



## Temporal trends of intestinal parasites in patients attending a tertiary care hospital in south India: A seven-year retrospective analysis

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**Background & objectives:** Intestinal parasitic infections and their associated complications are a major cause of morbidity in the developing world. This retrospective study was done to assess the prevalence of intestinal parasitic infections among patients in a tertiary healthcare setting and to analyze age-, gender- and time-related trends in the prevalence of these intestinal parasites over a seven year period (2006-2012).

**Methods:** The presence of various intestinal parasites in a tertiary care setting over a seven year period in different age groups was determined by performing routine stool microscopy. Modified acid-fast staining was performed for stool samples collected from children less than five years of age for the detection of intestinal coccidian parasites. Statistical analysis was carried out to analyze age-related trends in relation to the prevalence of commonly detected intestinal parasites. Seasonal fluctuations in parasite prevalence were evaluated by performing harmonic regression analysis.

**Results:** A total of 257,588 stool samples were received over the seven year period for examination. The highest percentage of intestinal parasites was in the 6-10 yr age group. Among the intestinal parasites, *Giardia intestinalis* had the highest prevalence across most age groups, except in those above 60 yr of age where hookworm became more prevalent. A significant decreasing trend with age was observed for *G. intestinalis*, whereas for hookworm and *Strongyloides stercoralis*, an increasing trend with age was seen. Significant linear temporal trends were observed for parasites such as *G. intestinalis*, *Entamoeba histolytica* and *Ascaris lumbricoides*.

**Interpretation & conclusions:** While *G. intestinalis* was more common in the younger age groups, certain soil-transmitted helminths such as hookworm and *S. stercoralis* showed a higher prevalence in the older populations. Significant temporal trends and seasonality were observed for some of the common intestinal parasites.

**Key words** Prevalence - soil-transmitted helminths - South India - temporal trends

According to the World Health Organization (WHO) estimates, approximately 21 per cent of India's population may be infected with intestinal parasites such as soil-transmitted helminths<sup>1</sup> and

the global disease burden attributed to intestinal helminths is 39 million disability adjusted life years<sup>2</sup>. Intestinal parasitic infections are important public health problems in developing countries. For example,

hookworm infection is a major cause of enteric blood loss and anaemia in tropical countries, and according to the WHO estimates, 44 million pregnancies face complications because of hookworm infestation<sup>3</sup>. The prevalence of intestinal helminths and intestinal parasites is estimated by the extrapolation from findings of population surveys carried out in particular communities and settings<sup>4</sup>. Compared to the prevalence data for helminthic intestinal infections, data regarding the epidemiological distribution and burden of disease due to intestinal protozoan parasites are limited, and global burden of many of these infections is not available.

In India, the estimates of intestinal parasitic infections differ in different parts of the country<sup>5,6</sup>. In some studies on soil-transmitted helminths (STHs), the prevalence in the Indian subcontinent is comparable to other countries in Southeast Asia and China<sup>7</sup>, which in absolute numbers translates to a huge burden of infected cases. According to the WHO estimates, although the disease burden due to intestinal helminths in India is considerable, only 9.97 per cent of the estimated population requiring preventive chemotherapy has received anthelmintics<sup>8</sup>.

Among the neglected tropical diseases, the STHs such as *Ascaris lumbricoides*, hookworm, *Trichuris trichiura* and *Strongyloides stercoralis* are most prevalent. In 2004, the WHO included intestinal parasites such as *Giardia intestinalis* and *Cryptosporidium* species in the WHO expanded list for the 'Tropical Diseases Initiative' with a call to classify them as pathogens associated with stunting as has been done with the intestinal helminths<sup>9</sup>.

Data on prevalence are important to plan effective interventions. Specific population groups at increased risk of intestinal parasite infections should be the target for intervention measures such as chemoprophylaxis<sup>10</sup>. Although community-based prevalence data are most informative from a public health point of view, hospitals can also act as sentinel facilities because they capture greater morbidity and help provide data on the presence and absence and, to some extent, the relative abundance of specific parasites. Apart from age-related trends, temporal trends and seasonality patterns might also be helpful in developing focussed prevention programmes against intestinal parasites. Seasonal trends also reflect the relationship of intestinal parasites, particularly those with shorter life cycles such as the protozoa, with abiotic factors such as temperature and relative

humidity which affect the survival of these parasites in the environment<sup>11</sup>.

In this study, a retrospective analysis was carried out to determine the prevalence of intestinal parasites and to identify any age-related or temporal trends over a period of seven years (2006-2012) among patients attending a tertiary care hospital in south India.

### Material & Methods

The study was carried out at the department of Gastrointestinal Sciences, Christian Medical College, Vellore, India. Microscopy data from all stool samples sent to the parasitology laboratory for routine diagnostic screening from January 2006 to December 2012 were analysed. Stool samples collected from patients known or suspected to be HIV positive were excluded. Samples were collected in laboratory prescribed clean, dry, leak-proof-labelled containers. On receipt, all samples were screened with saline and iodine wet mounts. For samples obtained from children less than five years of age, formalin ether concentration and modified acid-fast staining were performed to detect intestinal coccidian parasites. Demographic details of the patients were obtained from the computerized database of the hospital. Ethical clearance was obtained from the Institutional Review Board of the Christian Medical College, Vellore, for this retrospective study.

*Statistical analysis:* Prevalence (with 95% confidence intervals) of intestinal parasitic infections was determined and stratified based on gender and different age groups. The *Chi-square* test was used to determine any association of intestinal parasitic infections with sex and age ranges. The *Chi-square* test for trend was performed to detect any age-related trends in the prevalence of common intestinal parasites. Proportion distribution of different intestinal parasites in positive samples was analyzed by *Chi-square* test for trend. Monthly prevalence of intestinal parasites was calculated and plotted for the seven year period for six common intestinal parasites. To assess the seasonal fluctuations in the prevalence of intestinal parasites, a monthly time series of prevalence of each pathogen was compiled and a Poisson harmonic regression model with a single annual hump (accommodating one sine and one cosine function) was fitted. The month of highest prevalence was determined using methods described previously<sup>12</sup>. The linear trend was assessed by adding the month of the year as a covariate in the model.

All statistical analyses were carried out with GraphPadInstat version 3.0 (GraphPad Software, San Diego, CA, USA), with STATA (version 10, STATA Corp, TX, USA) used for the annual harmonic regression model.

## Results

A total of 257,588 stool samples were received over the seven-year period for routine examination. The number of samples received annually ranged from 29,746 in 2007 to 47,640 in 2012. The age of patients ranged from less than one year to a maximum of 98 yr. When stratified as five-year intervals, samples from patients in the age groups 36-40 and 41-45 yr comprised the majority of clinical specimens (12%) while children below the age of five years contributed to 1.8 per cent of the total samples. Number of samples from males (61.3%) was more than females (Table I).

The overall prevalence of intestinal parasitic infections did not vary much over the seven year period, with an overall positivity rate of 8.9 per cent (22,864/257,588), and the highest positivity was observed in 2009 (9.5%). The most common parasite identified was *G. intestinalis*, found in 3.5 per cent (9036/257,588) of all samples (Table I) and 40 per cent of all positive samples. This was followed by hookworm ova in 1.6 per cent (4055/257,588) of all samples screened and 17.7 per cent (4055/22,864) of all positive samples. *A. lumbricoides* was found in 11.2 per cent (2565/22,864) followed by *S. stercoralis* in 10 per cent

(2309/22,864), *Entamoeba histolytica/Entamoeba dispar* in 8.2 per cent (1873/22,864) and *T. trichiura* in 4.4 per cent (1015/22,864) of all positive samples. The other common parasites seen were *Hymenolepis nana* found in 3.3 per cent (755/22,864) and *Enterobius vermicularis* in two per cent (457/22,864) of all positive specimens.

Modified acid-fast staining was performed only for samples from children less than five years of age, and the prevalence of *Cryptosporidium* spp. was 0.73 per cent overall, comprising 8.2 per cent of all positive specimens in this age group. *Blastocystis* species, reported from 2009 onwards, was found in 0.22 per cent (335/155,012) of all samples screened for intestinal parasites and 2.9 per cent (389/13,520) of all stool specimens positive for intestinal parasites over the period 2009-2012. In 99 per cent (386/389) of these *Blastocystis*-positive samples, no other parasite was identified. No significant age associated trend was found for *Blastocystis* spp. and none of the samples in the age group 0-5 yr was positive for *Blastocystis*.

Other intestinal parasites which were found rarely in the stool specimens included *Taenia* spp. seen in only 24 samples over the seven year period and *Hymenolepis diminuta* in 28 samples. Two patients were positive for ova of *Capillaria* spp. One was a 31 yr old male patient with *Capillaria philippinensis*, also confirmed by histopathological examination of intestinal biopsy. The second was a 51 yr old female patient with a 'spurious' *Capillaria hepatica* infection with passage of unembryonated eggs.

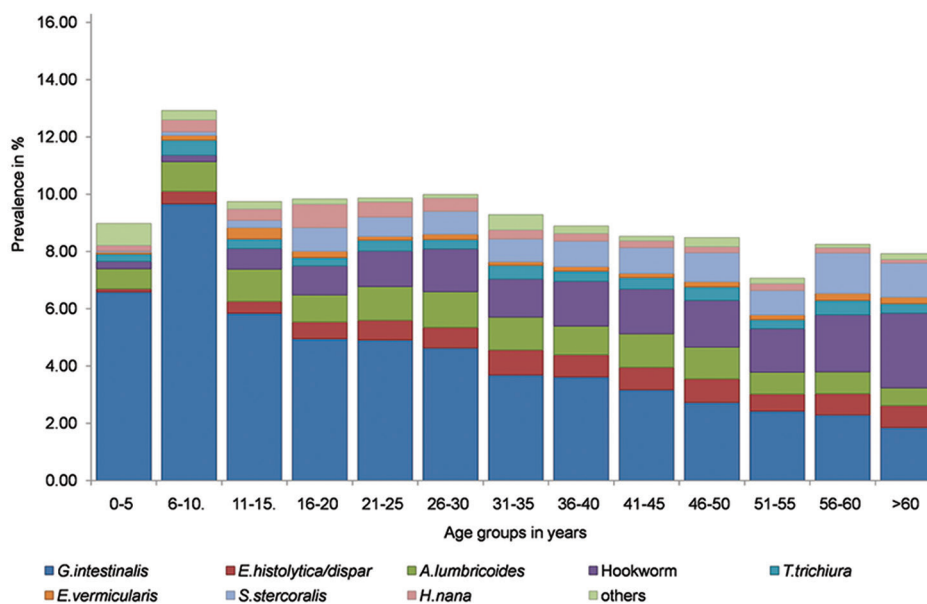


Fig. 1. Prevalence of intestinal parasites in different age groups.

Table I. Overall prevalence of intestinal parasites stratified by gender and age groups

Age stratified (yr)	Prevalence % (95% CI) n	Males (95% CI) n	Females (95% CI) n	<i>Giardia intestinalis</i>	<i>Entamoeba histolytica/Entamoeba dispar</i>	<i>Ascaris lumbricoides</i>	Hookworm	<i>Trichouris trichiura</i>	<i>Strongyloides stercoralis</i>	<i>Hymenolepis nana</i>	<i>Enterobius vermicularis</i>	Others
Overall (n=257,588)	8.9 (8.77-8.99) n=22,864	10.40 (10.25-10.55) n=16,391	6.47 (6.32-6.63) n=6,473	3.51 (3.44-3.58) n=9036	0.73 (0.70-0.76) n=1873	1 (0.96-1.03) n=2565	1.57 (1.53-1.62) n=4055	0.39 (0.37-0.42) n=1015	0.90 (0.86-0.93) n=2306	0.29 (0.27-0.32) n=759	0.18 (0.16-0.19) n=456	0.31 (0.29-0.33) n=795
0-5 (n=4612)	8.97 (7.94-9.57) n=414	9.21 (8.19-10.31) n=260	8.70 (7.44-10.13) n=154	6.59 (5.88-7.35) n=304	0.11 (0.10-0.56) n=5	0.69 (0.49-0.98) n=32	0.26 (0.13-0.45) n=12	0.26 (0.13-0.45) n=12	0.04 (0.01-0.17) n=2	0.20 (0.09-0.37) n=9	0.07 (0.01-0.20) n=3	0.76 (0.54-1.06) n=35
6-10 (n=5762)	12.9 (12.05-13.78) n=745	14.07 (12.9-15.27) n=475	11.31 (10.08-12.63) n=270	9.67 (8.94-10.46) n=557	0.43 (0.28-0.64) n=25	1.04 (0.80-1.34) n=60	0.23 (0.12-0.38) n=13	0.52 (0.35-0.74) n=30	0.12 (0.05-0.25) n=7	0.42 (0.28-0.62) n=24	0.17 (0.08-0.31) n=10	0.33 (0.21-0.52) n=19
11-15 (n=5061)	9.8 (9.01-10.65) n=493	10.66 (9.62-11.83) n=331	8.28 (7.08-9.59) n=162	5.85 (5.22-6.53) n=296	0.41 (0.26-0.63) n=21	1.13 (0.85-1.46) n=57	0.71 (0.50-0.98) n=36	0.34 (0.20-0.54) n=17	0.26 (0.14-0.44) n=13	0.40 (0.24-0.61) n=20	0.40 (0.24-0.61) n=20	0.26 (0.15-0.44) n=13
16-20 (n=7967)	9.9 (9.26-10.58) n=784	11.29 (10.39-12.28) n=510	7.82 (6.94-8.76) n=274	4.96 (4.51-5.46) n=395	0.59 (0.43-0.78) n=47	0.94 (0.74-1.18) n=75	1.00 (0.80-1.25) n=80	0.30 (0.19-0.45) n=24	0.82 (0.63-1.04) n=65	0.82 (0.63-1.04) n=65	0.23 (0.13-0.36) n=18	0.19 (0.11-0.31) n=15
21-25 (n=15,287)	9.9 (9.44-10.38) n=1508	11.59 (10.95-12.28) n=1133	6.48 (5.86-7.18) n=375	4.91 (4.59-5.26) n=751	0.69 (5.62-8.30) n=105	1.18 (1.01-1.36) n=180	1.24 (1.07-1.42) n=189	0.38 (0.29-0.49) n=58	0.67 (0.55-0.81) n=103	0.52 (0.41-0.65) n=80	0.14 (0.09-0.22) n=22	0.13 (0.07-0.24) n=20
26-30 (n=24,177)	9.9 (9.53-10.28) n=2415	11.64 (11.14-12.16) n=1796	7.08 (6.53-7.63) n=619	4.63 (4.36-4.89) n=1120	0.72 (0.62-0.84) n=175	1.24 (1.11-1.38) n=300	1.50 (1.35-1.66) n=363	0.33 (0.26-0.41) n=79	0.81 (0.70-0.93) n=195	0.46 (0.38-0.55) n=111	0.18 (0.13-0.24) n=44	0.12 (0.05-0.24) n=28
31-35 (n=28,956)	9.3 (8.97-9.64) n=2689	11.48 (10.15-10.95) n=2066	5.68 (5.70-6.58) n=623	3.69 (3.47-3.90) n=1068	0.88 (0.77-0.99) n=254	1.15 (1.02-1.28) n=332	1.33 (1.20-1.47) n=384	0.49 (0.41-0.57) n=141	0.81 (0.71-0.91) n=234	0.31 (0.25-0.38) n=91	0.11 (0.08-0.16) n=33	0.52 (0.40-0.69) n=152
36-40 (n=31,697)	8.9 (8.59-9.22) n=2818	10.55 (10.15-10.95) n=2085	6.15 (5.70-6.58) n=733	3.62 (3.43-3.83) n=1148	0.78 (0.68-0.88) n=246	1.00 (0.89-1.11) n=316	1.57 (1.43-1.71) n=497	0.36 (0.30-0.43) n=114	0.89 (0.79-0.99) n=281	0.27 (0.21-0.33) n=85	0.15 (0.11-0.20) n=49	0.26 (0.17-0.41) n=82
41-45 (n=31,598)	8.5 (8.20-8.81) n=2696	9.89 (9.46-10.35) n=1879	6.48 (6.06-6.94) n=817	3.17 (2.98-3.38) n=1003	0.78 (0.68-0.88) n=246	1.17 (1.05-1.30) n=370	1.56 (1.42-1.70) n=493	0.41 (0.34-0.48) n=128	0.90 (0.79-1.00) n=283	0.25 (0.20-0.30) n=78	0.15 (0.11-0.20) n=47	0.15 (0.08-0.27) n=48
46-50 (n=29,650)	8.5 (8.19-8.82) n=2518	10.08 (9.64-10.54) n=1716	6.35 (5.94-6.80) n=802	2.73 (2.55-2.93) n=810	0.82 (0.72-0.93) n=244	1.11 (0.99-1.23) n=328	1.63 (1.49-1.78) n=484	0.47 (0.40-0.56) n=140	1.01 (0.90-1.12) n=298	0.22 (0.17-0.27) n=64	0.19 (0.14-0.24) n=55	0.32 (0.22-0.48) n=95
51-55 (n=25,065)	7.1 (6.79-7.42) n=1936	9.12 (8.66-9.60) n=1325	5.80 (5.35-6.26) n=611	2.43 (2.25-2.63) n=610	0.59 (0.50-0.70) n=149	0.76 (0.66-0.88) n=191	1.52 (1.36-1.68) n=380	0.32 (0.25-0.39) n=79	0.85 (0.74-0.97) n=214	0.23 (0.17-0.30) n=58	0.17 (0.12-0.23) n=43	0.20 (0.11-0.34) n=49

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Age stratified (yr)	Prevalence % (95% CI)	Males (95% CI)	Females (95% CI)	<i>Giardia intestinalis</i>	<i>Entamoeba histolytica/Entamoeba dispar</i>	<i>Ascaris lumbricoides</i>	Hookworm	<i>Trichuris trichiura</i>	<i>Strongyloides stercoralis</i>	<i>Hymenolepis nana</i>	<i>Enterobius vermicularis</i>	Others
56-60 (n=19,804)	8.2 (7.83-8.59) n=1634	9.69 (9.14-10.21) n=1157	6.06 (5.55-6.63) n=477	2.29 (2.21-2.52) n=454	0.74 (0.63-0.87) n=147	0.76 (0.64-0.89) n=151	1.99 (1.73-2.28) n=395	0.50 (0.41-0.61) n=100	1.41 (1.25-1.58) n=279	0.19 (0.13-0.26) n=37	0.25 (0.18-0.33) n=49	0.11 (0.06-0.20) n=22
>60 (n=27,952)	7.9 (7.59-8.22) n=2214	9.02 (8.62-9.44) n=1658	5.81 (5.35-6.31) n=556	1.86 (1.71-2.03) n=520	0.76 (0.66-0.87) n=213	0.62 (0.53-0.72) n=173	2.61 (2.43-2.80) n=729	0.33 (0.27-0.41) n=93	1.19 (1.06-1.32) n=332	0.13 (0.09-0.18) n=37	0.23 (0.09-0.18) n=63	0.19 (0.11-0.32) n=54
Chi-square test for trend <i>P</i>	<0.001	-	-	<0.001	<0.001	<0.0001	<0.0001	0.605	<0.001	<0.001	0.0745	-

\*Others\* include *Taenia* spp., *Isospora belli*, *Cyclospora cayetanensis*, *Hymenolepis diminuta*, *Blastocystis* spp. and for the age-group 0-5 yr, also *Cryptosporidium* spp; CI, confidence interval

Multi-parasitism or presence of more than one species of intestinal parasite in a single specimen was seen in only 0.06 per cent (164/257,588) of all stool specimens screened and comprised 0.7 per cent (164/22,864) of all positive samples. The most common combination observed was *G. intestinalis* and hookworm (21/164) followed by hookworm and *S. stercoralis* (19/164), *G. intestinalis* and *A. lumbricoides* (16/164) and hookworm and *A. lumbricoides* (15/164). Multiple parasitic infections were rare in children, with only three of all samples from children less than five years yielding more than one intestinal parasite.

*Age-related trends:* Across age groups, the highest prevalence of intestinal parasites was found in the age group of 6-10 yr (13%) whereas the lowest prevalence (7.1%) was in the age group 51-55 yr (Table I). When common parasites, with prevalence more than 0.1 per cent, were analyzed, the proportions differed by age group (Fig. 1). *G. intestinalis* was the dominant parasite across all age groups, except >60 yr, in which hookworm had the highest prevalence (Table I). A significant linear trend was seen for overall prevalence of intestinal parasites across age groups. Among individual parasites, significant age-related trends were observed for *G. intestinalis* ( $P<0.001$ ), *E. histolytica/E. dispar* ( $P<0.001$ ), hookworm ( $P<0.0001$ ), *A. lumbricoides* ( $P<0.0001$ ) and *S. stercoralis* ( $P<0.0001$ ). The prevalence of *G. intestinalis* showed a decreasing trend with age while hookworm and *S. stercoralis* showed an increasing trend with age, with the maximum prevalence for both seen in the >60 yr age group (Table I). However, no age-associated trend was observed for *E. vermicularis* ( $P=0.0745$ ) or *T. trichiura* ( $P=0.605$ ).

*Gender association:* Although >60 per cent of samples were from males, there was a significant difference ( $P<0.001$ ) in the proportion of samples positive for parasites in males (16391/157,583; 10.4%) and females (6473/100,005; 6.5%). The overall prevalence of intestinal parasites in different age groups (Table I) showed significant differences between male and female patients in all age groups ( $P<0.01$ ), except the 0-5 yr age group ( $P=0.593$ ). The difference in the prevalence among females and males was significant for all the common parasites, except *A. lumbricoides* (Table II).

*Temporal trends:* *G. intestinalis* was found in a major proportion of positive samples in all years and the



**Table II.** Association of prevalence of common parasites with gender

Parasite	Total number of positive samples	Males (n=157,583), n (%)	Females (n=100,005), n (%)	$\chi^2$	<i>P</i>
<i>Giardia intestinalis</i>	9036	6591 (4.18)	2445 (2.44)	547.36	<0.001
Hookworm	4055	2857 (1.81)	1198 (1.20)	146.6	<0.001
<i>Ascaris lumbricoides</i>	2565	1585 (1.01)	980 (0.98)	0.527	0.467
<i>Strongyloides stercoralis</i>	2019	1636 (1.04)	383 (0.38)	341.96	<0.001
<i>Entamoeba histolytica/Entamoeba dispar</i>	1873	1293 (0.82)	580 (0.58)	48.5	<0.001
<i>Trichuris trichiura</i>	1015	666 (0.42)	349 (0.35)	7.485	0.006

The value in parentheses is the prevalence of intestinal parasites in percentage. The percentage prevalence is calculated out of the total number of samples from males (n=157,583) and females (n=100,005)

**Table III.** Proportion contributed by common intestinal parasites (2006-2012)

Year	Intestinal parasites						Total positives	Total number of samples
	<i>G. intestinalis</i>	<i>Entamoeba histolytica/Entamoeba dispar</i>	<i>Ascaris lumbricoides</i>	Hookworm	<i>Strongyloides stercoralis</i>	<i>Trichuris trichiura</i>		
2006	1219 (36.2)	178 (5.3)	624 (18.5)	667 (19.8)	352 (10.4)	136 (4.0)	3369	36,023
2007	1287 (38.1)	185 (5.5)	601 (17.8)	640 (18.97)	322 (9.5)	159 (4.7)	3373	36,556
2008	1071 (40.5)	223 (8.4)	327 (12.4)	456 (17.3)	271 (10.3)	127 (4.8)	2643	30,010
2009	1104 (38.7)	249 (8.7)	309 (10.8)	493 (17.3)	283 (9.9)	205 (7.2)	2851	30,139
2010	1559 (47.3)	263 (7.97)	269 (8.15)	544 (16.5)	366 (11.1)	112 (3.4)	3299	39,519
2011	1324 (41.4)	312 (9.7)	236 (7.4)	603 (18.8)	387 (12.8)	141 (4.4)	3201	37,916
2012	1602 (42.5)	475 (12.6)	244 (6.47)	675 (17.9)	382 (10.12)	135 (3.58)	3771	47,425
Chi-square test for trend ( <i>P</i> )	51.320 (<0.001)	151.29 (<0.001)	447.98 (<0.001)	3.929 (0.047)	2.674 (0.102)	3.612 (0.057)	-	-

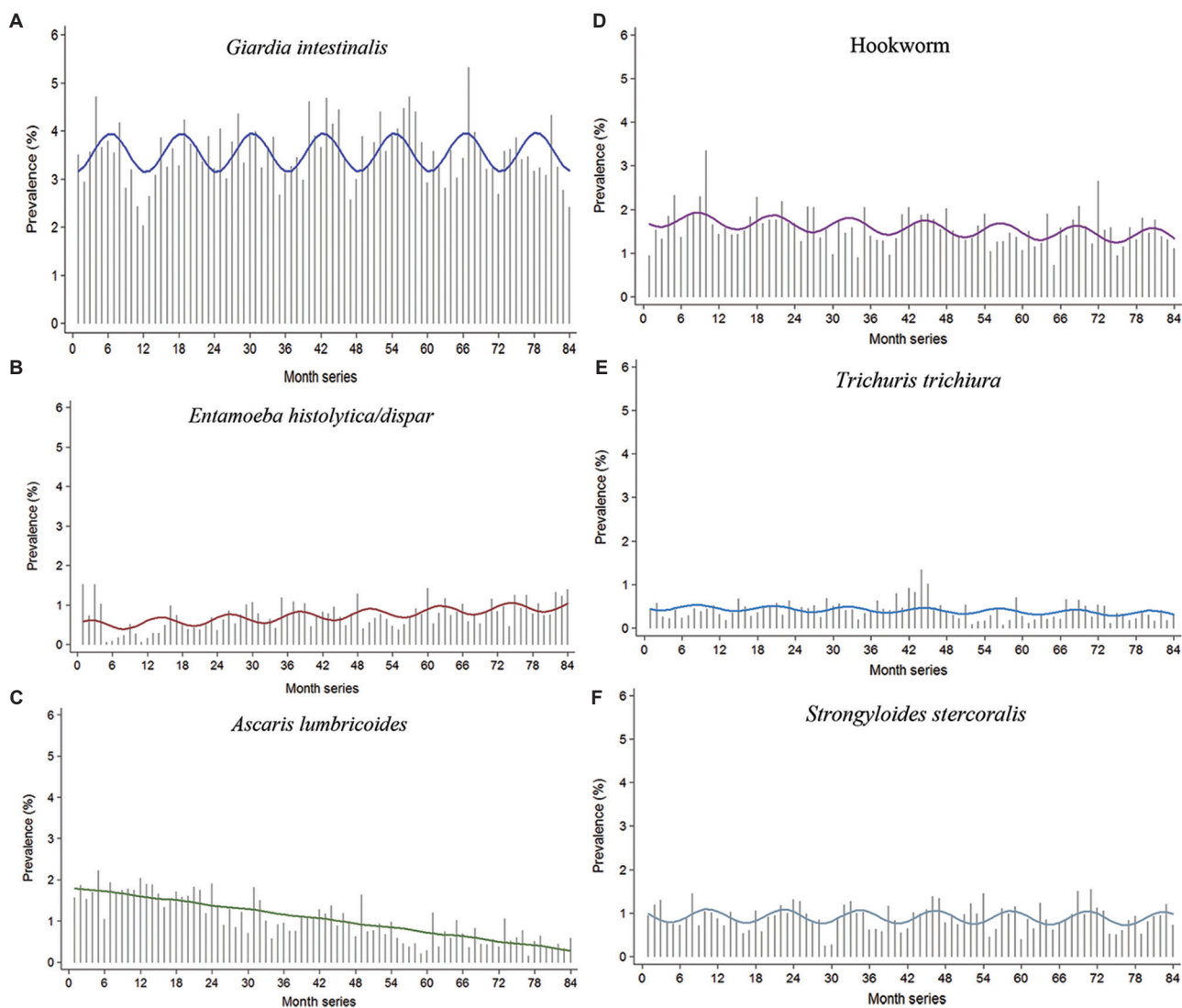
The value in parentheses for each of the years is the proportion of positive samples contributed by the particular parasite for that year expressed as percentage

proportion contributed by *G. intestinalis* showed a linear increasing trend over the years (Table III). The proportion contributed by *E. histolytica/E. dispar* also increased over time, whereas that contributed by *A. lumbricoides* declined. However, no significant linear trends were seen for the proportion contributed by *S. stercoralis* or *T. trichiura*.

Fig. 2 shows the monthly time series and annual regression analysis models for the six commonest intestinal parasites in relation to the actual monthly time series. Significant seasonality was seen for all the common intestinal parasites, except *A. lumbricoides*. A significant cosine function ( $P<0.001$ ) was seen for *G. intestinalis* with a peak in June. Using the same model, a significant decreasing monthly trend was seen for *A. lumbricoides* ( $P<0.001$ ) and hookworm ( $P=0.004$ ), whereas an increasing trend was observed for *E. histolytica/E. dispar* ( $P<0.001$ ).

## Discussion

The overall prevalence of intestinal parasitic infections over the seven year period (2006-2012) was 8.9 per cent. This was lower than reports from the Indian subcontinent and elsewhere in the developing world<sup>5,13</sup>. This difference could be because of the setting, where many patients might already have been treated in the community before the collection of stool samples. In a similar hospital-based study of intestinal parasites from Rome, Italy, Masucci *et al*<sup>14</sup> reported a total of 11.1 per cent of the total samples screened to be positive for intestinal parasites. Most studies from developing country settings have reported the prevalence of intestinal parasites from community settings and show wide variation. The reasons for such variation, apart from the actual difference in prevalence, could be because of different sampling methods employed, difference in the number of



**Fig. 2.** Annual harmonic regression models for intestinal parasites versus the actual time series. The gray vertical spikes depict observed prevalence of the specific pathogens; the coloured horizontal lines depict the predicted prevalence obtained from the Poisson harmonic regression model.

samples examined per patient, inclusion or exclusion of concentration techniques in the workup of samples.

In our study, the prevalence of intestinal parasites varied across age groups with the highest prevalence of 13 per cent seen in the age group 6-10 yr. Pre-school and school-going children have been found to be at the greatest risk for intestinal parasitic infections in some studies<sup>7</sup>, but others have reported higher prevalence in older age groups<sup>15</sup>.

The most common intestinal parasite among the patients screened was *G. intestinalis*. The prevalence of *Giardia* infection was higher in the younger age groups and it showed a decreasing trend with age. Data from

the Global Enteric Multi-Center Study indicate that *Giardia* occurs commonly in asymptomatic infection and may even play a role in protection from diarrhea<sup>16</sup>.

In our study, the highest prevalence of hookworm and *Strongyloides* infection was found in the older population (>60 yr) and a significant correlation was found between older age and infection with these parasites. Similar trends have been reported in other studies<sup>16</sup>, and the intensity of hookworm infection has also been found to be higher in adults compared to children in some other parts of the world where hookworm disease is endemic<sup>17</sup>. This association between increased prevalence of hookworm

infection and the elderly population in a region or setting might have important implications for the public health policies in countries/regions where such trends are seen, as efforts at control of STHs are usually focussed on the paediatric population<sup>17</sup>. Unlike hookworm and *Strongyloides*, there was no particular age-associated trend observed for *Trichuris* or *Ascaris*. The maximum intensity of infection with *A. lumbricoides* and *T. trichiura* was shown to be at about the age of 5-10 yr<sup>3</sup>. In our study, the highest prevalence of *T. trichiura* was in the age group 6-10 yr while that for *A. lumbricoides* was in the 26-30 yr age group, although the prevalence rates for *A. lumbricoides* were not significantly higher than that in younger age groups. Most studies in developing country settings have shown higher prevalence of *Ascaris* infections among children compared to older individuals<sup>18</sup>. However, some studies have reported higher prevalence in older age group when low-intensity infections were considered<sup>19</sup>. Similar trends of increasing hookworm infection prevalence with increasing age have been reported by Kaliappan *et al*<sup>20</sup> from a tribal area in southern India. However, these relationships between age and prevalence of particular intestinal parasites, especially helminths, vary in different geographical regions and susceptibility to any helminthic infection, in general, and intestinal helminthic infections, in particular, cannot be considered as a function of age alone.

In our study, a significantly higher proportion of samples from male patients was found to be positive compared to females (10.4 vs. 6.5%). Although males have generally been found to be more predisposed to be positive for intestinal parasites<sup>21</sup>, the differences observed in the prevalence rates of these infections have not always been significant<sup>22</sup>. This difference in the prevalence and intensity of parasite infections has been attributed to increased exposure to certain parasites in males because of occupation and activity and also hormonal and immunological mechanisms such as the immunomodulatory effects of testosterone in males which increases their susceptibility to certain parasitic infections<sup>23</sup>. It has been established that females generally exhibit higher levels of immune responses as compared to males<sup>24</sup>.

A limitation of this retrospective analysis was the inability to distinguish between cysts of *E. histolytica* and *E. dispar*, both of which look similar on microscopy. The WHO has recommended<sup>25</sup> the use of methods such as antigen detection and different molecular tools

such as nested multiplex polymerase chain reaction (PCR) or real-time PCR for the specific diagnosis of *E. histolytica*.

The prevailing opinion that *Blastocystis* spp. are non-pathogenic has undergone a change with studies showing an association with this parasite and clinical disease<sup>26</sup>. The prevalence of *Blastocystis* spp. in our study was low, but in the majority of the *Blastocystis*-positive samples, no other intestinal parasite was concurrently encountered. We were unable to evaluate the association of *Blastocystis* spp. with clinical disease as enough clinical details were not available to study association.

Multiple intestinal parasitic infections were not common in this study. In a study from Lao People's Democratic Republic, 86.6 per cent of all study participants were found to harbour two or more different intestinal parasites concurrently<sup>27</sup>. Mehraj *et al*<sup>28</sup> in a study from Karachi reported 10 per cent of all children screened for intestinal parasites had two or more than two intestinal parasites. Application of different diagnostic techniques and screening of multiple samples may be required to estimate the true burden of intestinal multiparasitism<sup>5</sup>.

Our study also looked at whether there was any seasonality in the prevalence of different intestinal parasites. For STH infections such as hookworm, *Strongyloides* and *T. trichiura*, the peak months of prevalence were during September and October, while for *Giardia* species, the peak was in June. These peaks corresponded to the months of high humidity levels and rainfall in our setting. Although our study was from a hospital setting, this seasonality was likely also a reflection of the picture in the community. For many intestinal parasites, variation in temperature, humidity and rainfall seems to affect the intensity of infections. This has especially been seen for parasites where there are external larval stages which develop in the soil<sup>29</sup>.

Temporal trend of decreasing STH infection over the seven year period could be a reflection of the same in the community because of the mass administration of albendazole and ivermectin as part of lymphatic filariasis control programmes in India for the past many years. The mass drug administration programme was introduced by the Government of India in 2004 and the population coverage for this programme has improved from 73 per cent in 2004 to 83 per cent in 2013<sup>30</sup>. A study from Tamil Nadu by Kattula *et al*<sup>31</sup> done in community settings among school children also reported much lower rates of



STHs compared to previous reports from the area.

The major strength of this study was the long duration coverage and the number of samples analyzed. The limitations of this study included the lack of clinical correlation and the lack of complete testing on all samples, including concentration techniques. Since modified acid fast staining for intestinal coccidian parasites was performed only for samples from children less than five years of age, the actual burden of infection with these parasites could not be correctly estimated. The effect of antiparasitic treatment on the estimation of parasitic infections was also not taken into account as the information was not available for all the patients included in the analysis. Further, since this was a study from a tertiary care centre and a major proportion of patients screened for intestinal parasites were referred from other hospitals and healthcare centres, referral bias could be possible.

In conclusion, our study showed certain age-related trends such as higher prevalence of hookworm and *Strongyloides* in elderly age groups. Significant seasonality and overall decreasing temporal trends were seen for STHs such as hookworm and *A. lumbricoides*. Some of these findings may have public health implications.

**Conflicts of Interest:** None.

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