

Study on Related Factors of Giardiasis in Nangarhar

**Teaching Assistant Dr. Shah Agha Salehi,
Teaching Assistant Dr. Ahmadgul Azamee,
Assistant professor Dr. Mohammad Azim azimee**

Lecturer of Microbiology Department, Medical Faculty, Nangarhar University.

Lecturer of Histopathology Department, Medical Faculty, Nangarhar University.

Lecturer of Biochemistry Department, Medical Faculty, Nangarhar University.

Abstract

Giardia lamblia is the leading cause of water born diarrhea in developing countries that can increase the morbidity and mortality of children. Due to that fact, this study aims to investigate the common risk factors for mentioned issue in Nangarhar province.

A descriptive study was conducted from May 2018 to October 2018 through properly designed and self-administered questionnaires on 152(89male and 63 Female) patients with 1-18 years' age in Nangarhar Teaching Hospital and Nangarhar Regional Hospital Central Laboratory, located in Jalalabad city. After stool exam, positive individuals were interviewed, and registered with their lab records in questionnaires. Data were collected, organized and analyzed using SPSS Statistics 20(IBM, USA).

The median age for over all 152 included participants were 10(range 1-18) that 57.9% were males, children with 1.5-year age had the lowest incidence which was 0.7% and children of 12 years were the highest parasite incidence in the study (11.8%). 65.1% participants were from Jalalabad city. Majority of them were unmarried and illiterates. Highest percentage of them had not a job and many of them had a moderate economic status. 35.5% of the participants had animals in their home including cows, cats, sheep and dogs. 41.6% had used the dairy products in their daily routine meals. 15.8percentage of them had other parasites in their stool samples which were H Nana, Entamoeba histolytica and Ascaris lumbricoidus.

According to the study results, G lamblia is the prevalent infection in Nangarhar province, which increases with age. This parasite has a high incidence in school age Children. Therefore, serious measures are required to educate the population properly for their social and environmental Hygiene.

Keywords: Giardia lamblia, age, sex, Jalalabad

Introduction

In the history of microbiology in 1681, Antoine Van Leeuwenhoek examined his own diarrheal stool and found a flagellated parasite ^{(1), (2)}.

After that Vilém D.Lambl Czech physician explained a little more in 1859 he called it Cercomonas intestinalis and finally this parasite was named as G lamblia by Blanchard in 1888 for D.Lambl'sonor (the name of Giardia lamblia is given for the honor of two scientists Prof. Giard and Dr. Lambl ⁽²⁾).

This parasite was neglected by physicians ⁽⁶⁾ and other epidemiologists for a couple of years because for its pathogenic modifications. However, in early 1970s when several visitors of Soviet Union returned with symptomatic Giardiasis consideration for this parasite increased globally ⁽⁵⁾. Giardiasis is a global infection caused by a flagellated protozoan Giardia duodenalis (syn G intestinalis, G lamblia) that is estimated about 280 millions of its infections annually worldwide ⁽³⁾. Approximately 6-8% of children and 2% of adults in developed countries are infected with G lamblia parasite ^{(7), (4)}. However, the prevalence of Giardiasis is about 15% in children of developing countries ⁽⁷⁾.

Poor environmental sanitation, improper garbage disposal, drinking of contaminated water, unhygienic life style and low educational level cause to increase infectivity of Giardiasis among the people. Presence of asymptomatic carriers in the community who can be a source of infection by continuously excreting the cyst forms of the parasite in feces is another problem⁽¹²⁾.

We know that Giardia duodenalis is a major cause of water born infectious gastroenteritis of humans not only in developing countries as well as in developed countries too⁽¹⁰⁾. In United States Giardia, lamblia caused intestinal water borne disease in person who was drunken contaminated water, in childcare centers and in homosexual males^{(8), (9)}. In addition, Giardia lamblia parasite can affect both domestic and wild animal's causes a joint public and veterinary challenge worldwide. This is an endemic infection in the world significantly in tropical and subtropical areas⁽⁵⁾.

Clinic of disease varies from asymptomatic carriage to symptomatic acute and chronic clinical manifestation depending on some host related factors such as duration of parasite in host, immunity and other parasite related factors. In big number of cases this is a self-limiting infection and in rare cases it will be changed to a chronic clinical problem. Incubation Period of the infection is between 9 to 15 days. Initial clinical presentation begins with atypical gastrointestinal symptoms like intestinal discomfort (70.27%), nausea and vomiting (56.76%), Low-grade fever, loss of appetite (58.11%) and chills. Typical clinic is associated with watery, foul-smelling diarrhea, abdominal distention (36.49%)⁽¹⁵⁾, belching, epigastric tenderness and changed taste sense. In addition, there may be extra intestinal and post infectious complications with Giardiasis infection⁽¹³⁾. Extra intestinal feature like urticaria, anterior uveitis and arthritis is described which are caused by giardiasis. Giardia is the parasite of proximal small bowel and does not transmit through blood stream^{(9), (14)}.

We confirm Giardiasis infection by stool examination. Three different stool samples should be examined separately during different ten days. Sometimes we cannot recognize Giardiasis parasite by examination of even six or more stool samples. Laboratory stuff needs to find Giardia lamblia cysts and trophozoites forms in stool samples. Repeated negative stool results cannot rule out Giardiasis infection. Different plans for water sanitation are seriously recommended to prevent Water borne Giardiasis infection⁽⁵⁾.

Despite the development of technology and remarkable implementation of preventive measure intestinal parasites, significantly Giardia intestinalis still has a higher incidence in the children worldwide. Misdiagnosis of the mentioned protozoan parasites is due to old diagnostic procedures in the laboratories⁽¹²⁾. In our community poor sanitation, lack of safe water canalization system and eating of street meals by children facilitates to be faced with intestinal parasites, so the population need to be investigated for intestinal parasites for taking of massive preventive measures.

Afghanistan is one from the countries where majority of people live in overcrowded urban areas with poor life condition and close contact to animals, which can be another rout for its transmission. There is a big number of Giardia cases especially in children in Nangarhar province. When children are infected with Giardiasis, they become involved to various clinical problems like dehydration and malnutrition that will be fatal to children. As mentioned before Nangarhar province is one of the cross border province in Afghanistan, which has a long border with Pakistan. This province has second population in the country in 22 districts. There was not a research about Giardiasis in Nangarhar province to indicate the exact number of Giardiasis. The social awareness for the disease is decreased. Involvements to the Intestinal parasitic infections among Afghan refugees are common and serious preventive measures are recommended to promote a healthy lifestyle for these people⁽⁶⁾. Research outcomes will be applied in the society for implementation of better solutions to the issue.

The study was conducted in Nangarhar Principal Hospitals to understand the risk factors of Giardia infestation as well as for detection the high-risk people in the society and to obtain the primary information about the disease in the area because it is the first research in the field about relevant issue. It can establish new ways to further researchers to investigate any more about the

topic and provide sufficient data for governmental and non-governmental health care administrations and other stockholders.

Materials and Methodology:

Study area and study site

The study was conducted at Nangarhar Teaching Hospital and Nangarhar Regional Hospital. These hospitals are the principal hospitals at the area, which are located in Jalalabad center of Nangarhar. Nangarhar is an eastern province of Afghanistan. Nangarhar province is the second overcrowded province with three millions of estimated population that has border with Pakistan. The climate of the center Jalalabad is tropical and temperature varies from 20 to 44°C at different seasons of the year.



Figure-1: Study area, Nangarhar Province
Source: <http://www.google.com/Nangarhar map>.

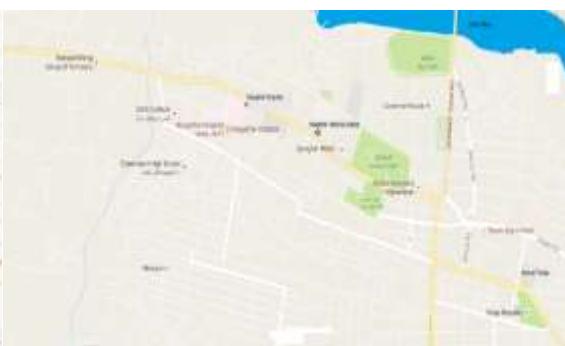


Figure-2: Study Site Source:
www.google.com/maps.

Study population

The study was conducted on 152 participants (89 male and 63 Female) in Nangarhar Teaching Hospital and Nangarhar Regional Hospital.

All of 152 participants were fulfilled to included criteria. Patients were from Nangarhar province, aged between 1- 18 years. The study was performed from May 2018 to October 2018. Verbal informed consent was obtained from all included population. After that comprehensive questionnaires consisting of complete medical, family and living history comprising questions about rearing the animals in home, using of dairy products, sources of drinking water, socioeconomic status, level of education, weight and occupation. The other main component of the questionnaire was consent from the participants and laboratory investigation record. Questionnaires were filled by researchers through direct interviews and observation with all participants of the study.

Inclusion and exclusion criteria

Inclusion Criteria

- ✓ Residence of Nangarhar province
- ✓ Age from 1 to 18 years

Exclusion Criteria

- ✓ All who are not in included criteria

Fecal samples Collection and processing

A small clean and disposable plastic container was given to each participant for fresh stool collection; after that, containers were immediately transferred by patients themselves or by their attendants having their request sheets. A single sample was tested for a single participant. The specimens were examined in parasitology laboratories of the mentioned centers by OLYMPUS CH20 microscope of CH20 BIM F200 model. In the procedure first of all the specimen were

labeled with patient's name, Microscopic slide was divided into two parts the first one was stained with one drop of Lugol's iodine and the other with normal saline. A small proportion of stool was mixed with normal saline and Lugol's iodine on the slide.

The samples were examined on the slide with 10 Objective of the microscope and again with 20 Objective. The active form of Giardia will be seen in normal saline and the cyst form will be seen in the Lugol's iodine stained portion of the slide. In some cases when the results were not recognized perfectly the senior parasitology lab technician was requested to confirm or re-apply the test.

Statistical Analysis

After the completion of Data collection process all those filled questionnaires are arranged according to dates they were obtained and cleaned from errors before being entered in data base. Data were entered in Nangarhar Medical Faculty, Biochemistry and Microbiology Department. Statistical analysis was performed using SPSS Statistics Version20 (IBM, USA). Database was prepared according to collected data and questionnaires. NPar Chi-Square test was used for categorical organizing of the data and variables. Tables and charts were used for better description of the data.

Results

This study was conducted on 152 participants which 88(57.9%) males and 64(42.1%) were females (P Value=0.052). Mean age was 10.0 years (range 1-18).

Table-1: Distribution of Giardiasis according to Sex.

(n=152)

Sex	Frequency	Percent	P Value
Male	88	57.9	
Female	64	42.1	0.052
Total	152	100.0	

Male population was 88(57.9%) and females were 64(42.1%) according to statistical analysis there was not a prominent significance (P Value= 0.052).

Table-2: Distribution of participants according to demographic characteristics

Demographic characteristics (n=152)		Number	Percentage	P Value
Sex	Male	88	57.9	0.052
	Female	64	42.1	
Address	Jalalabad City	99	65.1	
	Behsod	14	9.2	
	Sorkhrod	24	15.8	
	Kama	2	1.3	
	Khewa	2	1.3	
	Shenwari	2	1.3	
	Mohmandara	2	1.3	
	BatiKot	1	0.7	
	Khogyani	2	1.3	
	Koot	2	1.3	
	Hesarak	1	0.7	
	Gushta	1	0.7	

Marital Status	Married		5	3.3	
	Unmarried		147	96.7	
Education	Literate		26	17.1	0.000
	Illiterate		126	82.9	
Occupation	Present	No	139	91.4	
		Yes	13	8.6	
	Past	No	140	92.1	0.000
		Yes	12	7.9	
Economic Status	Good		40	26.3	0.000
	Fair		87	57.2	
	Bad		25	16.4	

According to address, many of the population were from Jalalabad city (65.1%), Sorkhrod (15.8%) and Behsod (9.2%) districts. Participants from each of Kama, Khiwa, Shenwar, Mohmandara, Khogyani and Koot districts were(1.3%) and there were total 3 participants(one from each of them) of Hesarak, Gushta and BatiKot districts. Many of them(96.7%) were unmarried while (3.3%) were married. from all 152 participants only 26(17.1%)were literates and 126(82.9%)were illiterates (P=0.000). Approximately all of them(91.4%) had not occupation in the period close to interview and 8.6% of them had occupation during study period however 92.1% of them were jobless in the past but a lower percentage(7.9%)had an occupation before study period(P=0.000). According to economic status majority of the participants (57.2%) had a moderate (average) economic condition, 26.2% had good and 16.4% had bad economic status (P=0.000).

Table-3: Distribution of patients according to other associated diseases with Giardiasis.

Disease	Patient		Patient's family	
	Number	Percentage	Number	Percentage in all cases
Amoebiasis	6	3.9	2	1.3
Intestinal Worm	5	3.3	3	2.0
Diabetes Mellitus	1	0.7	3	2.0
Tuberculosis	1	0.7	1	0.7

Table-3 indicates that the leading associated diseases were Amoebiasis 3.9% in Patient (P Value=0.000) and 1.3% was in patient's family, Intestinal worms 3.3% in patient and 2% in patient's family (P Value=0.000)., in addition other chronic diseases such as Diabetes Mellitus (0.7% in patient and 2% in patient's family) as well as Tuberculosis (0.7% in patient and 0.7% in patient's family) were the other associated diseases

Table-4: Distribution of patients according to animal number in the home

	Number of animal	Frequency	Percent
0	99	65.1	
1	11	7.2	
2	22	14.5	
3	8	5.3	
4	4	2.6	
5	4	2.6	
6	2	1.3	
7	2	1.3	
Total	152	100.0	

This table indicates that a big percentage (66.4%) of participants had not animals in their homes, 14.5% had 2 animals, 6.6% had one, 5.3% had three, 2.6% had four or five and 1.3% had six or seven animals (P Value=0.000).

Table-5: Distribution of patients according to type of animals

Type of animal	Frequency	Percent
No	101	66.4
Cow	44	28.9
Cat	4	2.6
Dog	2	1.3
Sheep	1	0.7
Total	152	100.0

As we mentioned before 66.4% of study population had no animals in their homes but in people who had animal the animals were cows (28.9%), cats (2.6%), dogs (1.3%) and sheep 0.7% (P Value=0.000).

Table-6: Distribution of patients according to dairy products they had used

Name of product	Frequency	Percent
No	89	58.6
Milk	53	34.9
Yogurt	9	5.9
cheese	1	0.7
Total	152	100.0

Data in table-6 shows that 89(58.6%) of people had not used dairy animal products in their routine meal however participants who had used animal products 34.95% had used milk, 5.9% yogurt and 0.7% cheese (P Value=0.000).

Table-7: Distribution of study population according to stay of animals in their homes.

Place of animal	Frequency	Percent
No place	101	66.4
between the house	29	19.1
far to the house	11	7.2
near from house	11	7.2
Total	152	100.0

In table-7 all 152 patients majority (66.4%) of them had no animals therefore they had not space for animals but 19.1% participants who had animals had a space for keeping the animals between the house where 7.2% individuals had a space for animals far from house and same percentage (7.2%) was for people who had place near to home (P Value=0.000).

Table-8: Distribution of study population according to source of drinking water

Source	Frequency	Percent
Pipe	80	52.6
Well	68	44.7

	running water	4	2.6
	Total	152	100.0

Table-8 indicates that 52.6% of all participants had used pipe water as a source for drinking water while 44.7% was well and 2.6 % was running water(P Value=0.000).

Table-9: Distribution of study population according to form of Giardia in Stool Examination

	Giardia form	Frequency	Percent
	Cyst	88	57.9
	Trophozoite	64	42.1
	Total	152	100.0

In all 152 stool samples which were examined for Giardia parasites 88(57.9%) were Cyst forms and 64(42.1%) were trophozoites.

Table-10: Distribution of study population according to other associated parasites.

	Parasite	Frequency	Percentage
	None	131	86.2
	H nana	11	7.2
	E. amoeba histolytic	7	4.6
	Ascaris lumbricoidus	3	2.0
	Total	152	100.0

In 152 participants 131(86.2%) had no other parasite in their stool specimen but 11(7.2%) had H nana, 7(4.6%) had E Histolytica and 3(2%) Ascaris lumbricoidus in their stool sample.

Table-11: Frequency of Giardia incidence according to months.

	Month	Frequency	Percent
	May	31	20.4
	June	13	8.6
	July	45	29.6
	August	39	25.7
	September	24	15.8
	Total	152	100.0

This table shows that high incidence of Giardia infestation was recorded in July (29.6%), August (25.7%), and May (20.4%).the incidence rate in September was 15.8% and in June 8.6%.

Figure-6: shows monthly variation of Giardia parasite in our study

Discussion

In our study total 152 individuals were participated which 88(57.9%) were males and 64(42.1%) were female population (P value=0.052), mean age was 10 years (range1-18). In our study 21(13.8%) had mixed infection which 11(7.2%) H nana, 7 (4.6%) E histolytica and three (2%) was Ascaris lumbricoidus. Fabiana Lora-Suarez et al had observed in his study at Colombia that 55.3% of study populations were males and remaining 44.7% were females (18).

A study which was conducted by Krzysztof Korzeniewski et al at Ghazni Province of Afghanistan with mean age of 6.9 years which 67.7% were males (P value=0.0001). Mixed invasion was 37(45.2%) in 81 children. Ascaris lumbricoidus and H nana were the common associated parasites

(¹⁶). In another study, which was conducted by M. S. Prado et al at Salvador, Brazil, indicated incidence of Giardia parasite was lower in children less than two years age (²⁰). Our study also provides a significant lower number in 1-2 year period. Study of M.S Prado et al has indicated that incidence of Giardia is higher in population who didn't use piped connection as a water supply; in our study big percentage of participants had piped connection as a source for drinking water. Mohammad Younas et al had described that higher number (39.13%) of Giardia positive cases were detected in age period between 8-10 years (¹⁷), as well as in our study that number (42%) was in 6-12 year period.

A study which was conducted by Ahsan-ul-Wadood et al at Quetta indicated that the maximum incidence of Giardia parasite was in 9-12 years and 1-2 year period. Mean age for participants was 5 years. The frequency was higher in males than females. Mixed infestation was seen in 18 % of patients, which were *H nana* and *E histolytica* (²²).

Bernard Nkrumah et al had obtained that the higher frequency of Giardia infestation was in 15-17 years period while in our study the higher incidence is in 6-12 years age (¹¹). Luis Enrique Jerez Puebla et al mentioned in his study at Cuba that in 45 Giardia positive cases 33 of them had dogs in their homes otherwise remaining 12 were individuals who had not dogs in their homes, according to residing area 90.7% of their study population were from urban area and 9.3% were in rural area (²¹), in comparison to our study in 51 Giardia positive population who had animals in their homes 2(1.3%) of them had dogs in their homes. In addition, our study population were 65.1% in urban area and 34.9% were from rural areas.

Cláudia Júlio et al had mentioned in his study at Portugal which was conducted on children with age range between 0-15 years had explained that 54.3% of study population with Giardia positive infestation were males and 45.7% of them were females, this study also indicated that 77.8% of Giardia positive population were urban residents and 22.8% were residents of rural areas, in addition this study had detected that 17.6% of the positive cases with Giardia parasite had dogs and 3.5% of included population had cats in their homes (¹⁹). If we compare these findings with our study results that our age range for participants was from 1 to 18 years, 57.9% were males and 42.1% were females, 65.1% were residents of urban area while 34.9% were from rural areas, 1.3% of the participants had dogs and 2.6% cats in their home but they had other animals like cows and sheep in their homes too.

Conclusion

According to the study results, *G lamblia* is the prevalent infection in Nangarhar province which increases with age. This parasite has a high incidence in school age children. Therefore, serious measures are required to educate the population properly for their social and environmental Hygiene.

In Nangarhar especially in Jalalabad city the temperature raises up to 44° C in summer in this temperature the children need more water to drink so they usually use unsafe water supplies available in streets this study has detected that the higher incidence of giardiasis were in July month but further studies should be done to follow up the exact cause for this issue. In addition, 1/3 of the study population had livestock and other domestic animals in their homes and a big percentage of them had used dairy animal products in their routine diet.

Suggestions and Recommendation

- ✓ The incidence rate is higher in school age children therefore serious preventive measure are required to be implemented.
- ✓ Contamination of water is a big cause of Giardia outbreaks so municipality should care about the water supplying system by on time chlorination of water reservoirs.
- ✓ Close contact to animals should be saved by using of gloves, masks and other essential equipment.
- ✓ Water should be boiled or filtered before drinking and fresh and well-cooked foods should be eaten.
- ✓ Animal dairy products should be cleaned and well pasteurized before consumption.
- ✓ Due to the prevalent incidence of Giardia parasite further studies should be conducted in the area to establish an improved strategy for lowering the incidence.

References

1. Hawrelak Jason. ND (2003). *Giardiasis Pathophysiology and Management* Vol 8 (2), P 129.
2. L. Rockwell Robert. *Giardia lamblia and Giardiasis*, (2003). PubMed article, p 2.
3. Ekdahl Karl, Andersson Yvonne. *Imported Giardiasis: Impact of international travel, immigration and adoption*, Department of Epidemiology, Swedish Institute for Infectious Disease Control (SMI).published in 2005 by American Society of Tropical Medicine and Hygiene. Pp 825–830.
4. Wensaas, Knut-Arne (2011). *Giardiasis in Bergen Outbreak and clinical consequences*, Department of Public Health and Primary Health Care, University of Bergen, Norway.p-13.
5. Martine S Wolf. *Giardiasis* (1992), American Society for Microbiology, Vol.5 (1), Pp. 93-100.
6. Minetti Corrado, Lamden Kenneth and Durband Caroline, Cheesbrough John, Platt Katherine, Charlett Andre, J.O'Brien Sarah, Fox Andrew and M. Wastlinga Jonathan (2015). *Case-Control Study of Risk Factors for Sporadic Giardiasis and Parasite Assemblages in North West England*. Institute of Infection and Global Health, University of Liverpool, Journal of Clinical Microbiology, www.jsm.asm.org, Vol 53(10) Pp31-33.
7. Larson Kerry Leedom, Spickler Anna Rovid and Pleva Ariel (2013). *Giardia Enteritis Lambliasis or Beaver Fever*, Center for Food Security and Public Health, Iowa State University, P 10.
8. Fauci Braunwald, Kaspar Longu, Hauser Jameson and Loscalzo Harrison's (2008) *Principles of Medicine 17th edition*, McGraw-Hill, chapter. 208.
9. A. Papadakis Maxine and J. McPhee Stephen (2016), Current Medical Diagnosis and Treatment, Mc Graw Hill, pp.1523-24.
10. Minetti Corrado; Lamden Kenneth; Durband Caroline et al (2015). *Determination of Giardia duodenalis assemblages and multi-locus genotypes in patients with sporadic giardiasis from England*. Parasites & Vectors, 8: 444, DOI 10.1186/s13071-015-1059-z.
11. Nkrumah Bernard and Nguah Samuel Blay (2011). *Giardia lamblia: a major parasitic cause of childhood diarrhea in patients attending a district hospital in Ghana*, Parasite and Vectors, 4:163, <http://www.parasitesandvectors.com/content/4/1/163>.
12. KATZ D. E, HEISEY-GROVE D, BEACH M. *Prolonged outbreak of giardiasis with two modes of transmission*, Cambridge University Press, Epidemiol Infect (2006), 134, pp. 935–941.
13. A. Cotton James, B. Amat Christina and G. Buret Andre. *Disruptions of Host Immunity and Inflammation by Giardia Duodenalis: Potential Consequences for Co-Infections in the Gastro-Intestinal Tract*, ISSN 2076-0817 www.mdpi.com/journal/pathogens. 2015, 4, 764-792.
14. A NIKOLIĆ, I KLUN, B BOBIĆ, V IVOVIĆ, M VUJANIĆ, T ŽIVKOVIĆ and O DJURKOVIĆ-DJAKOVIĆ. Human Giardiasis in Serbia: asymptomatic vs symptomatic infection, Parasite, 2011, 18, 197-201.
15. A. Ali Syed and R. Hill David. *Giardia intestinalis*, Source: PubMed article, 2003.

16. Korzeniewski Krzysztof, Augustynowicz Alina and Lass Anna. *Prevalence of intestinal parasites in Afghan community on the example of patients treated in Ghazni Provincial Hospital*, www.intmarhealth.pl, 2014; 65, 2: 68–72, ISSN 1641–9251.
17. Younas Mohammad, Shah Saminullah, Talaat Azmat. *Frequency of Giardia lamblia Infection in Children with Recurrent Abdominal Pain*, Department of Pediatrics, Postgraduate Medical Institute, Hayatabad Medical Complex, Peshawar, Vol. 58, No. 4, ,2008, pp.171.
18. Lora-Suarez Fabiana, Marin-Vasquez Carolina, Loango Nelsy, Gallego Martha, Torres Elizabeth, Gonzalez Maria Mercedes, Castaño-Osorio John Carlos and Gómez-Marín Jorge Enrique. *Giardiasis in children living in post-earthquake camps from Armenia (Colombia)*, BMC Public Health 2002, 2:5, pp.1-6.
19. Júlio Cláudia, Vilares Anabela, Oleastro Mónica, Ferreira Idalina, Gomes Salomé, Monteiro Lurdes, Nunes Baltazar, Tenreiro Rogério and Ângelo Helena. *Prevalence and risk factors for Giardia duodenalis infection among children: A case study in Portugal*, Parasite and Vectors Publication, 2012, 5:22, p.1-8.
20. M. S. Prado, A. Strina, Barreto M.L, Marlucia Ana, Assis Oliveira, Maria Paz Livia and S. Cairncross. *Risk factors for infection with Giardia duodenalis in pre-school children in the city of Salvador, Brazil*, Cambridge University Press, Epidemiol. Infect. (2003), 131, P. 899–906.
21. Enrique Luis, Puebla Jerez, A. Núñez Fidel, Martínez Silva Isabel, Rojas Rivero Lázara, González Marta Martínez, Sutil Yuliet Méndez, Valdés Lucía Ayllón, Millán Iraís Atencio and Müller Norbert. *Molecular Characterization and Risk Factors of Giardia duodenalis among School Children from La Habana, Cuba*. Hindawi Publishing Corporation, Journal of Parasitology Research Volume 2015, Article ID: 378643, 8 pages.
22. Ahsan-ul-Wadood, Abdul Bari, Aziz urRhman and Qasim Khawaja Farooq Frequency of Intestinal Parasite Infestation in Children Hospital Quetta2005 Article Vol. 44, No.2.