



“Freezing” parasites in pre-Himalayan region, Himachal Pradesh: Experience with mini-FLOTAC



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ABSTRACT

Background: Helminths and protozoa infections pose a great burden especially in developing countries, due to morbidity caused both by acute and chronic infections. Data on distribution of intestinal parasitic infections among the native and expatriates populations in Himachal Pradesh are scarce. The aim of our survey was to analyze the intestinal parasitic burden in communities from Dharamsala, Kangra district, in clinical and public health settings. We also field-tested the mini-FLOTAC, an innovative diagnostic device.

Methods: Subjects referring to the Tibetan Delek Hospital for abdominal discomfort and all children of the Tibetan Primary School in Dharamsala were screened for intestinal parasitic infections with direct smear, formol-ether concentration (FEC) method and mini-FLOTAC, their clinical history was recorded, and correlations between clinical symptoms and infections analyzed.

Results: 152 subjects were screened for intestinal parasites, of which 72 subjects in the outpatients department (OPD) (36 expatriates and 36 natives) and 80 in the school. 60% of schoolchildren and 57% of OPD patients were found positive for any infection, the most represented were protozoa infections (50%), whereas helminthic infections accounted only for 13% and 20% in OPD patients and schoolchildren, respectively. The most prevalent among helminths was *Ascaris lumbricoides* (11%). *Giardia intestinalis* was more present among schoolchildren than the OPD patients (20% vs 6%) and *E. histolytica/dispar* was more prevalent among the OPD patients (42%) than the school children (23%). Correlations were found between nausea and loose or watery stools and parasitic infections, particularly in expatriates, whereas schoolchildren, despite being as infected as adults, were completely asymptomatic. Mini-FLOTAC detected higher number of helminth infections whereas FEC method was more accurate for the diagnosis of protozoa.

Conclusions: This study presents an accurate snapshot of intestinal parasitic infections in Dharamsala, and their high prevalence calls for more awareness and control measures. Mini-FLOTAC is a promising and simple technique for the diagnosis of helminth infections.

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1. Introduction

Soil-transmitted helminths (STH) (*Ascaris lumbricoides*, *Trichuris trichiura*, *Ancylostoma duodenale*/*Necator americanus*) together with intestinal protozoa (*Giardia intestinalis*, *Entamoeba histolytica/dispar*) are widely represented in developing countries and their transmission is usually linked to favourable humid-warm environmental conditions and sustained by lack of sanitary facilities and poverty. Acute infections are especially symptomatic in travellers, such as tourists, business people but also immigrants and

expatriates (Ansart et al., 2007; Fryauff et al., 1999) compared to the indigenous population in which the greater burden is represented by chronic infections, that can impair growth and mental development of children, and adult productivity (Hotez et al., 2006; Savioli et al., 2006).

Although not many data are available on intestinal parasitic infections in North India, it is widely acknowledged that STH and intestinal protozoa are responsible for almost half of diarrhoea and abdominal symptoms and the most represented infections are due to *A. lumbricoides* and *G. intestinalis*. (Kaur et al., 2002; Wani et al., 2007; Nitin et al., 2007; Baba et al., 2009). Albeit most of the intestinal parasites are spread all over the country, surveys conducted across the Indian subcontinent demonstrated that the distribution of intestinal parasites differs, for instance hookworms

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are present in the South (Ragunathan et al., 2010), though not everywhere (Rayan et al., 2010) but almost absent in central India (Dambhare et al., 2010) or in the North (Wani et al., 2010). In India, as well as in other endemic countries, helminth control is now under the umbrella of the neglected tropical disease (NTD) control programmes and deworming programmes are carried out in many schools together with health education (Ananthakrishnana and Das, 2001; Awasthi et al., 2008); however, STH control in India is very much driven by Lymphatic Filariasis Elimination Programme which uses albendazole in co-administration with diethylcarbamazine citrate (Padmasiri et al., 2006; Rajendran et al., 2003; Raju et al., 2010).

The aim of our survey, within a project for transferring knowledge and building capacity to improve the diagnostic facilities of the Tibetan Delek hospital, was to analyze the intestinal parasitic burden in communities from Dharamsala, together with a comparison in terms of rate of infection and clinical symptoms, between indigenous population and expatriates. This survey was also an opportunity to field-test the mini-FLOTAC, an innovative device for the diagnosis of intestinal parasitic infections.

2. Materials and methods

2.1. Study site and population

Dharamsala is a small town in Himachal Pradesh, North India, of almost 20,000 inhabitants (census 2001), in Kangra district at an altitude of 1750 m. The climate of the region is monsoon-influenced: summer starts in early April till mid-June (max $T=36^{\circ}\text{C}$), the monsoon season follows from July to mid-September with heavy rainfall, autumn is mild and lasts from October to the end of November (average $T=16\text{--}17^{\circ}\text{C}$). Winter starts in December and continues until late February, snow and frost are common during this season which is followed by a short, pleasant spring until April. Our parasitological survey was conducted in the first quarter of 2012 during winter and spring and temperatures registered a minimum of -5°C to maximum of 25°C , rainfall and snow were experienced during the first two months of our study.

As it is the home town of the Dalai Lama, Dharamsala represents a popular destination of pilgrims, tourists and many expatriates that embrace the Buddhism.

The Tibetan Delek Hospital was founded in 1971 to provide health care to the Tibetan and local Indian communities in Dharamsala. The Italian Association for solidarity among people (AISPO) is an NGO that has recently supported the Delek hospital with special focus on a TB programme (2009–2012). Our project was conducted within the AISPO support in order to introduce a new diagnostic method (mini-FLOTAC) and conduct a survey on intestinal parasites in this area, as direct smear on a single faecal sample was the only technique used in the hospital laboratory.

We analyzed faecal samples of patients who visited the outpatient department (OPD), for the presence of intestinal helminths and protozoa and examined also all children from the nearby Tibetan primary and secondary school, a college where they live and study.

2.2. Sample size and randomization

A cross-sectional survey was conducted between January and April 2012 in the study area described above. A sample size of at least 50 schoolchildren, was selected, based on the WHO recommendation that such number should represent a homogeneous ecological zone (WHO, 2011). For ethical reasons we selected all children attending the Tibetan school (80 children). In the study period, approximately the same number of patients attending the

outpatients clinic of the Tibetan Delek hospital for any abdominal symptom were also recruited in the study. No randomization was necessary as all children in the Tibetan school and all patients attending the outpatients clinic for gastro-intestinal complaints in the study period were enrolled in the parasitological survey.

2.3. Interview and questionnaire

All subjects were interviewed before stool collection and a questionnaire was filled to collect information about age, gender, housing and associated symptoms (nausea, abdominal pain, diarrhoea, vomit, abdominal bloating), previous deworming treatment, and stool discharge. The questionnaire was pre-tested in a small sample and translated in local language. A local health worker was trained to assist in the administration of the questionnaire.

2.4. Parasitological methods

All stool samples were collected and analyzed within 24 h with three different techniques: direct smear, formol-ether concentration (FEC) method and mini-FLOTAC. Prevalence of infections was calculated as any positive subject by any of the three methods over the total samples examined.

FEC method and the direct smear were carried out according to WHO guidelines (WHO, 1994).

Mini-FLOTAC is an evolution of FLOTAC techniques (Cringoli et al., 2010), and conceived in order to be performed in limited resources laboratories, so neither centrifugation nor expensive equipment is needed. It comprises two components, namely the base and the reading disc. The device includes two 1 ml flotation chambers, which are designed for optimal examination of faecal sample suspensions (total volume = 2 ml) and which permit a maximum magnification of $\times 400$. The stools were processed as follows for the mini-FLOTAC basic technique (analytic sensitivity = 10 eggs/larvae/cysts per gram of faeces). Eight grams of stool were placed in the fill-FLOTAC, a plastic device part of the kit which facilitates filtration, dilution and homogenization of the sample, suspended with 8 ml of formalin 5%, and thoroughly homogenized and filtered. Two millilitres of the suspension (1 gr of stool + 1 ml of formalin) were directly added to 18 ml of each of the two flotation solutions, namely FS2 (saturated sodium chloride; density = 1.20) and FS7 (zinc sulphate; density = 1.35). The flotation solutions are the same described in the FLOTAC protocols (Cringoli et al., 2010). Two mini-FLOTACs were performed for each sample, one filled with the faecal suspension in FS2 and the other with the faecal suspension in FS7. Before reading the slide and translating the reading disc, 10 min were needed for the eggs, larvae and (oo)cysts to float. Eggs, larvae and cysts of intestinal parasites were detected and read within the grid.

2.5. Statistical analysis

Data were entered into an Excel spread sheet and validated through double checking. Statistical analyses were performed using SPSS 13 software for Windows (SPSS Inc. Headquarters, Chicago, IL, USA). The associations between clinical symptoms and intestinal parasitic infections were analyzed by univariate (Pearson's chi-squared test for independence) statistical analysis. This analysis was performed for all subjects and for subjects divided in three different groups (OPD patients, both expatriates and indigenous, and schoolchildren).

The level of significance was set at a p value of <0.05 and the confidence interval (CI) was calculated at a level of 95%.

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