Prevalence of Giardia intestinalis and Hymenolepis nana in Afghan refugee population of Mianwali district, Pakistan.

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Abstract

Background: Present study aimed to investigate prevalence of Giardia intestinalis and Hymenolepis nana in Afghan refugees visiting Central Health Unit (CHU), Kot Chandana (Mianwali, Northern Punjab) during two years period (February 2007 to December 2009).

Methods: A total of 687 stool samples were collected from different age groups of both genders. Samples were processed under sterile conditions after gross examination. Microscopic examination was done on same day along with eggs (H. nana), cyst and trophozoites (G. intestinalis) detection after staining.

Results: The prevalence of G. intestinalis was significantly higher ($x2=59.54$, $p<0.001$) than that of H. nana. Females were found more likely to be infected as compared to males (OR: 1.40, 95% CI=1.03-1.92). Prevalence of both parasites decreased with age and highest prevalence was observed in young individuals belonging to 1-15 years of age group (41.8% and 48.7% respectively for H. nana and G. intestinalis, $p<0.001$). Abdominal distress (OR: 1.13, 95%CI=0.83-1.53), vomiting (OR: 1.13, 95%CI=1.13-1.81) and rectal prolapse (OR: 4.26, 95%CI=1.38-13.16) were the gastro-intestinal clinical symptoms observed in G. intestinalis. Whereas, bloody diarrhea (OR: 1.56, 95%CI=1.00-2.43) and rectal prolapse (OR: 5.79, 95%CI=1.87-17.91) were associated with H. nana infections.

Conclusions: Intestinal parasitic infections are common among Afghan refugees and serious preventive measures should be implemented to promote the safety and healthy lifestyle of these people.

Keywords: Giardia intestinalis, Hymenolepis nana, Prevalence, Afghan Refugees, Punjab.

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Introduction

Intestinal parasites infect approximately 3 billion people worldwide, leading to augmented risk of developmental deficiencies, and even deaths[1]. In tropical countries parasitic infection is a common cause of morbidity along with mortality[2]. Its incidence is predominantly high in developing countries owing to the poor sanitary conditions, usage of contaminated drinking water and poor personal hygiene[3]. The prevalence rate in the range of 9% to 80% was reported from different countries and target populations[2-4].

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Giardia intestinalis (synonymous with Giardia duodenalis, Giardia lamblia and Lamblia intestinalis) is a zoonotic protozoan which causes gastroenteritis (giardiasis) in humans[5,6]. Giardiasis affects humans and domestic as well as wild animals[7]. World Health Organization[1] had reported that, since 1988, there were over 280 million new cases of Giardia intestinalis (G. intestinalis) infection observed every year in Africa, Latin America and Asia. The infection might be asymptomatic or symptomatic and G. intestinalis trophozoites hamper nutrient absorption from the intestine, generating various degrees of malabsorption[8]. Giardiasis is associated with socioeconomic level of a country and its prevalence ranges from 2 to 7% in most of industrialized regions and reaching 40% in developing countries[9].

In developing countries, food and water, the primary source of infection, are mainly contaminated by human excrement and consequently play an important role in transmission of human infection. However, high incidence of G. intestinalis infection in developed countries could not be explained by this fact alone and it might comprise of zoonotic transmission as well by fecal contamination from animal sources[10,11]. High occurrence of Giardia has also been documented in children in an epidemiological investigation conducted in children of Spain and Colombia[12,13].

Hymenolepis genus constitutes approximately 400 species, almost all of which infect higher vertebrates[14]. Hymenolepis nana (H. nana) generally known as dwarf tapeworm, is most commonly found in children and is more generally present in the warmer climates[15]. The mature parasite resides in host intestine. H. nana varies from almost all the rest of tapeworms in being capable of completing its intact life cycle in a single host. On ingestion of eggs by humans, mice or rats, the oncospheres start crawling inside their shells and eventually reach the lumen of the intestine[16].

There is broad diversity of clinical symptoms in patients with parasitic infection. Sullivan et al.[17] established a high prevalence of G. intestinalis in children with chronic diarrhea along with malnutrition. Mild infections of H. nana are asymptomatic but severe infections cause abdominal pain, headache, diarrhea and dizziness among other vague symptoms. Cases associated with neurological symptoms have also been reported from ex-Soviet Union[18]. Deaths from these infections have not been reported. In juveniles the infection tends to clear spontaneously[19]. The method of infection and the development of immunity are interconnected[20].

Though investigations have been conducted on prevalence of H. nana in different parts of Pakistan[21-23] yet no study has been carried out on G. intestinalis and H. nana among Afghan refugees residing in District Mianwali. The present study was designed to investigate the overall prevalence and the relationship between sex and age of the host with these intestinal parasitic infections. Outcomes of the present investigation were therefore projected to be helpful for devising control strategies and future research programs on these parasites in Pakistan.

Materials and methods

The study was conducted in District Mianwali (Northwest) of Punjab that is neighboring to Khaber Pkhtunkhwa Province. After the Soviet invasion of Afghanistan in 1979, millions of Afghan families took refuge in neighboring Pakistan. Most of Afghan refugees (reaching approximately 3.2 million in 1990) were initially hosted in Khyber Pakhtunkhwa and Balochistan Provinces but later on camps were also established at Kot Chandana, district Mianwali in 1982 (Commissionerate for Afghan Refugees - CAR, 2013). Central Health Unit (CHU) is the only medical care unit working for Afghan Refugees at Kot Chandna camp, under the supervision of CAR Government of Pakistan. A passive study was conducted from February 2007 to December 2009 at Central Health Unit (CHU), to access the prevalence of H. nana and G. intestinalis in the refugees. Patients visiting the unit with primarily gastrointestinal complaints were sampled for prevalence of intestinal parasites.

A total of 687 stool samples were collected to examine prevalence of G. intestinalis and H. nana. Written informed consent was obtained from all of the patients comprising of complete medical and living history including; economic status, hygiene behavior, source of drinking water and tools pertaining to sanitation and primary complaint. The samples were collected (one sample from one patient) using sterile plastic containers and gross examination was made for density and presence of worms or their segments. The samples were then transferred in 0.85% saline solution[24] and microscopic examination was done on the same day by direct wet smears in 0.85% saline and 5% Lugol's iodine. Two types of direct wet slides were attained for each sample. Further pathogen detection was done with trichrome staining. Prevalence (%) along with respective 95% confidence interval by Binomial exact method was calculated. Chi square analysis was conducted to test the association of prevalence with age and sex. Odds ratio along with 95% confidence interval was calculated to express the strength of association among sex, age, clinical signs and diagnostic results.

Results

The overall prevalence of G. intestinalis and H. nana along with prevalence in both genders and odds ratio (from February 2007 to December 2009) is presented in Table I.

Table I: Overall prevalence of *Giardia intestinalis* and *Hymenolepis nana*:

Parasite	Male			Female			Total	
	Pos./ Tested	prev. % (95% CI)	OR (95%CI)	Pos./ Tested	prev. % (95% CI)	OR (95%CI)	Pos./ Tested	Prev. % (95% CI)
H. nana	89/306	29.1 (24.1-34.5)	0.85 (0.61-1.18)	124/381	31 (27.6-34.6)	1.18 (0.85-1.63)	213/687	31 (27.6-34.6)
G. intestinalis	102/306	33.3 (28.1-38.9)	0.71 (0.52-0.98)	157/381	41.2 (36.2-46.3)	1.40 (1.03-1.92)	259/687	37.7 (34.1-41.4)

In total 213 (31.0%) samples were found positive for H. nana and 259 (37.7%) for G. intestinalis, ($\chi2$=59.54, p<0.001). Higher prevalence of both parasites was observed in females. This difference in prevalence was not significant in case of H. nana (x2=0.95, p=0.33) and significant in case of G. intestinalis (x2=4.48, p=0.034). Univariate analysis indicated that females were more likely to be infected by H. nana (1.18, 95% CI=0.85-1.63) and G. intestinalis (1.40, 95% CI=1.02-1.92). The number of positive individuals for both parasites decreased with age (Table-II).

Table II: Relationship of age and parasitic infestation:

Age Group	*H. nana*			*G. Intestinalis*		
	Pos./ Tested	prev. % (95% CI)	OR (95% CI)	Pos./ Tested	prev. % (95% CI)	OR (95% CI)
0	109/261	41.8 (35.7-48)	4.24 (2.14-8.40)	127/261	48.7 (42.4-54.9)	3.29 (1.82-5.94)
1	58/213	27.2 (21.4-33.7)	2.22 (1.09-4.48)	79/213	37.1 (30.6-44)	2.05 (1.12-3.75)
2	35/137	25.5 (18.5-33.7)	2.03 (0.96-4.27)	36/137	26.3 (19.1-34.5)	1.24 (0.64-2.39)
3	11/76	14.5 (7.5-24.4)	1.00 (0.41-2.45)	17/76	22.4 (13.6-33.4)	1.00 (0.47-2.13)

NB. Age groups represent as; 0 = 1-15 years, 1 = 16-30 years, 2 = 31-45 years, 3 = above 45 years

The highest prevalence of H. nana was observed in age group-1 (41.8%), followed by group-2 (27.2%), group-3 (25.5%) and group-4 (14.5%), $\chi2$=27.15, p<0.001. Similarly, the highest prevalence of G. intestinalis was recorded in age group-1 (48.7%) followed by group-2 (37.1%), group-3 (26.3%) and group-4 (22.4%), $\chi2$=28.56, p<0.001. Odds ratio for association between prevalence and age in various age groups regarding each parasite was calculated and presented in Table II.

The most common clinical presentation was recurrent abdominal discomfort (51.8%), diarrhea (28.1%), upper and lower respiratory symptoms (14.7%), blood in stool (14.0%) and vomiting (11.9%). Less common clinical features were recurrent pneumonia (4.1%) and rectal prolapse (2.0%). Clinical data indicated that majority of the clinical symptoms were not specific for diagnosis of both parasites (Table III). Abdominal distress (OR: 1.13, 95%CI=0.83-1.53), vomiting (OR: 1.13, 95%CI=1.13-1.81) and rectal prolapse (OR: 4.26, 95%CI=1.38-13.16) were more specific gastro-intestinal clinical signs observed in G. intestinalis positive individuals. Bloody diarrhea (OR: 1.56, 95%CI=1.00-2.43) and rectal prolapse (OR: 5.79, 95%CI=1.87-17.91) were the specific gastro-intestinal symptoms associated with H. nana infection. Respiratory problems (OR: 2.04, 95%CI=1.33-3.11) and pneumonia (OR: 2.28, 95%CI=1.08-4.84) were also found associated with patients infected with G. intestinalis. Moreover, patients suffering from respiratory problems were found more likely to be positive for H. nana infection (OR: 1.49, 95%CI=0.96-2.30).

Table III: Clinical presentations of the patients:

Clinical Sign	*G. intestinalis*			*H. nana*		
	observed (% of Total Positive)	OR (95% CI)	p value	observed (% of Total Positive)	OR (95% CI)	p value
Abdominal discomfort	139 (53.7%)	1.126 (0.827, 1.534)	0.451	103 (48.3%)	0.82 (0.59, 1.13)	0.233
Diarrhea	65 (25.1%)	0.785 (0.554, 1.113)	0.174	59 (27.7%)	0.97 (0.68, 1.39)	0.878
Respiratory problems	53 (20.5%)	2.037 (1.331, 3.118)	0.001	39 (18.3%)	1.49 (0.96, 2.30)	0.073
Bloody Diarrhea	33 (12.7%)	0.846 (0.538, 1.330)	0.469	38 (17.8%)	1.56 (1.00, 2.43)	0.05
Vomiting	33 (12.7%)	1.129 (0.705, 1.809)	0.613	19 (8.9%)	0.64 (0.37, 1.09)	0.102
Pneumonia	16 (6.2%)	2.28 (1.08, 4.84)	0.03	7 (3.3%)	0.73 (0.31, 1.73)	0.483
Rectal prolapse	10 (3.9%)	4.26 (1.38, 13.16)	0.009	10 (4.7%)	5.79 (1.87, 17.91)	0.001

Discussion

The prevalence of G. intestinalis and H. nana in the excrements of patients suffering from abdominal discomfort was examined in the present manuscript. High prevalence of both intestinal parasites was observed in the studied population. Mehraj et al.,[25] reported prevalence of intestinal parasites to be 52.8% in Karachi. Siddiqui et al.,[26] detected 47.5% incidence of intestinal parasites in a rural area of Karachi. Similar prevalence has been reported from Bangladesh and Yemen[20]. Botero et al.,[13] also identified high prevalence of parasites as strong predictor of stunting in Colombia.

In the present study, higher prevalence of G. intestinalis (37.7%) was recorded than H. nana, which is in accordance with investigations from Nawabshah (Sindh), Argentina and rural Southern India[27-29]. The infection of G. intestinalis in this study was found more than (14.7%) of Rajeswori et al[30]. This difference could be due to variation in climate and living conditions. The prevalence of G. intestinalis was notably much higher among labor community having very low economic status. This result is in consistence with the finding of Goldin et al.[31].

The prevalence of H. nana was also found at a high level (31%), similarly to the study conducted by Wadood et al.[23] Ramesh et al.[32] estimated the prevalence of H. nana at 12.4% in Chandigarh (Northern India). Our results differ from those described by Omar et al.[33], showing H. nana (3.0%) in the city of Abha, South Western, Saudi Arabia. Jalili and Cerven[34] reported H. nana (6.0%) in the province of Baghlan (Afghanistan). Machado and Costacruz[35] reported 6.7% prevalence of H. nana in the city of Uberlandia, State of Minas Gerais. Azazy and Aitiar[36] reported 2% prevalence of H. nana in urban areas of Sana province (Yemen). Tasawar et al.[22] reported 1.81% prevalence of H. nana in and around Multan, Pakistan. These variations may be due to comparatively better living conditions and relatively dry climate leading to lower incidence in these areas.

Higher incidence of both parasites was detected in females. Statistical analysis also specified that females were more likely to be infected by these parasites. The relationship between sex of the host and H. nana had also been established in different parts of the world. Results of this study differ from that of Menan et al.[20], who reported that the male subjects were commonly infected than female and Tasawar et al.[22], who found that H. nana was more prevalent in male patients.

The group of 1-15 year old patients was most vulnerable to both parasites. Steinmann et al.[37] observed similar high prevalence rate (41%) in children aged 6-15 years from Kyrgyzstan. Worldwide distribution and prevalence rate of these parasites has been reported at 15% to 20% among children of less than 10 years of age[1]. Incidence rate of the G. intestinalis, at the level of 12.5 % and 14.5 %, has been reported among kindergarten and preschool children of Warsa and Saudi Arabia respectively[20]. The contamination of water used for hand washing before meals could also be the source for the G. intestinalis infection[38]. This infection was also widespread among the children with the habit of consuming street food available at roadways; it could be due to the contamination of street food with pathogenic microorganisms. This finding supports the observations of already published reports that, contaminated water or unhygienic handling of food and poor health awareness of street vendors lead to parasitic infection through consumption of contaminated food[39,40.] Similar high prevalence (27.25%) of H. nana was reported by Afzal [21] in children of Mansehra (Pakistan). Whereas, Omar et at.[33] reported the prevalence of H. nana (3.0%) in school boys between the ages of 5-13 years in the city of Abha.

It can be concluded from this study that intestinal parasitic infections are common among Afghan refugees of (Mianwali) Northern Punjab. Prompt preventive measures should be taken for the eradication of high infection rate that should include ensuring clean water supply, public health education, sanitation facilities, promoting personal hygiene and especially periodic deworming of the children.

Conflict of interests:

The authors affirm that they have no conflict of interest.

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