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Epidemiological study on Intestinal Parasitic Infections among Children attending Day-Care Centers, Quetta, Pakistan

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Abstract Background: Intestinal parasitic infections (IPIs) are globally endemic affecting the health, growth and development of children world-wide including Pakistan. This cross-sectional study aimed to investigate the current epidemiological status of IPIs and identify associated risk factors among children from day-care centers in Quetta, Pakistan.

Methods: The study involved pre-structured questionnaire and stool tests to obtain epidemiological and disease data. Stool specimens were collected from 150 children [87 males (58%), 63 females (42%)], aged ≤5 years (3.8±1.6). Data were statistically analyzed using descriptive statistics and univariate logistic regression methods. Specimens were examined for parasitic infections using saline and Lugol's wet mount preparation and formol-ether concentration technique.

Results: The overall prevalence of IPIs was 28.7% (43/150) (95% CI:21.5-35.9). Children infected with single parasite were 22.7% while 6% represented ployparasitism. The prevalence of protozoan parasitic infections were higher 21.3% (32/150) than helminthic infection 15.3% (23/150). The most common parasite was *Entamoebahistolytica* 14% (21/150), followed by *Hymenolepis nana* 8.7% (13/150), *Giardia lamblia* 7.3% (11/150). Other parasites with lower rates of occurrence were *Ascarislumbricoides* (4.0%), *Taeniaspp* (1.3%), and *Trichuristrichiura* (1.3%). Age (OR=3.5;95% CI:1.56 –8.08), Maternal education (OR=2.5;95% CI:1.2-5.2), type of drinking water (treated/untreated) (OR=2.44;95% CI:1.14 –5.26), hand-washing practice (OR=2.19, 95% CI:1.0-4.6), and soil-eating habit (OR=4.5;95% CI:2.0-10.0) were significantly associated with IPIs. However, no significant difference was found with gender and family size (p>0.05).

Conclusion: Due to high occurrence of IPIs among children in Quetta day-care centers, the need for screening, deworming programmes, treatment and health education is advocated.

Keywords: Epidemiology; Ipis; Day-Care Center; Deworming; Quetta.

1. INTRODUCTION

Intestinal parasitic infections (IPIs) are responsible for high morbidity and mortality worldwide, especially in low-income countries (Nyarango *et al*., 2008). IPIs are amongst the most common infections and significant causes of illnesses and diseases among the disadvantaged population globally (Ngui *et al*., 2011). Theycause malabsorption, malnutrition and impair children's growth and development (Munis *et al*., 2002). These infections are ubiquitous in hot and humid environments and among the poor and socioeconomically deprived communities where over crowding, poor sanitation, low level of education and lack of access to safe water are prevalent (Mehraj *et al*., 2008).

Soil-transmitted helminthes (STHs) are the most common intestinal parasites that include *Ascarislumbricoides*, *Trichuristrichiura* and hookworms (Bethony *et al*., 2006). Approximately one third of the world's population is infected with at least one species of STH, with *A.lumbricoides* infecting 1,450 million, hookworm 1,300 million and *T.trichiura*1,050 million, while Schistosomiasis infects over 200 million people worldwide (WHO, 2002). Among the protozoa, *Giardia lamblia* and *Entamoebahistolytica* have been associated with persistent and acute diarrhea (Utzinger, *et al*., 1999). It is also estimated that *G.lamblia* and *E.histolytica* infect around200 million and 60 million people worldwide, respectively (Murray *et al*., 2002). The fecal-oral route is crucial for the transmission of intestinal parasites to humans via improper hygiene and environmental conditions such as contamination of water sources and soil and with faeces (Nyarango *et al*., 2008).

Children are significantly at high risk of protozoan and helminth infections. Patients with intestinal parasites (IPs) present a range of symptoms, such as chronic diarrhea, malnutrition and anemia (Miller *et al*., 2003). Death has also been reported from infection with *E.histolytica*, a causative agent of amebic dysentery,

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around the world (Davoudi *et al*., 2004). Daycare centers are places where children are more likely to acquire intestinal parasites due to poor hygiene, facility of interpersonal contact (child-child, child-functionary), poorly-trained staff (de Carvalho *et al*., 2006), direct contacts and sharing toys with other children (Davoudi *et al*., 2004, Cañete *et al*., 2012). The prevalence of IPIs in children under five years in countries, such as; Cuba 45.2% (Cañete *et al*., 2012), Sri Lanka 24% (de Silva *et al*., 1994), Argentina 54% (Gamboa *et al*., 1998), and children in day care centers of Venezuela 62% (Miller *et al*., 2003), as well as Iraq 72% (Nadham and Naael, 2002) has been reported. There is scarcity of literature on prevalence of IPIs among day-care center children in Pakistan. To our knowledge this is the first study to investigate the epidemiological status of the diseases caused by helminthes and protozoans and identify the associated risk factorsamong day-care children in Quetta, Pakistan.

2. MATERIAL AND METHODS

Study area and Study population

This cross-sectional study was conducted in four different day care centers located at different regions of Quetta city i-e. Gahwara daycare center Brewry road, Gahwara daycare center Patail road, Daycare center Jinnah road and SBK Women's University daycare center and preschool. A total of 150 children consisting of 87 (58%) males and 63 (42%) females aged ≥5 years were recruited in the study.

Ethical considerations

The study was approved by Ethical Review Committee of the SardarBahadur Khan Women's University, Quetta. Only those children were included whose parents'/guardians' consent was sought to participate in the study.

Data collection

After the parents' consent was obtained, data on the independent variables, socio-demographic and epidemiologic data were collected by means of a pre-structured questionnaire, covering the important relevant aspects such as name, age, gender, maternal education, child's habit of eating soil (geophagia), hand-washing habit after defecation and before taking meal, family size and type of drinking water (treated/untreated). Parents were given instructions on how to collect the stool samples.

Stool specimen collection and parasitological diagnosis

At the time of interview, sterilized, screw-capped containers pre-labeled with name and specific identification number were provided to the parents of children. Stool samples were collected the following day from the participants and were appreciated for their participation. About 2-3g of fresh stool was collected from each participant. Stool samples were microscopically examined immediately by direct saline (0.85% NaCl solution) and Lugol's iodine wet mounts on grease-free slides using 40X and 100X bright field to observe trophozoites, cysts and eggs of parasites. Lugol's iodine staining was used for the identification of different protozoan cysts. Stool examinations were subsequently performed using the formalin ether concentration technique. 1g of stool was well mixed with 7ml of 10% formalin and processed according to the protocol previously described (Lindo, *et al*., 1998). A minute amount of sediment was placed onto a microscope slide and stained with Lugol's iodine and examined thoroughly for diagnosis of protozoan cysts and helminth ova. An experienced laboratory technician confirmed the presence and type of parasites.

Statistical analysis of data

Descriptivestatistics was performed by computing proportion for categorical variables, andmean and standard deviation for continuous variables. The association between the prevalence of IPIs and the contributable variables was explored using Pearson's Chi square test or Fisher's exact test whenever required. Logistic regression analysis was used to assess the strength of association between risk factors and intestinal parasitic infections. Odd ratios (OR) and 95% confidence interval (CI) were calculated for the different risk factors. Data were analyzed descriptively and inferentially using SPSS Statistics 20 software package.*P* values ≤0.05 were considered as significant.

3. RESULTS

Out of 150 stool samples analyzed for presence of protozoal and helminthic intestinal parasites, 43 (28.7%) were found positive for at least one parasite (95% CI: 21.5–35.9). About 34 (22.7%) samples contained single parasite while 9 (6%) samples contained more than one parasites. Thirty two (21.3%) and twenty three (15.3%) children harbored protozoan and helminthic intestinal parasites, respectively. In this study, two protozoan and four helminth species were identified. *Entamoebahistolytica* was the most prevalent parasite with infection rate of 14% (21/150), followed by *Hymenolepis nana* 8.7%(13/150), *Giardia lamblia* 7.3% (11/150). Some other parasites with lower rates of prevalence were also observed such that *Ascarislumbricoides* 6 cases (4%), *Taeniasaginata*in 2 cases (1.3%), *Trichuristrichiura*in2 cases (1.3%) **(Table 1).**

Table 1: Distribution of intestinal parasite infection among day care center children, ≤ 5 years, residing in Quetta, Pakistan.

Characteristics	N	%	95% CI[a] Upper	Lower
Intestinal parasitic infection	43	28.7	21.5	35.9
Mono parasitism[b]	34	22.7	16.0	29.4
Poly parasitism[c]	9	6.0	2.2	9.8
Nil	107	71.3	64.1	78.5
Protozoal infection	32	21.3		
Entamoebahistolytica	21	14.0	8.4	19.6
Giardia lamblia	11	7.3	3.1	11.5
Helminth infection	23	15.3		
Hymenolepis nana	13	8.7	4.2	13.2
Ascarislumbricoides	6	4.0	0.9	7.1
Teaniasaginata	2	1.3	–	–
Trichuristrichiura	2	1.3	–	–

[a] Confidence interval; [b] Infection with one parasite only; [c] Infection with two or more parasites.

In the present study, a total of 150 children 87(58.0%) males and 63 (42.0%) females] aged ≤5 years were examined for intestinal parasitic infections with an average of 3.8±1.6 (Mean±SD). As indicated in **(Table 2),** stool sample analysis revealed that the infection rate was higher in males 33.3% (29/87) than females 28.6% (18/63). The males were at higher risk of IPIs with OR of 1.25 (95% CI: 0.62–2.53). However, the difference in the prevalence of IPIs between males and females was statistically non-significant (χ^2=0.385, df=1, p=0.54). A major proportion of children (62.7%) in the study were aged between 3 to 5 years while 37.3% children were below 3 years of age. Children with age 3–5 years were 3.5-fold (95% CI: 1.56–8.08) at higher risk of acquiring IPs as compared with children aged below 3 years (40.4% vs. 16.1%). The prevalence was significantly different between the two age groups (χ^2= 9.674, df=1, p=0.002).

Table 2:Univariate regression analysis of risk factors correlated with intestinal parasite infections among children in day care centers, residing in Quetta, Pakistan.

Risk Factor	Parasite status Positive N=43	Negative N=107	Total N=150	OR	95%CI	*P*
Gender						0.535[ns]
Female	18 (28.6)	45 (71.4)	63 (42.0)	(ref.)	–	
Male	29 (33.3)	58 (66.7)	87 (58.0)	1.25	0.62 –2.53	
Age (Years)						0.002*
> 3	9 (16.1)	47 (83.9)	56 (37.3)	(ref.)	–	
3–5	38 (40.4)	56 (59.6)	94 (62.7)	3.544	1.56 –8.08	
Mother's education						0.012*
Above Primary	16 (20.0)	64 (80.0)	80 (53.3)	(ref.)	–	
Primary & below	27 (38.6)	43 (61.4)	70 (46.7)	2.512	1.21 –5.21	
Family size						0.754 [ns]
≤4	16 (32.7)	33 (67.3)	49 (32.7)	(ref.)	–	
5-7	19 (26.8)	52 (73.2)	71 (47.3)	0.75	0.27 –2.05	
≥8	8 (26.7)	22 (73.3)	30 (20.0)	0.995	0.38 –2.61	
Drinking water						0.021*
Treated	12 (18.8)	52 (81.2)	64 (42.7)	(ref.)	–	
Untreated	31 (36.0)	55 (64.0)	86 (57.3)	2.44	1.14 –5.26	
Hand washing habit						0.036*
Yes	14 (20.3)	55 (79.7)	69 (46.0)	(ref.)	–	
No	29 (35.8)	52 (64.2)	81 (54.0)	2.19	1.04 –4.60	
Geophagia						<0.001*
No	24 (20.9)	91 (79.1)	115 (76.7)	(ref.)	–	
Yes	19 (54.3)	16 (45.7)	35 (23.3)	4.503	2.02 –0.05	

OR: Odds Ratio; 95% CI: Confidence interval at 95% of the estimated odds ratio; (ref.): Reference category for OR; *: Statistically significant at P < 0.05; [ns]=non-significant.

Maternal education plays a prominent role in the health of their children. Eighty out of 150 (53.3%) mothers were educated above primary level. A higher prevalence of IPIs was observed in children whose mothers had primary and below primary school education (38.6%) than those whose maternal education was above primary (20.0%), increasing the risk 2.5 times (95% CI: 1.2-5.2) for IPIs. Statistically significant association (χ^2= 6.297, df=1, p=0.012) between mother's level of education and parasite infection was observed. The proportions of children with family size categorized as ≤4, 5–7 and ≥8 were observed 32.7%, 47.3% and 20.0%, respectively. Family size of participants didn't affect the rate of intestinal parasites (χ^2= 0.566, df=1, p=0.75).

More than half of the children (57.3%) used untreated drinking water who were found more likely to

be infected (OR=2.44, 95% CI: 1.14 –5.26) than those who used treated drinking water (36.0% vs. 18.8%, P <0.05).The risk of infection increased about two-fold (OR=2.19; 95% CI: 1.0–4.6) in children who didn't wash hands before eating meal and after defecation. Statistically significant association (p<0.05) was found between presence of IPIs and soil-eating and hand-washing habit.Approximately one forth of children 23.3% (35/150) had soil-eating habit (geophagia) whereas hand-washing habit was observed in 46.0% (69/150) study subjects. 54.3% (19/35) children with geophagia were infected with intestinal parasites while 20.9% (24/115) children with no habit of soil eating were found infected. Soil eating by children increased the risk for developing protozoal and helminthic infections 4.5 times higher (95% CI: 2.0–10.0).

4. DISCUSSION

Human infections caused by protozoan and helminth parasites are important threats to healthy living in developing countries (Kia *et al*., 2008). These infections extensively contribute to health and problems worldwide and more than a billion people are infected by at least one species of intestinal parasites (Nematian *et al*., 2004).

In this study, the intestinal parasites, such as *Entamoebahistolytica*, *Giardia lamblia*, *Hymenolepis nana*, *Ascarislumbricoides*, *Taeniasaginata* and *Trichuristrichiura*were identified from the stool sample analysis. The overall prevalence rate of IPIs was 28.7%. Our finding is in agreement with the studies reported in Muzaffarabad district, Pakista 29.26% (Chaudhry *et al*., 2004), Brazil 29.3% (Goncalves *et al.* 2001), Kenya 25.6% (Mbae *et al*., 2013), Wonji, Ethiopia 24.3% (Ghiwot *et al*., 2014). However, this prevalence rate is lower when compared with a study from Karachi, Pakistan 52.8% among children aged 1-5 years (Mehraj *et al*., 2008) and studies in other countries, namely, Jos, central Nigeria 57.8% (Chirdan *et al*., 2010) and Cuba 71.1% (Cañete *et al*., 2012). Moreover, the infection rate of the current study is higher than those reported in Nigeria (13.8%) by Okpala *et al*., (2014), Iran (14%) by Abdi *et al.* (2014), Zahedan 19.1% by Davoudi *et al*., (2004) among children in day-care centers. These variations in prevalence rate could be attributed to differences in geographical area, time of survey, hygiene practices, seasonal differences, socio-economic status of parents and parasite diagnostic technique.

Globally, the distribution and prevalence of IPIs is not uniform and is an indicator of hygiene condition, sanitation facilities, water contamination, poverty, socio-economic status, and high population density. Our results indicated that protozoan parasites were more predominant than helminth parasites. Infection rate with *E. histolytica* (14.9%) was the highest followed by *G. lamblia* (7.3%) which is almost in line with a study in South Ethiopia by Wegayehu *et al.* (2013) who reported that *E. histolytica* (11.4%) had the highest prevalence followed by *G. lamblia* (10.6%). However, infection rate by *Entamoeba*is higher compared with 2.92% (Abdi *et al*., 2014). *H. nana* (8.7%) was the predominant IP detected among helmintheswhich is comparable with the studies in Rural Peshawar, Pakistan 8% (Ullah*et al*., 2003) and Iran day-care centers 7.84% (Abdi *et al*., 2014). However, infection by *H. nana* was higher than 2.3% reported by Goncalves *et al.* (2001) and lower than 24.5%reported by Aleka *et al*. (2015).

Children are more susceptible to infection by helminth and protozoal parasites particularly in day-care centers. The males were more infected with IPs than females, though the statistical difference was non-significant (p=0.054) which is similar to previous studies (Quihui *et al*., 2006; Okpala *et al*., 2014). In contrast, Adamu *et al.* (2006) in Addis Ababa found association of sex (p=0.035) with IPIs.Age is implicated as an important risk factor for the occurrence of IPs(Ngui *et al*., 2011) and the preschool and school going children being at elevated risk (Bethony *et al*., 2006). Children aged3-5 years were at increased risk of IPIs with odds ratio of 3.5 compared with <3 years old children and significant association was observed (p<0.05). This direct relationship of the age with IPIs might be due to increased exposure of children (3-5 years) to the faeacally- contaminated soil environment and play grounds.

Maternal education is significantly associated with high occurrence of IPIs. This shows that children whose parents are highly educated are at lower risk of acquiring parasitic infections. We found maternal education to be positively associated with IPIs (p<0.05). Several other studies conducted in Pakistan and other parts of the world highlighted that the higher the maternal educational level, the lower the risk of IP infection among children (Wamani *et al*., 2004; Nematian *et al*., 2004; Mehraj *et al*., 2008). On the contrary, Aleka *et al*., (2015) found no significant association of Parents' education with IP infection. Like in other studies; family size was not statistically associated with parasite infection (p>0.05) (Chaudhry *et al*., 2004; Quihui *et al*., 2006; Nxasana *et al*., 2013).

Untreated water supply is an important risk predictor of IPIs (Ngui *et al*., 2011). Majority of the participants in the study used boiled or chlorinated drinking water. Our study revealed that drinking treated water and hand washing practice significantly decreased the infection rate among children (p=0.021 and p=0.036, respectively). This finding is consistent with the studies

conducted in Belize, Central America (Aimpum and Hshieh, 2004), and Jos, Central Nigeria (Chirdan *et al*., 2010) where use of drinking water (treated versus untreated) was highly correlated with IPIs. Similarly, more children were infected ($p<0.001$) in Turkey who did not wash hands regularly (Östan *et al*., 2007). Children with soil-eating habit carried 4.5 times increased risk of harboring infection in the current study ($p<0.001$) which is similar to Mumtaz *et al*., (2009) but inconsistent to Mehraj *et al*. (2008).

Day-care centers are the environments where children are more susceptible to IPIs and have gained importance in the care of preschoolers, since women's participation has raised in the labor market. According to Gurgel and his colleagues (2005),

5. CONCLUSION

IPIs are major health problem in Pakistan like other developing countries. High occurrence of IPIs among children indicated that these infections are important public health issues in day-care centers. Age, education level of mothers, personal hygiene (hand washing and drinking treated water), geophagia were implicated as significant factors associated with IPIs.

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