

Original Article

Food handlers: an important reservoir of protozoans and helminth parasites of public health importance

Manipuladores de alimentos: um importante reservatório de protozoários e helmintos parasitas de importância para a saúde pública

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Abstract

Food handlers plays a primary role in the transmission of pathogenically important protozoans and helminth parasites. This study was aimed to evaluate the prevalence of intestinal pathogenic protozoans and helminth parasites among food handlers in and around University of Malakand, Lower Dir, Pakistan. Stool samples were collected from 642 food handlers (all of male) in a cross-sectional study from January to November, 2017. Wet Mount Techniques and concentration methods by using salt and formol-ether solutions. Three hundred and eighty four cases (59.8%) were found infected with one more parasites. Most of the individuals were found infected with helminth (47.6%) as compared to intestinal protozoans (0.93%). Seventy two cases (11.2%) of the cases presented mixed infection with both intestinal protozoan and helminth parasites. The order of prevalence for intestinal helminth was *Ancylostoma duodenale* (n = 258, 40.1%), followed by *Taeniasa ginata* (n=96, 14.9%) *Ascaris lumbricoides* (n = 54, 8.40%) and *Trichuris trichura* (n=30, 4.60%). For intestinal protozoa, *Entamoeba histolytica/dispar* (n = 36, 5.64%) was the only protozoan detected. Mono-parasitism was higher than poly-parasitism. Family size income and education level were the factors significantly (P<0.05) associated in the parasites prevalence. Current research showed that IPIs are primarily the foodborne pathogens still an important public health problem in Pakistan. Effective control programs on parasitic diseases transfer and their associated factors are recommended.

Keywords: foodborne pathogens, zoonosis, soil transmitted helminthiasis, gastroenteritis, sanitary practices.

Resumo

Os manipuladores de alimentos desempenham um papel fundamental na transmissão de protozoários e helmintos parasitas patogenicamente importantes. Este estudo teve como objetivo avaliar a prevalência de protozoários patogênicos intestinais e helmintos parasitas entre manipuladores de alimentos na Universidade de Malakand, Lower Dir, Paquistão. Amostras de fezes foram coletadas de 642 manipuladores de alimentos (todos do sexo masculino) em um estudo transversal de janeiro a novembro de 2017. Técnicas de montagem úmida e métodos de concentração usando soluções de sal e formol-éter. Trezentos e oitenta e quatro casos (59,8%) foram encontrados infectados com mais um parasita. A maioria dos indivíduos foi encontrada infectada por helmintos (47,6%) em comparação com protozoários intestinais (0,93%). Setenta e dois casos (11,2%) dos casos apresentavam infecção mista com protozoários intestinais e helmintos parasitas. A ordem de prevalência de helmintos intestinais foi *Ancylostoma duodenale* (n = 258, 40,1%), seguido por *Taeniasa ginata* (n = 96, 14,9%) *Ascaris lumbricoides* (n = 54, 8,40%) e *Trichuris trichura* (n = 30, 4,60 %). Para protozoários intestinais, *Entamoeba histolytica / dispar* (n = 36, 5,64%) foi o único protozoário detectado. Monoparasitismo foi maior do que poliparasitismo. A renda familiar e o nível de escolaridade foram os fatores significativamente (P <0,05) associados na prevalência de parasitos. A pesquisa atual mostrou que os IPIs são principalmente os patógenos de origem alimentar, ainda um importante problema de saúde pública no Paquistão. Programas eficazes de controle da transferência de doenças parasitárias e seus fatores associados são recomendados.

Palavras-chave: patógenos de origem alimentar, zoonose, helmintíase transmitida pelo solo, gastroenterite, práticas sanitárias.

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Received: May 31, 2020 – Accepted: July 23, 2020



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1. Introduction

Food protection is a global concern and a substantial number of food borne illnesses is due to unsafe food-handling practices. These diseases were originated to affect more than 30% of the people in industrialized nations. The problem is estimated to be even more complicated in unindustrialized countries. Thus, improving the consumer understanding of safety rules would reduce pathogenic microorganisms in food. Most of the academic awareness has been given to consider the knowledge on practices of food safety throughout world (Ahmed et al., 2017). Information about food handlers is a required factor in controlling the food borne diseases. Government of Pakistan has issued 22070 certificates to international and 8857 to local food handlers (Ahmed et al., 2017).

The infection caused by intestinal parasites is an important health issue in unindustrialized countries, ranges from 30 to 60% as compared to 2% in the developed nations of the world (Shahnazi et al., 2009). Intestinal parasite prevalence is mainly depend on nutrition, culture, socio environmental factors and hand washing. Intestinal parasitic infection is common disease worldwide. According to WHO two billion people are affected by helminth parasites in the world and closely two third is infected with one species of intestinal parasites (Tulu et al., 2016).

The infection caused by protozoan parasite, the *Giardia* spp., and nematode worms such *Ascaris lumbricoides* and *Trichuris trichiura*, infect one fourth of the population in the globe (Harhay et al., 2010) as the most common intestinal parasites in the world. The infection cause by *A. lumbricoides* in some parts of Pakistan has reached to 55.8 percent (Khan et al., 2017a).

Pakistan is one of the countries endemic for intestinal parasites. Some of the studies conducted previously on intestinal parasitic infection in various parts of Pakistan reflects high prevalence rates as food handlers 83.1% (Khan et al., 2017a), occupational groups 73.2% (Khan et al., 2017b), education departments 65.9% (Khan et al., 2015); medical students 70.5% (Farooki, 1965); shepherd 79.2% (Nisa et al., 2012); intestinal protozoal and helminth infection 12.4% (Arshad et al., 2019); 28.8% (Khan et al., 2019).

Several studies conducted previously in Pakistan were focused on particular areas and targeted populations, however information on prevalence of intestinal protozoan and helminths in food handlers in the study area is lacking. Current study was therefore designed to determine the prevalence of intestinal parasite in food handlers in and around the University of Malakand, Lower Dir, Khyber Pakhtunkhwa, Pakistan.

2. Materials and Methods

2.1. Study area and participants studied

Current research was conducted around the University of Malakand, Pakistan between January to November 2017. Dir Lower is located 263km in northwest of Islamabad (capital of Pakistan). It has a Lat= 35° 11'51.57" North and Long=71°52'29.72 East, characterized by raining in summer

than in winters. The average temperature in Dir is 22.8 °C. Lower Dir lies at 8500ft above sea level and shows 1588mm annual precipitation rate. The food handlers participated in current research were showing the symptoms related to intestinal parasitic infections however some of them were asymptomatic. These participants had belong to different sites and location of the region serving in these food stations.

2.2. Ethical approval and inform consent

The study was approved by the ethical review board, University of Malakand. A written consent was obtained from all the participants on the purpose and importance of the study. Each of the participants was interviewed on their life style i.e family size, number of individuals living in each family, income per month and education status of the parent's parents/guardians. The interview was conducted in local language (Pashto) and then translated into the English.

2.3. Stool sample collection

The consented food handlers were provided with a clean plastic bottle containing 10% preservatives (Formaldehyde and Merthiolate iodine) a wooden spatula, with an identification number. During collection of stool sample a questionnaire for each food handler was also filled. The collected faecal specimens were brought to the laboratory of Parasitology, Department of Zoology, University of Malakand and processed as below:

2.4. Physical examination

Each of the faecal samples collected were observed through naked eyes or by using the hand lens for the presence of proglottids of the tapeworm or any adult parasitic worm.

2.5. Microscopic examination

Using normal saline and iodine preparation the sample was examined under microscope. The slide was studied for at least 10 minutes. Initially under low power lenses then under high power bright field. The procedure of Formalin Ethyl Acetate is used (Mali et al., 2008).

2.6. Direct smear method

One up to two grams of feces was mixed in one to three drops of normal saline or Lugol's iodine solution. A cover-slip was placed on the slide and examined under 10X and 40X power objective lenses of a microscope. Saline direct smear method is found useful for the detection of movement of trophozoites stages and helminths ova. Iodine direct smear method was useful for the identification of the typical features of protozoa cyst (*E. histolytic/dispar*: the intestinal pathogenic protozoan). Parasitological assessment was performed by the first author of this manuscript.

2.7. Formol-ether concentration

In formol-ether concentration method, about one gram of feces was mixed in 10% formalin solution and formol-ether concentration technique was applied (Williams, 1992).

2.8. Statistical analysis

Collected data was analysed using the Graph Pad version 5. The P value less than 0.05 at 95% CI was considered significant (P <0.05).

3. Results

Out of the 642 food handlers examined, 48.5% (n=612) were infected with single or multiple species of parasites. These were including intestinal protozoa 0.93% (n=6) and 47.6% (n=306) with helminths and 72 food handlers (11.2%) had mixed infection with both helminth and protozoa. Mono-parasitism was higher as compared to poly-parasitism. The prevalence of mono-parasitism 312/642 (48.5%) including 306/642 (47.6%) were infected with helminths, while only six individuals 6/642 (0.93%) were found infected with *Entamoeba histolytica* (the intestinal protozoan). Out of the total infected food handlers 54/642 (8.4%) were found with mixed infection including 42/642 (6.5%) were infected with two helminthic parasites and 12/642 (1.86%) were infected with one protozoan and one helminthic parasite. The remaining 18/642 (2.8%) were infected with three parasitic species, two helminths and one protozoan (Table 1).

The order of prevalence for intestinal helminth detected was *Ancylostoma duodenale* (n = 258, 40.1%), followed by *Taenia saginata* (n=96, 14.9%) *A. lumbricoides* (n = 54, 8.40%) and *Trichuris trichura* (n=30, 4.60%). Intestinal protozoa,

E. histolytica/dispar (n = 36, 5.64%) was the only protozoan detected (Table 2).

In total, 642 food handlers participated in this study, including 300 (46.7%) were inside and 342(53.2%) were outside the University. The overall prevalence was noted as 59.8% (384/642). It was 28.9% (186/300) and 57.8% (198/342) among inside and outside food-handlers, respectively. No significantly difference (P = 0.5958) among these two groups was noted (Table 3). However, family size, income and education level are significantly associated with the distribution of infection (P <0.05) (Table 3).

4. Discussion

Food safety can prevent food from contacting food borne pathogens during preparation and storage of food in different ways. Food handlers links the food from industry to the market and then to consumer. In this way food can transmit food borne pathogens. All the food handlers should be referred to a healthy centre (Medical Diagnostic Laboratory) to diagnose for intestinal parasitic infection prior to receive health certificate.

Infection caused by intestinal parasites is endemic in the world and have designated as one of the main sources of disease and illness (Keiser and Utzinger, 2010). Intestinal parasitic infections are usually found in the people of poor population, in the people of lacking access to safe water supply and lacking of hygiene. All these factors are variably associated in the distribution of intestinal

Table 1. Pattern of mono-parasitism and poly-parasitism among food handlers in University of Malakand.

Type of infection	Number of species	Associated species	Case (%)
Mono-parasitism	One species (n=312)	Hookworms	192(29.9)
		<i>Ascaris lumbricoids</i>	42(6.5)
		<i>Tania species</i>	60(9.34)
		<i>Trichuris trichura</i>	12(1.86)
		<i>Entamoeba histolytica</i>	6(0.93)
Total mono parasitism			312(48.59)
Poly-Parasitism	Two species (n=54)	Hook worms + <i>Taenia species</i>	24(3.73)
		Hook worms + <i>T. Trichura</i>	12(1.86)
		Hook worms + <i>E histolytica</i>	6(0.93)
		<i>A. lumbricoids</i> + <i>Ehistolytica</i>	6(0.93)
		Hookworms + <i>A. lumbricoides</i>	6(0.93)
Sub-total			54 (8.41)
	Three species (n=18)	Hookworms + <i>Taenia species</i> + <i>E.histolytica</i>	12(1.86)
		Hookworms + <i>E histolytica</i> + <i>T. trichura</i>	6(0.93)
Sub-total			18(2.80)
Total poly parasitism			72(11.2)
All infected individuals			384(59.81)
Total Sample examined			642
Samples medically unfit			270
Total sample collected			912

Table 2. Frequency distribution of intestinal protozoan and helminth parasites found in stool of food handlers at university of Malakand Lower Dir, Pakistan.

Parasite species	Population infected	Prevalence (%)	R ²	P-Value
Helminths			0.02292	0.9978
Hookworms	258/642	40.1		
<i>Ascaris lumbricoides</i>	54/642	8.40		
<i>Trichuris trichura</i>	30/642	4.60		
<i>Taenia</i> species	96/642	14.9		
Sub-total	438/642	68.2		
Protozoa				
<i>Entamoeba histolytica</i>	36/642	5.64		
Sub-total	36/642	5.64		
Gross-total	474	73.8		

Table 3. Association of socio-economic characteristics and intestinal parasitic infections among food handlers at university of Malakand Lower Dir, Pakistan.

Parameters	No. examined (%)	No. infected (%)	P value (at 95% CI)
Site			
Inside the University	300(46.7)	186(28.9)	0.5958 (P<0.05)
Outside the University	342(53.2)	198(57.8)	
Total	642	384	
Age			
16-32	462(71.9)	294(45.7)	0.4104 (P<0.05)
32-48	144(22.4)	72(50)	
48-64	36(5.60)	18(50)	
Total	642	384	
Family size (No. of family members)			
<4	90(14.0)	36(40)	0.0004 (P>0.05)
4-8	184(28.6)	144(43.1)	
8-12	140(21.8)	102(72.8)	
>12	228(35.5)	102(72.8)	
Total	642	384	
Family income (PKR/ month)			
<10000	108 (16.8)	90(83.3)	0.0001 (P>0.05)
10000-15000	114(17.7)	84(73.6)	
15000-20000	168(26.1)	114(67.8)	
>20000	252(39.2)	96(38.0)	
Total	642	384	
Education Level			
<Matriculation	366(57.0)	246(67.2)	0.0183 (P>0.05)
Matriculation	180(28.0)	102(56.6)	
>Matriculation	96(14.9)	36(37.5)	
Total	642	384	

parasitic infections in the globe (Zaglool et al., 2011). The information on intestinal parasitic spectrum of a community is important for planning the control programs. Current study focused on the prevalence of intestinal parasite infection among food handlers inside and outside University of Malakand, Lower Dir, Pakistan. Present study showed 59.8% of the examined food-handlers were infected with intestinal parasites. These intestinal parasitic disease affected both inside and out-side University food-handlers. Both inside and outside food-handlers coming mainly from districts Swat, Peshawar, Dir (lower and upper) Malakand (Dargai and Sakhakot. No significant difference ($P < 0.05$) was noticed between inside and outside of the University food-handlers.

Various surveys on the prevalence of intestinal parasites have been conducted including general survey 30.4% (Bilqees et al., 1982); different occupations 30.5% (Khan et al., 2018); medical laboratory 31% (Shaikh et al., 2000); General population 33.1% (Shaikh et al., 2003); education departments 65.9% (Khan et al., 2015); medical students 70.5% (Farooki, 1965); occupational groups 73.2% (Khan et al., 2017b) and food handlers 83.1% (Khan et al., 2017a).

The highest 83.1% rate of prevalence was observed in present study (Khan et al., 2017a) and lowest in general survey 30.4% (Bilqees et al., 1982). Nevertheless, by comparing the findings of the current research to the studies conducted previously, we noticed that our finding is somewhat higher as compared to other studies conducted in Pakistan.

The highest prevalence of intestinal parasitic infection was reported in India 97.4% (Kang et al., 1998), Ecuadoria 90% (Sackey et al., 2003) and Swat district, Pakistan 83.1% (Khan et al., 2017a). The higher rates of prevalence in these parts of the world may be due to inadequate hygiene and rural backgrounds.

E. histolytica/dispar was the frequently detected intestinal protozoan parasite in this study. Similar values have also been reported by Haleem et al. (1965) Karachi; Bilqees et al. (1982) Karachi; Chaudhry et al. (2004) Muzaffarabad, Pakistan; Tasawar et al. (2006) Vehari. *E. histolytica/dispar* may end in immune-compromised patients (Cacciò et al., 2005) which can be transferred through direct contact or drinking parasite contaminated water.

Comparatively high prevalence of helminth parasites was seen in the present study findings. This variation may likely be explained by the climate of the study area which is known to be moist in the entire year. This condition might be suitable for development of infective stages of helminth eggs that become infective if ingested. *A. duodenale* was the most prevalent helminth in present study. Safe water supply provision, latrines development, hygiene improvement and awareness about transmission of parasitic infection should be ensured to minimise these infections.

Acknowledgments

The authors thanks to the food handlers for their cooperation during stool specimen collection. The authors

also extend their appreciation to the researchers supporting project number (RSP-2020/94) King Saud University Riyadh, Saudi Arabia.

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