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The changing pattern of enteric protozoan infections in the Los Angeles area between 1996 and 2013

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Abstract

This is the first study of seasonal prevalence of parasitic infections over a period of 18 years in an urban/suburban setting anywhere in the world. We examined parasitological test results of 7766 fecal specimens from 3883 patients in the Los Angeles County, from 1996 to 2013 tested at Parasitology Center, Inc. (PCI), Scottsdale, Arizona. During this period, 1629 (41%) of patients were found infected with one or more protozoan parasites. The most prevalent parasites were *Blastocystis hominis* (19%), *Entamoeba histolytica/ E. dispar* (6%), *E. hartmanni* (6%), and *Cryptosporidium parvum* (5%). *Blastocystis hominis* made up 45% of all infections. The prevalence of infections with *Blastocystis hominis* showed progressive decline through 2013. The reverse pattern was observed in infections with *C. parvum.* Infections with *B. hominis* were more prevalent in the cold months and lowest in August and September. Infections with *C. parvum* were most prevalent from March to June and lowest in August. The overall monthly prevalence rates for all protozoan parasites varied between 34% in August and 51% in February. An interpretation of the above findings is provided. Notable similarities and differences from other studies are noted, especially regarding the composition of the parasitic fauna diagnosed, annual prevalence rates, and seasonality.

Keywords: Prevalence, protozoan infections, Los Angeles, USA, 1996-2013

INTRODUCTION

Parasitological studies of large patient populations are rare in the United States. Such studies are more common in third-world countries where endemic parasitosis are more readily documented (Amin, 1997). At the Parasitology Center, Inc. (PCI), in Scottsdale, Arizona, we routinely monitor and report on the patterns and trends of human parasitosis in the US. The seasonal prevalence of 19 species of intestinal parasites infecting 916 of 2,896 (32%) examined patients from 48 states in the year 2000 was reported (Amin, 2002). Three hundred and fourteen of 859 examined patients (36%) from California were infected (Amin, 2002). Infections with helminth parasites such as *Ascaris lumbricoides* and non-major protozoans were rare and are not included in this study. The seven reported species of protozoan parasites constituted 91.5% of 18 species of parasites reported in the United States. Multiple infections with 2-4 parasitic species constituted 10% of the infected cases (Amin,

2002). A large scale investigation of the epidemiology of *Blastocystis hominis* in 48 states and the District of Columbia in 2002-2004 reported on the trends in annual, seasonal, geographical and host distribution, and symptomology by age, sex and season (Amin, 2006). In that report, 16% of 10,582 fecal specimens from 5,291 patients tested positive for *B. hominis*; in California, 263 of 1,328 examined patients (20%) were positive. In a similar study of 9,856 fecal specimens from 4,928 patients from all states and the District of Columbia tested between 2003 and 2005, 279 (6%) were positive for *Cryptosporidium parvum* infections. In that study, the seasonal prevalence, parasite and host distribution by sex and age in single and multiple infections, enteric and extra-intestinal symptoms were reported over a period of three years. Studies of this magnitude are not known in the US. Few other studies of relatively large patient populations in the US (Kappus et al., 1994; Church et al.,

2010) or in more geographically limited populations addressing *B. hominis* only, e.g., California (Conteas et al., 1998) or Ontario (Senay and Macpherson, 1990) have been reported. The present investigation is the first to cover the span of 18 years. Evaluating the patterns and trends of parasitic infections in studies of such a long duration is a great tool for understanding the epidemiological characteristics, disease burden, improving the reporting of cases, planning prevention, therapeutic, and other public health measures to be considered to manage these infections. Nevertheless, an overview of patterns from comparable urban/suburban area in developing and developed nations are included.

MATERIALS AND METHODS

A total of 7766 specimens from 3883 patients (two specimens per patient) were collected, preserved, and transported to Parasitology Center, Inc. (PCI) in Proto-fix ™ (Alpha-Tec Systems, Inc. Vancouver, Wash.) or SAF (sodium acetate–acetic acid–formalin mixture) in plastic vials provided in mailable kits. Patients were referred to PCI by 187 doctors in Los Angeles County from January 1996 through December 2013. Specimens were processed and stained with CONSED™ according to the manufacturer's (Alpha-Tec Systems) directions. This procedure was used in thousands of specimens at PCI, evaluated, and described previously (Amin, 2000). Briefly, specimens are filtered, mixed with CONSED and ethyl acetate, vortexed, centrifuged, and decanted. The resulting fecal plug was mixed with CONSED diluting reagent, transferred to, and mounted on a slide for microscopic examination as wet mounts. All samples were evaluated by the same observer blinded to patient information, e.g., symptoms, travel history, etc. The reliability of diagnosis is indicated by the consistency of detection of different parasites at different levels of infection during the same period of time. Differences in the number of patient samples tested in different years reflect changing patterns of patient traffic from Los Angeles County over time. Positive results were quantified (number of organisms per high power field on a scale of 1-4) from duplicate samples from each patient. The term prevalence rate used in this study refers to the number of parasite species identified (not the number of patients infected) from the number of patients examined.

About 10% of infected patients had 2-4 parasitic species each which is the same prevalence rate of concurrent infections noted in our earlier study (Amin, 2002). A prevalence rate based on the number of infected/examined patients would be about 10% lower but would not reflect the activity of individual parasitic species. The number of samples tested underwent a gradual decrease over the years corresponding to changing patterns of patients and practitioners' traffic in the Los Angeles area while increasing elsewhere in the US and internationally.

Diagnostic issues

We recognize the possible presence of *Blastocystis* and *Cryptosporidium* organisms from animal sources in human infections with *B. hominis* and *Cryptosporidium parvum*, reviewed in Tan (2008) and Garcia (2001) respectively. Fletcher et al. (2012) provided a comprehensive, well referenced diagnostic coverage of intestinal protozoan infections in developed countries. Human, mammalian, avian, and reptilian isolates of *B. hominis* have been assigned to 13 subtypes. *Blastocystis* subtype 3 is most commonly associated with illness in human prevalence studies. *Blastocystis hominis* refers to about 10 different genetic populations that are indistinguishable microscopically and the term is used for parasites isolated from humans while *Blastocystis* spp. is used for isolations from animal hosts (Fletcher et al., 2012). Most animals are not infected with human pathogenic cryptosporidiosis. However, zoonotic transmission from direct contact with infected animals or their feces through indirect sources including drinking of contaminated water occurs. The invasive *Entamoeba histolytica* trophozoites is less common than the morphologically identical non-pathogenic *E. dispar* and *E. moshkovskii* but distinguishable from them by using isoenzyme analysis. *Giardia intestinalis* infections are detected microscopically by us and also by various antigen assays demonstrating seven genetically distinct genotypes (A-G). Assemblages A and B infect humans as well as other mammalian species and are considered zoonotic. See Fletcher et al. (2012) for a discussion of above diagnostic issues.

RESULTS

In the 7766 specimens collected from 3883 patients in the Los Angeles Area, a total of 1629 parasitic infections (41%) were identified. *Blastocystis hominis* was the most frequent parasitological finding. It was identified in 19% of samples, and represented 45% of all parasitological infections (Table 1). The next most common protozoan was *E. histolytica/dispar*. It was found in 6% of samples constituting 15% of all infections. The prevalence of *E. hartmanni*, *C. parvum* and *E.coli* was 6%, 5%, and 4%, respectively. *Dientamoeba fragilis* and *Giardia intestinalis* were found in 4% and <1% of the samples examined, in the same order. These parasites constituted 91.5% of 18 species of intestinal parasites reported from 5792 fecal specimens tested from throughout the US in 2000 (Amin, 2002).

The prevalence of all parasitic infections was highest during 1996-97 (63%) then gradually declined through 2012-1013. *Giardia intestinalis* was the only protozoan that was consistently identified in less than 1% of tested

Table 1. Prevalence of protozoan infections diagnosed from 3883 patients*
examined from Los Angeles County between 1996 and 2013.

Parasite species	Patients infected	Prevalence	Percent of infections
Blastocystis hominis	732	19%	45
Entamoeba histolytica/E. dispar **	234	6%	15
Entamoeba hartmanni **	226	6%	14
Cryptosporidium parvum	201	5%	12
Entamoeba coli **	156	4%	10
Dientamoeba fragilis	60	1%	3
Giardia intestinalis **	20	0%***	1
Total	1629*	41%	100

*About 10% of infected patients were concurrently infected with more than 1 species of parasite.

**Trophozoites and cysts

***0% indicates values of less than 1%.

samples throughout the study period. The prevalence of *Cryptosporidium* sp. increased and decreased cyclically reaching the lowest level of 2% in 1996-1997 (the period of most prevalent infections with other protozoans) to 11% in 2008-2009 before declining again. The prevalence of *B. hominis* infections progressively declined from 21% in 1996-2001 to 7% in 2012-2013 (Table 2). The total number of samples submitted from Los Angeles County was highest in 1996-1997 but declined then stabilized afterwards as did the total prevalence rates of all parasites.

The prevalence of all parasites was highest (43-51%) between December and March but lower during the rest of the year reaching a low of 34-36% in August and September. This pattern was clearly influenced by a corresponding pattern in the seasonal prevalence of *Blastocystis hominis*, the most common parasite, which reached 20-23% then declined to 13-15%, during the same time periods, respectively (Table 3). The seasonal prevalence of *Cryptosporidium parvum* infections was more or less stable throughout

the year (4-7%) but declined to a low of 3% in August. The prevalence of *D. fragilis* was highest in February (3%) and November (4%). The prevalence of *E. coli* cysts and trophozoites was highest in July (8%) but lower (3-4%) between December and May. The prevalence of each of *E. Hartmanni* and *E. histolytica/dispar* cysts and trophozoites was highest (10%) in February and lowest (2%) in November. The prevalence of the amoeba trophozoites was usually relatively higher than that of cysts during most months. The numbers of detected *G. intestinalis* were too small to produce credible seasonal data (Table 3).

DISCUSSION

Prevalence in the USA

The overall prevalence of infection with all investigated protozoans was 41% of double fecal samples examined between 1996 and 2013 from 3,883 patients in the Los Angeles County.

Infections with B. *hominis* made up roughly half (45%) of all protozoans studied and noted in Table 1. Los Angeles is an urban/suburban area and a 41% prevalence rate is markedly higher than the 32% and 36% prevalence rates reported earlier in the United States and California, respectively (Amin, 2002). The testing of our laboratory procedures employed in this study produced a prevalence rate of 32.6% (3,373 infected of 10,358 examined patients throughout the United States) between 1996 and 1998 (Amin, 2000). An almost identical prevalence of 32% was reported in our comprehensive study of the prevalence of intestinal parasites in 5,792 fecal specimens from 2,896 patients in the United States (Amin, 2002). Our results reflect our most efficient methods of parasite detection (Amin, 2000) which show a considerably higher prevalence rates than others across the country. For instance, Kappus et al. (1994) reported US prevalence of 20% (from 216,275 stool specimens) compared with 19.7% (from 178,786 stool specimens) reported by state diagnostic

Table 2. Number and prevalence (%) of infections with major protozoan parasites diagnosed from 3883 patients examined from Los Angeles County between 1996 and 2013 by two year period increments.

Combined 2 year periods	96-97	98-99	00-01	02-03	04-05	06-07	08-09	10-11	12-13
Blastocystis hominis	315(21)*	203(21)	112(21)	29(15)	26(14)	18(9)	12(8)	9(9)	8(7)
Cryptosporidium parvum	30(2)	72(8)	18(3)	14(7)	14(10)	16(8)	17(11)	9(9)	6(4)
Dientamoeba fragilis	47(3)	1(0)	1(0)	1(1)	0(0)	2(2)	1(1)	0(0)	5(5)
Entamoeba coli cysts	50(3)	17(2)	6(1)	3(2)	4(2)	3(1)	2(1)	1(1)	1(0)
E.coli trophozoites	62(4)	3(0)	4(1)	1(1)	1(1)	0(0)	0(0)	0(0)	0(0)
E. hartmanni cysts	89(6)	9(1)	0(0)	0(0)	1(1)	0(0)	0(0)	0(0)	1(0)
E. hartmanni trophozoites	122(8)	0(0)	0(0)	0(0)	1(1)	0(0)	0(0)	0(0)	0(0)
E. histolytica/dispar cysts	57(4)	14(1)	4(1)	1(0)	0(0)	0(0)	0(0)	1(1)	0(0)
E. histolytica/dispar trophozoites	145(10)	12(1)	1(0)	2(1)	0(0)	0(0)	0(0)	0(0)	0(0)
Giardia intestinalis cysts	9(1)	5(1)	2(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
G. intestinalis trophozoites	1(0)**	1(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
Total # of infections	927	337	138	51	47	39	32	20	21
Total examined	1483	947	530	193	187	195	155	92	101
Prevalence	63%	36%	26%	26%	25%	20%	21%	22%	21%

*No. of infections (% of examined patients per each 2 year period). Percentages are rounded.

**0% indicates values of less than 1%.

Table 3. Seasonal distribution of infections with major protozoan parasites diagnosed from 3883 patients examined from Los Angeles County between 1996 and 2013.

Parasite species	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	OCT	NOV	DEC	TOTAL
Blastocystis hominis	75(23)*	72(21)	73(22)	80(20)	63(16)	62(18)	53(18)	41(13)	47(15)	58(19)	58(20)	50(20)	732(19)
Cryptosporidium parvum	17(5)	18(5)	20(6)	16(4)	22(6)	19(6)	12(4)	9(3)	22(7)	17(6)	13(5)	16(6)	201(5)
Dientamoeba fragilis	4(1)	11(3)	4(1)	9(2)	5(1)	4(1)	1(0)	2(1)	1(0)	7(2)	11(4)	1(0)	60(2)
Entamoeba coli C**	6(2)	8(2)	3(1)	8(2)	8(2)	9(3)	12(4)	6(2)	5(2)	6(2)	8(3)	6(2)	85(2)
E. coli T**	7(2)	3(1)	5(2)	7(2)	3(1)	6(2)	12(4)	5(2)	2(1)	8(3)	10(3)	3(1)	71(2)
E. hartmanni C	11(3)	13(4)	11(3)	8(2)	10(3)	10(3)	7(2)	11(4)	5(2)	5(2)	3(1)	9(4)	103(3)
E. hartmanni T	10(3)	20(6)	9(3)	12(3)	9(2)	10(3)	9(3)	10(3)	12(4)	8(3)	4(1)	10(4)	123(3)
E. histolytica/dispar C	6(2)	10(3)	8(2)	2(1)	10(3)	10(3)	8(3)	4(1)	4(1)	5(2)	2(1)	6(2)	75(2)
E. histolytica/dispar T	15(5)	24(7)	10(3)	14(4)	15(4)	19(6)	15(5)	19(6)	10(3)	6(2)	3(1)	9(4)	159(4)
Giardia intestinalis C	2(1)	1(0)	1(0)	3(1)	2(1)	0(0)	1(0)***	2(1)	3(1)	2(1)	0(0)	1(0)	18(0)
G. intestinalis T	0(0)	0(0)	0(0)	(0)0	0(0)	0(0)	0(0)	0(0)	1(0)	0(0)	1(0)	0(0)	2(0)
Total # of infections	153	180	0	0	0	0	0	0	0	0	0	0	0
Total examined	322	348	333	397	386	339	301	312	307	301	286	251	3883
Prevalence	47%	51%	43%	40%	38%	43%	43%	34%	36%	40%	39%	44%	41%

*No. of infections (% of examined patients per each month). Percentages are rounded.

**C=cysts, T=-trophozoites.

*** 0% indicates values of less than 1%%.

laboratories in 1987. Similarly, Garcia et al. (1984) reported a prevalence of 20.6% from 2,360 US patients. Differences in test populations or in the composition of the component parasite may be involved. Church et al. (2010) reported a low prevalence of 6.4-7.2% of 2,604 fecal specimens from Colorado, Montana, New Mexico, and Utah were infected with parasites between August, 2006 and April, 2007. We (Amin, 2002) reported 19%, 50%, 39%, and 29% from the same states in the same order. Church et al. (2010) attributed their low prevalence rates, in part, to their inability to detect infections with *C. parvum* and *C. cayetanensis*; Quest Diagnostics, Denver tested their specimens.

Prevalence in developing countries

In comparable urban/suburban areas in Africa, Asia and South America, the prevalence of parasitic infections was mostly similar to ours (Table 1) but occasionally somewhat lower or markedly higher. It was 21.4% of 5,990 patients in Madhya Pradesh, India (Marothi and Singth, 2011), 23.14% of 350 patients in Dhakka University, Bangladesh (Khanum et al., 2013), 29.26% of 287 patients in Muzaffaraband, Pakistan (Chaudhry et al., 2004, who reviewed prevalences in 14 Pakistani cities from 40,096 subjects), 33% of 199 patients in Chennai, India (Fernandez et al., 2002), 33.4% of 1,127 patients in Izmir, Turkey (Aksoy et al., 2007), 42.9% of 2,400 patients in Kumasi, Ghana (Walana et al., 2014), 47% of 293 patients in Varamin, Iran (Aminzadehe et al., 2007), 47.2% of 1267 patients in León, Nicaragua (Téllez et al., 1997), 50.5% of 93 patients in Central Nigeria (Ikeh et al., 2005), 62% of 195 patients (protozoans only) in Tamil Nadu, India (Rayan et al., 2010), and 75.1% of 1,227 patients in Bioko, Equatorial Guinea (Roche and Benito, 1999). On rare occasions, the overall prevalence of intestinal infection was very low reaching 5.92% of 5,743 patients in Eghbalieh City, Iran (Sadeghi et al., 2013). We believe such differences in prevalence to be attributable to demography, diet, environmental exposure, social habits, urbanization, and zoonotic relationships as was apparent from the above articles.

Prevalence in developed countries

Fletcher et al. (2012) summarized 33 prevalence studies from cities in developed countries, 23 in Europe and 10 from the US, Canada, Australia, and Korea. The prevalence rate of *B. hominis* varied between 1% in Danish counties, Denmark to 16.9% in Sydney, Australia. For *C. parvum*, it varied between 0.4% in Melbourne, Australia to 9.1% in Helsinki, Finland. For *E. histolytica/dispar*, it varied between 0.4% in Helsinki to 3.5% in Noumea, New Caledonia. For *G. intestinalis*, it varied between 0.3% in Melbourne, Australia to 29% in Helsinki. *Entamoeba coli* was reported only once from Brussels, Belgium at a rate of 5.4% and *D. fragilis* 6 times

between 0.4% in Melbourne, Australia and 14.6% in Holland.

Prevalence of individual protozoan species

In the United States, *B. hominis* was the most dominant protozoan parasite. Its reported prevalence was 19% (45% of all infections) (Table 1), 23% in 2000 (Amin, 2002), 20-30% (Lee, 1995), and 12.2% (Garcia et al., 1984). It was also the most dominant parasite species reported by Kappus et al. (1994) and Church et al. (2010) but at surprisingly lower prevalences of only 2.6% and 4.3%., respectively. In developed countries, *B. hominis* appears to also be the dominant intestinal parasite, e.g. Izmir, Turkey (Aksoy et al., 2007), Amsterdam, Holland (Fernandez et al., 2002) (24.2%), Sydney, Australia (Stark et al., 2007) (18-21%), Thessaloniki, Greece (Papazahariadou et al., 2004) (5.3-16.8%), Stockholm, Sweden (Svenungsson et al., 2000) (4.0%), Berlin, Germany (Jansen et al., 2008) (7.6%), Helsinki, Finland (Fletcher et al., 2012) (Table 1; 13%), Rome, Italy (Marothi and Singth, 2011) (7.5-14.1%), and Brussels, Belgium (Fletcher et al., 2012) (Table 1; 9.8%). In most developing countries in Asia, Africa and South America, however, *E. histolytica/dispar* and /or *G. intestinalis* appear to be the dominant parasites, e.g., Jordan (Ali-Shtayeh et al., 1989); India (Patel, 1986; Marothi and Singth, 2011; Fletcher et al., 2012); Saudi Arabia (Imam et al., 2012); Lebanon (Araj et al., 2011), Nicaragua (Téllez et al., 1997), Ghana (Walana et al., 2014), Equatorial Guinea (Roche and Benito, 1999), Pakistan (Chaudhry et al., 2004), Iran (Aminzadehe et al., 2007), and Bangladesh (Khanum et al., 2013). Fletcher et al. (2012) concluded that "while some enteric protozoa, such as *Entamoeba* sp., *Cryptosporidium*, and *Giardia* are isolated frequently from diarrheal patients in developing regions such as Asia and sub-Saharan Africa, others, such as *Blastocystis* spp. and *Dientamoeba fragilis* are isolated mainly in developed countries." We concur.

In the present study, the next highest prevalences to *B. hominis* were 6%, 5%, 5%, and 41.% noted for *E. histolytica/dispar*, *E.hartmanni*, *C. parvum,* and *E. coli*., respectively. Only 20 patients (<1%) were infected with *G. intestinalis* (Table 1). This ranking was not consistent in some other studies in the United States. For example, in the Rocky Mountain states, the prevalence of *Endolimax nana* and *G. intestinalis* ranked second (1.5%) and third (1.4%) to *Blastocystis* infections (Church et al., 2010). The prevalence of *E. histolytica/dispar* of 6% (Table 1) is markedly higher than the 0.9% reported in a large 1987 survey than the estimated prevalence of 4% in the United States (Kelsall and Ravdin, 1994). The prevalence of *C. parvum* of 5% (Table 1) is higher than 0.6-4.3% reported elsewhere in North America but less than the 3-20% known from other area of the world (Asia, Australia, Africa, and Central and South America) (Garcia, 2001). *Cryptosporidium parvum* appears to be

underdiagnosed in the western hemisphere; its seroprevalence in Europe and North America is usually between 25% and 35% and may reach 64% in South America (Current and Garcia, 1991). *Cryptosporidium* oocysts were observed in 27% of drinking water sampled from 66 surface water treatment plants in 14 states and one Canadian province (LeChevallier et al., 1991). Differences in the prevalence and composition of the intestinal parasite fauna in different geographies are probably attributable to demography, diet, environmental exposure, social habits, urbanization, and zoonotic relationships as well as to the structure of the edaphic conditions and weather affecting the extra-human stages of the parasites particular to each location.

Annual prevalence

The total number of samples submitted from the Los Angeles area was highest in 1996-1997 but declined then stabilized afterwards corresponding with changing patterns of patient traffic. The prevalence of most parasitic infections was highest during 1996-97 (63%) then gradually declined reaching 21% in 2012-1013 agreeing with that of *B. hominis*, the most common parasite detected, being 21% and 7%, in the same order (Table 2). The prevalence of all other protozoans, except *C. parvum*, was highest in 1996-1997. The prevalence of *C. parvum* progressively increased from 2% in 1996-1997, when prevalence of all other infections were at a minimum, to a high of 11% in 2008-2009 then declined afterwards. *Giardia intestinalis* was the only protozoan that was consistently identified in 1% or less of the samples throughout the study period. The general decline in the prevalence of all parasites and especially of the dominant *B. hominis* over the years was similar to declines over time reported in other studies. In 10,582 fecal specimens from U.S. general population (Amin, 2006), reported declining *B. hominis* prevalence rates of 23%, 20%, 15%, and 11% between 2000 and 2004, respectively. A similar study of *C. parvum* from 9,856 fecal specimens from US general population between 2003 and 2005 noted an almost even prevalence of 5-6% (Amin, 2008). Annual prevalence rates of microsporidiosis from fecal specimens of 8,550 HIV-infected patients in Southern California demonstrated a decline from 8.8% in 1993, 9.7% 1994, 6.6% in 1995, and 2.9% in 1996 which was attributed to "the use of multi-drug antiretroviral regimens and the use of protease inhibitors, a new class of antiretroviral agents, the first of which was licensed in 1995" (Conteas et al., 1998). In Izmir, a Turkish Mediterranean coastal city with a climate similar to that of Los Angeles, the prevalence of intestinal parasites was 42.5% in 2003 (Senay and Macpherson, 1990) and 65% five years earlier in the same area (Akisu et al., 2000). Of 18,563 hospital patient records studied in Qatar, the prevalence of intestinal parasites decreased from 13.4% in 2005-2008 to 6.6% in 2009-2011 (Abu-Madi et al.,

2013). During this period, the prevalence of *B. hominis*, *G. intestinalis*, and pathogenic amoeba decreased from 4.3% to 2.9%, 1.9% to 1.4%, and 0.29% to 0.25%, respectively. This decline was attributed, in part, to improved screening of foreign workers. In Madhya, Pradesh, India, the prevalence of intestinal parasites (mostly *Giardia* and *E. histolytica*/dispar), during 2003, 2006, 2007, 2008, 2009, 2010, and 2011 was 59.5% (Rao et al., 2003), 24.1%, 22.3%, 20.3%, 19.9%, and 20.4%, 21.4% (Marothi and Singth, 2011), respectively. In Lebanon, Araj et al. (2011) reported decreasing prevalence from 14% to 12% in *E. histolytica*/*dispar* and from 16% to 6% in *G. intestinalis* between 1997-1998 and 2007-2008 in 14,771 and 7,477 fecal specimens tested, respectively. We can attribute the overall decline in prevalence of parasitic protozoan infections over time to improved health education, better preventive measures, and more effective drug therapies.

Seasonal prevalence

The seasonal prevalence of all infections in our study populations did not show a dramatic seasonal periodicity (Amin, 2002; Amin, 2006; Amin, 2008). However, in the present investigation, the seasonal prevalence of all protozoan infections was highest in February (51%) and lowest in August (34%) corresponding with the pattern in *B. hominis* (Table 3). In 2000, the seasonal prevalence of all infections from 48 states and the District of Columbia was highest in September and October (42% and 43%) and lowest in February (22%) (Amin, 2002). In 2000 (Amin, 2002) and 2002-2004 (Amin, 2006), the seasonal prevalence of *B hominis* in the general US populations also did not show any marked seasonality but was highest in September (23%) and lowest in February (13%). The seasonal prevalence of *C. parvum* in the Los Angeles area (this paper) was more or less stable throughout the year (3% in August to 7% in September).

The prevalence of microsporidiosis in 8,439 fecal specimens from persons with diarrhea and human immunodeficiency syndrome in southern California showed no seasonal variation (Conteas et al., 1998). The prevalence of *C. parvum* from throughout the US was low (3-6%) during the colder months of the year (October to March) but higher during the warmer months of April and May, reaching 9% of 9,856 fecal specimens examined between 2003-2005 (Amin, 2008). "Peaks in *C. parvum* prevalence appear to correspond with warmer seasons in temperate and tropical climates especially when associated with rain. During the rainy seasons, the runoff from cattle farms readily contaminate surface waters feeding into water treatment plants as happened during the March-April, 1993 Milwaukee outbreak (Amin, 2008). This pattern of seasonal waterborne fecal contamination has also been reported by other observers throughout the world, e.g. US (Hlavsa et al., 2005), New Orleans (Inungu

et al., 2000), Peru (Bern et al., 2002), England (Howe et al., 2002), Korea (Chai et al., 2001), Uganda (Tumwine et al., 2003), Jordan (Mahgoub et al., 2004), Guatemala (Bern et al., 2000), Indonesia (Katsumata et al., 1998), and Zambia (Nchito et al., 1998). *Cryptosporidium* infections from 2000 HIV-positive patients in Benin City, Nigeria were reported to also be associated with the rainy season (Akinbo et al., 2011). The infectious stages of these soil-based intestinal parasites released into the environment are clearly vulnerable to seasonal variations in temperature, rainfall and humidity before they encounter other hosts. Transmission depends on the production of and host encounters with parasite stages in the environment. Seasonal variations in host immune system being weaker in the winter may also be involved (Altizer et al., 2006).

The seasonal prevalence of other soil-based infections, e.g., *E. histolytica/dispar* and *G. intestinalis* was also highest in the rainy season and lowest in winter or summer in 350 fecal specimens tested in Dhakka University Medical Center, Bangladesh (Khanum et al., 2013). Similar results were reported for the same two protozoans from 23,278 fecal specimens in Qassim region of Saudi Arabia and from 22,970 stools in the Nablus area, Jordan by Imam et al. (2012) and Ali-Shtayeh et al. (1989), respectively. Patel (1986), however, reported "no significant" seasonal differences in the prevalence of *E. histolytica/dispar*, *G. intestinalis*, and *E. coli* from 36,000 reports of inpatients and outpatients of Bombay hospital, India analyzed between 1966 and 1975.

Non-water sources of seasonal *Cryptosporidium* infections (food stuffs, drinks, animal to person, person to person contact, exposure to contaminated recreational water, among others) are known in arid desert countries mostly in the coldest season and may reach as high as 50% of total exposures (Anonymous, 1999). This pattern is best illustrated in the desert country of Kuwait where 77% of the infections occur between November and April (Gaitei et al., 2006). Seasonal prevalence of other protozoans investigated (Table 3) were more or less stable throughout the year.

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