calves excreted many cysts per gram of feces, with higher excretion peaks among younger animals, which had intermittent with the development of adaptive immunity. The association between diarrhea and excretion of *Giardia* spp. in all animals were analyzed by Toledo *et al.* (2017) who reported that there was no significant differences, and only 11/97 (12.6%) of animals with diarrhea excreted cysts.

In the present study, the frequency increased to 31.4% only with small calves of 0 to 2 months-ages with significant difference. Despite the uncertainty of *Giardia* as a primary cause of the diarrhea in cattle, others in fecal samples identified *Giardia* spp. cysts in 36% (Geurden *et al.*, 2007), and 36.7% (Ouchene *et al.*, 2014). Also, a significant association was reported between the presence of giardiasis and diarrhea, beyond immunization, even among school-children and travelers (El-Bahnasawy and Morsy, 2015). Therefore, the presence of calves infected with a zo-

onotic strain of *C. parvum* and/ or *G. intestinalis* favored the environmental contamination as water, vegetables and even fruits consequently, even the farmers and field workers were risky group (Martins-Vieira *et al*, 2009). In addition, the infectious dose was strongly depended on the management system, with superior influence in intensive farming systems (Geurden *et al*, 2010).

In the present study, the lower prevalence of giardiasis by using PCR compared to microscopy regardless of risk factors may be due to time passed from sample collection to DNA extraction. This was negatively influenced the amplification success in the specimens, particularly those preserved directly for several months or even a year before being extracted due to the degradation of cysts and parasite DNA over time (Minetti *et al*, 2015). The use of the *bg* gene for the identification followed by both sequencing and phylogenetic analysis produced two genotypes of assemblages (A & B). They may be the only prevailing genotypes in the current study. However both assemblages have been isolated from sheep and cattle, and the small sample size performed to sequencing may determine the findings of this study. The generated fragments from goats were not sharp enough thus excluded from sequencing. The association between Assemblage and symptomology has existed (Gelanew *et al*, 2007). However, the clinical manifestations of the disease in ruminants are quite variable, ranging from asymptomatic to acute or chronic diarrhea, dehydration, and weight loss. These assemblages primarily seemed to be linked with the animal-animal transmission, while Assemblage B was more coupled with human-to-human transmission (Faria *et al*, 2016). The investigation source of the two infections may be difficult in absence of sub-Assemblage analysis, but in the present study, we may explain the prevalence of Assemblage A & B in fecal samples (Molina *et al*, 2011). Close contact with a human in agriculture and rural communities provide the opportunity for transmission

from animals to humans as reported in the previous studies (Wegayehu *et al*, 2016).

In the present study, it was clearly homogenous with each other within each assemblage. The degree of variability between the two different assemblages identified showed remarkable similarities with previous studies in Egypt (Ismail *et al*, 2016). It was complicated by the heterogeneous positions that were distributed in 3 groups belonging to 2 sub-clusters whereas sequences were in assemblages A isolate showed homogenous in a group branched from another sub-cluster (Tembo *et al*, 2020). The higher prevalence of Assemblage A than Assemblage B was reported worldwide among human patients (Zajackowski *et al*, 2021), and in Egypt (Fahmy *et al*, 2015). Prevalent genotype in asymptomatic patients infected with *Giardia* Assemblage A, without significant association between *Giardia* genotype and clinical pictures, though El-Badry *et al*. (2017) in Sharkia Governorate found that *G. intestinalis* Assemblage B was in diarrheic children.

Conclusion

It is the first to detect and genotype giardiasis in asymptomatic and symptomatic ruminants in South Sinai, Egypt. Cattle were the preferable hosts than either goats and/or sheep, but without significant differences between sexes, ages, and clinical pictures. Also, the study showed only the presence of *G. intestinalis* Assemblages A & B among the edible animals in South Sinai Governorate. The diarrheic status was associated with *G. intestinalis* infections. The causative genotypes of *G. intestinalis* (assemblages A and B) elucidated a possible correlation between genotype and clinical status.

Authors' contribution: ST, SB supervised study, read, revised, and approved the final manuscript. SB designed the plan of work and share it with SS in collected field samples, carried out the molecular studies, analyzed the results, drafted and wrote the manuscript. SS carried out microscopic examination and ER carried out statistical analysis.

Conflict of interest: The authors declare

that they didn't have conflict of interest in publishing this article.

Acknowledgments

The authors gratefully acknowledge the financial support of the Academy of Scientific Research and the scientific support of the Desert Research Center and Faculty of Science, Zagazig University. Thanks are also due to the Staff, South Sinai Directorate of Veterinary Medicine.

References

- Abd El-Latif, NF, El-Taweel, HA, Gaballah, A, Salem, AI, Abd El-Malek, AHM, 2020:** Molecular characterization of *Giardia intestinalis* detected in humans and water samples in Egypt. *Acta Parasitol.* 65, 2:482-9.
- Ahmad, AA, El-Kady, AM, Hassan, TM, 2020:** Genotyping of *Giardia intestinalis* in children in upper Egypt using assemblage-specific PCR technique. *PLoS One* 15(10): e0240119. <https://doi.org/10.1371/journal.pone.0240119>
- Al-Saad, R, Al-Emarah, G, 2014:** Giardiasis in Cattle: Clinical assessment of *Giardia lamblia* in cattle at Basrah, Iraq. *Int. J. Sci. Wld.* 2, 2:80-3.
- Altschul, SF, Gish, W, Miller, W, Myers, EW, Lipman, DJ, 1990:** Basic local alignment search tool. *J. Mol. Biol.* 215:403-10.
- Benhassine, S, Baroudi, D, Hakem, A, Thomas, M, Laatamna, A, et al, 2020:** Occurrence and molecular characterization of *Giardia intestinalis* in lambs in Djelfa, the central steppe of Algeria. *Parasitol. Res.* 119:2965-73.
- Cacciò, SM, Lalle, M, Svärd, SG, 2018:** Host specificity in the *Giardia intestinalis* species complex. *Infect. Genet. Evol.* 66:335-45.
- Caccio, SM, Ryan, U, 2008:** Molecular epidemiology of giardiasis. *Mol. Bioch. Parasitol.* 160: 75-80.
- Cao, L, Han, K, Wang, L, Hasi, S, Yu, F, et al, 2020:** Genetic characteristics of *Giardia intestinalis* from sheep in Inner Mongolia, China. *Parasit.* 27:60. <https://doi.org/10.1051/parasite/2020060>.
- Carmena, D, Cardona, GA, Sánchez-Serrano, LP, 2012:** Current situation of *Giardia* infection in Spain: Implications for public health. *World J. Clin. Infect. Dis.* 2:1-12.
- Chen, D, Zou, Y, Li, Z, Wang, SS, Xie, SC, et al, 2019:** Occurrence and multilocus genotyping of *Giardia intestinalis* in black-boned sheep and goats in southwestern China. *Parasit. Vectors* 12:1-9.
- Eckmann, L, 2003:** Mucosal defenses against *Giardia*. *Parasit. Immunol.* 25:259-70.
- El-Badry, A, Mohammed, F, Abdul Gawad, E, 2017:** Predominance of *Giardia intestinalis* assemblage B in diarrhoeic children in Sharkia, Egypt. *Parasitol. United J.* 10:39-43.
- El-Bahnasawy, MM, Morsy, TA, 2015:** Beyond immunization: Travelers' infectious diseases. 1- Diarrhea. *J. Egypt. Soc. Parasitol.* 45, 1:29-42
- Eldeek, HEM, Attia, RAH, Nageeb, MM, Sakla, A, 2019:** Comparative evaluation of multiple staining techniques for identification of different developmental stages of *Acanthamoeba* and *Naegleria*. *J. Egypt. Soc. Parasitol.* 2:409-22.
- Elhadad, H, Abdo, S, Tolba, M, Salem, AI, Mohamed, MA, et al, 2021:** Detection of *Giardia intestinalis* assemblages A and B among children from three villages in the West Delta region, Egypt using assemblage specific primers. *J. Parasit. Dis.* 45, 3:655-63
- El Shazly, AM, Elsheikha, HM, Soltan, DM, Mohammad, KA, Morsy, TA, 2007:** Protozoal pollution of surface water sources in Dakahlia Governorate, Egypt. *J. Egypt. Soc. Parasitol.* 37, 1:55-64.
- Fahmy, H, El-Serougi, A, El Deeb, H, Hussein, H, Abou-Seri, H, et al, 2015:** *Giardia intestinalis* assemblages in Egyptian children with diarrhea. *Eur. J. Clin. Microbiol. Infect. Dis.* 34:1573-81.
- Fayer, R, Santin, M, Trout, JM, 2007:** Prevalence of *Cryptosporidium* species and genotypes in mature dairy cattle on farms in eastern United States compared with younger cattle from the same locations. *Vet. Parasitol.* 145: 260-6.
- Fayer, R, Xiao, L, 2007:** *Cryptosporidium* and cryptosporidiosis. CRC Press.
- Feng, Y, Gong, X, Zhu, K, Li, N, Yu, Z, et al, 2019:** Prevalence and genotypic identification of *Cryptosporidium* spp., *Giardia intestinalis* and *Enterocytozoon bieneusi* in pre-weaned dairy calves in Gu-angdong, China. *Parasit. Vectors* 12:1-9.
- Gelanew, T, Lalle, M, Hailu, A, Pozio, E, Cacciò, SM, 2007:** Molecular characterization of human isolates of *Giardia intestinalis* from Ethiopia. *Acta Trop.* 102:92-9.
- Geurden, T, Berkvens, D, Martens, C, Casarot, S, Verrecruysse, J, et al, 2007:** Molecular epidemiology with subtype analysis of *Cryptosporidium* in calves in Belgium. *Parasitol.* 134:1981

-7.

- Geurden, T, Thomas, P, Casaert, S, Vercruyssen, J, Claerebout, E, 2008:** Prevalence and molecular characterization of *Cryptosporidium* and *Giardia* in lambs & goat kids in Belgium. *Vet. Parasitol.* 155, 1/2:142-5. doi: 10.1016/j.vetpar.2008.05.002.
- Geurden, T, Vercruyssen, J, Claerebout, E, 2010:** Is *Giardia* a significant pathogen in production animals? *Exp. Parasitol.* 124: 98-106.
- Helmy, YA, Klotz, C, Wilking, H, Krücken, J, Nöckler, K, et al, 2014:** Epidemiology of *Giardia intestinalis* infection in ruminant livestock and children in the Ismailia Province of Egypt: Insights by genetic characterization. *Parasit. Vectors.* 7:1-11.
- Hooshyar, H, Rostamkhani, P, Arbabi, M, Delavari, M, 2019:** *Giardia lamblia* infection: Review of current diagnostic strategies. *Gastroenterology and hepatology from bed to bench.* 12:3-9.
- Ismail, MA, El-Akkad, DM, Rizk, EM, El-Askary, HM, El-Badry, AA, 2016:** Molecular seasonality of *Giardia lamblia* in a cohort of Egyptian children: A circannual pattern. *Parasitol. Res.* 115:4221-7.
- Jin, Y, Fei, J, Cai, J, Wang, X, Li, N, et al, 2017:** Multilocus genotyping of *Giardia intestinalis* in Tibetan sheep and yaks in Qinghai, China. *Vet. Parasitol.* 247:70-6.
- Kiani-Salmi, N, Fattahi-Bafghi, A, Astani, A, Sazmand, A, Zahedi, A, et al, 2019:** Molecular typing of *Giardia intestinalis* in cattle, sheep and goats in an arid area of central Iran. *Infect. Genet. Evol.* 75:104021.
- Lee, YJ, Ryu, JH, Shin, SU, Choi, KS, 2019:** Prevalence and molecular characterization of *Cryptosporidium* and *Giardia* in pre-weaned native calves in the Republic of Korea. *Parasitol. Res.* 118: 3509-17.
- Martins-Vieira, M, Brito, L, Heller, L, 2009:** Oocistos de *Cryptosporidium parvum* em fezes de bezerro infectado experimentalmente. *Arq. Bras. Med. Vet. Zootec.* 61:1454-8.
- McHardy, IH, Wu, M, Shimizu-Cohen, R, Couturier, MR, Humphries, RM, 2014:** Detection of intestinal protozoa in the clinical laboratory. *J. Clin. Microbiol.* 52:712-20.
- Minetti, C, Taweanan, W, Hogg, R, Featherstone, C, Randle, N, et al, 2014:** Occurrence and diversity of *Giardia intestinalis* assemblages in livestock in the UK. *Transbound. Emerg. Dis.* 61:e60-7.
- Molina, N, Minvielle, M, Grenovero, S, Salomon, C, Basualdo, J, 2011:** High prevalences of infection with *Giardia intestinalis* genotype B among children in urban and rural areas of Argentina. *Ann. Trop. Med. Parasitol.* 105:299-309.
- Mueller-Doblies, D, Giles, M, Elwin, K, Smith, RP, Clifton-Hadley, FA, et al, 2008:** Distribution of *Cryptosporidium* species in sheep in the UK. *Vet. Parasitol.* 154:214-9.
- Naguib, D, El-Gohary, AH, Roellig, D, Mohamed, AA, Arafat, N, et al, 2018:** Molecular characterization of *Cryptosporidium* spp. and *Giardia intestinalis* in children in Egypt. *Parasit. Vectors* 11: 403. <https://doi.org/10.1186/s13071-018-2981-7>
- O'Handley, RM, Ceri, H, Anette, C, Olson, M E, 2003:** Passive immunity and serological immune response in dairy calves associated with natural *Giardia intestinalis* infections. *Vet. Parasitol.* 113:89-98.
- Ouchene, N, Ouchene-Khelifi, N, Zeroual, F, Benakhla, A, Adjou, K, 2014:** Study of *Giardia* spp., *Cryptosporidium* spp. and *Eimeria* spp. infections in dairy cattle in Algeria. *J. Parasitol. Vector Biol.* 6:61-5.
- Paz e Silva, F, Lopes, R, Bresciani, K, Amaranthe, A, Araujo, J, 2014:** High occurrence of *Cryptosporidium ubiquitum* and *Giardia intestinalis* genotype E in sheep from Brazil. *Acta Parasitol.* 59:193-6.
- Paz e Silva, FM, Lopes, RS, Araújo Jr, JP, 2012:** Genetic characterisation of *Giardia intestinalis* in dairy cattle in Brazil. *Folia Parasitol. (Praha).* 59:15-9.
- Peng, JJ, Zou, Y, Li, ZX, Liang, QL, Song, H Y, et al, 2020:** Prevalence and multilocus genotyping of *Giardia intestinalis* in Tan sheep (*Ovis aries*) in northwestern China. *Parasitol. Int.* 77: 1021-6.
- Rayani, M, Unyah, NZ, Hatam, G, 2014:** Molecular identification of *Giardia intestinalis* isolates from Fars Province, Iran. *Iran. J. Parasitol.* 9, 1:70-8.
- Sahraoui, L, Thomas, M, Chevillot, A, Mammeri, M, et al, 2019:** Molecular characterization of zoonotic *Cryptosporidium* spp. and *Giardia intestinalis* pathogens in Algerian sheep. *Vet. Parasitol. Reg. Stud. Repts.* 16:100280.
- Santin, M, 2020:** *Cryptosporidium* and *Giardia* in ruminants. *Vet. Clin. North Am. Food Anim. Pract.* 36:223-8.
- Santín, M, Trout, JM, Fayer, R, 2009:** A longitudinal study of *Giardia intestinalis* genotypes

- in dairy cows from birth to 2 years of age. *Vet. Parasitol.* 162:40-5.
- Savioli, L, Smith, H, Thompson, A, 2006:** *Giardia* and *Cryptosporidium* join the neglected diseases initiative. *Trends Parasitol.* 22:203-8.
- Skirnisson, K, Hansson, H, 2006:** Causes of diarrhoea in lambs during autumn and early winter in an Icelandic flock of sheep. *Icel. Agric. Sci.* 19:43-57.
- Sudré, AP, do Couto, MCM, do Bomfim, TC B, 2014:** Occurrence of *Giardia intestinalis* in dairy goats and evaluation of risk factors for infection: Research note. *R. Bras. Ci. Vet.* 19, 3: 149-53.
- Tamura, K, Stecher, G, Peterson, D, Filipski, A, Kumar, S, 2013:** MEGA6: molecular evolutionary genetics analysis version 6.0. *Mol. Biol. Evol.* 30:2725-9.
- Tembo, SJ, Mutengo, MM, Sitali, L, Changula, K, Takada, A, et al, 2020:** Prevalence and genotypic characterization of *Giardia intestinalis* isolates from asymptomatic school-going children in Lusaka, Zambia. *Food Waterborne Parasitol.* 19:e00072.
- Thompson, JD, Higgins, DG, Gibson, TJ, 1994:** CLUSTAL W: Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucl. Acids Res.* 22:4673-80.
- Thompson, RA, Koh, WH, Clode, PL, 2016:** *Cryptosporidium*, what is it? *Food Waterborne Parasitol.* 4:54-61.
- Trout, JM, Santín, M, Greiner, E, Fayer, R, 2005:** Prevalence and genotypes of *Giardia intestinalis* in post-weaned dairy calves. *Vet. Parasitol.* 130:177-83.
- Trout, J, Santín, M, Greiner, E, Fayer, R, 2007:** Prevalence and genotypes of *Giardia intestinalis* in adult dairy cows. *Vet. Parasitol.* 147:205-9.
- Toledo, RdS, Martins, FDC, Ferreira, FP, de Almeida, JC, et al, 2017:** *Cryptosporidium* sp. and *Giardia* spp. in feces and water and the associated exposure factors on dairy farms. *PLoS One* 12: e0175311.
- Trout, JM, Santín, M, Greiner, EC, Fayer, R, 2006:** Prevalence and genotypes of *Giardia intestinalis* in 1–2 year old dairy cattle. *Vet. Parasitol.* 140:217-22.
- Tokoro, M, Mizuno, T, Hendarto, J, Matey, E J, Songok, EM, et al, 2020:** Sub-cloning approach reveal intra-sub-genotype diversity of *Giardia intestinalis* (GenBank, Direct Submission).
- Tzanidakis, N, Sotiraki, S, Claerebout, E, Ehsan, A, Voutzourakis, N, et al, 2014:** Occurrence and molecular characterization of *Giardia intestinalis* and *Cryptosporidium* spp. in sheep and goats reared under dairy husbandry systems in Greece. *Parasite.* 21:45. doi: 10.1051/parasite/2014048.
- Utaaker, KS, Myhr, N, Bajwa, RS, Joshi, H, Kumar, A, Robertson, LJ, 2017:** Goats in the city: Prevalence of *Giardia duodenalis* and *Cryptosporidium* spp. in extensively reared goats in northern India. *Acta Vet. Scand.* Dec 22; 59, 1:86. doi: 10.1186/s13028-017-0354-4.
- Vargas, SF, Marcolongo, C, Adrien, MdL, Fiss, L, Molarinho, KR, et al, 2014:** Surto de criptosporidiose em bezerros no Sul do Rio Grande do Sul. *PVB* 34:749-52.
- Wang, H, Qi, M, Zhang, K, Li, J, Huang, J, et al, 2016:** Prevalence and genotyping of *Giardia intestinalis* isolated from sheep in Henan Province, central China. *Infect. Genet. Evol.* 39:330-5.
- Wang, R, Li, N, Jiang, W, Guo, Y, Wang, X, et al, 2019:** Infection patterns, clinical significance, and genetic characteristics of *Enterocytozoon bieneusi* and *Giardia intestinalis* in dairy cattle in Jia-ngsu, China. *Parasitol. Res.* 118: 3053-60.
- Wang, Y, Cao, J, Chang, Y, Yu, F, Zhang, S, et al, 2020:** Prevalence and molecular characterization of *Cryptosporidium* spp. and *Giardia intestinalis* in dairy cattle in Gansu, northwest China. *Parasit.* 27: 62. doi: 10.1051/parasite/2020058.
- Wegayehu, T, Karim, MR, Li, J, Adamu, H, Erko, B, et al, 2016:** Multilocus genotyping of *Giardia intestinalis* isolates from children in Oromia Special Zone, central Ethiopia. *BMC Microbiol.* 16:1-10.
- Wilke, H, Robertson, LJ, 2009:** Preservation of *Giardia* cysts in stool samples for subsequent PCR analysis. *J. Microbiol. Methods.* 78:292-6.
- Ye, J, Xiao, L, Wang, Y, Guo, Y, Roellig, DM, et al, 2015:** Dominance of *Giardia intestinalis* Assemblage A and *Enterocytozoon bieneusi* genotype BEB6 in sheep in Inner Mongolia, China. *Vet. Parasitol.* 210: 235-9.
- Yu, F, Amer, S, Qi, M, Wang, R, Wang, Y, et al, 2019:** Multilocus genotyping of *Giardia duodenalis* isolated from patients in Egypt. *Acta Trop.* DOI:10.1016/j.actatropica.2019.05.012.
- Zajaczkowski, P, Lee, R, Fletcher, SM, Alexa-**

nder, K, Mahimbo, A, *et al*, 2021: The controversies surrounding *Giardia intestinalis* assemblages A & B. CRPVD. 100055.
 Zhang, W, Zhang, X, Wang, R, Liu, A, *et al*,

2012: Genetic characterizations of *Giardia intestinalis* in sheep and goats in Heilongjiang Province, China and possibility of zoonotic transmission. PLoS Negl. Trop. Dis. 6, 9: e1826.

Explanation of figures

Fig. 1: Map of Sinai Peninsula showed locations of collected samples from different ruminants (cattle, sheep, and goats). Two main sites: El Tur and Ras Sudr, (with yellow shadows). Sites with red dot (Al-Arish, Nukhul, and Al-Hassana) where animals come from.

Fig. 2: Microscopic examination of *Giardia* cyst by power (40X magnification) using Iodine stained smears. It appeared an oval in shape, in yellow color with 2 nuclei.

Fig. 3: Agarose gel electrophoresis on 1.5% showed positive and negative results using nested PCR targeted *Beta-giardin* (*bg*) gene. Positive samples amplified producing 511 bp and 753 bp, respectively using semi and nested-PCR. L=ladder of 100-1000bp. Lanes P & N positive and negative controls, respectively. Lanes 1, 2, & 5 positive results.

Fig. 4: Phylogenetic tree constructed based on the nucleotide sequence data of *Beta-giardin* (*bg*) gene (753 bp) of submitted and reference *G. intestinalis* in GenBank. Five submitted isolates grouped in two main clusters belonging to assemblages A & B. MW925063, MW925066, and MW925067 categorized as Assemblage A, whereas, MW925064 and MW925065 clustered together as Assemblage B. Those isolates closely related to some Kenyan and Egyptian isolates than others.

