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Prevalence and Epidemiology of Gastrointestinal parasitosis among the people of Dehradun, Uttarakhand

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ABSTRACT

Gastrointestinal parasites play crucial roles in mortality and morbidity. They are more prevalent in rural areas than the urban areas. Parasitosis affects all age groups of people but in children, it causes slow mental and physical growth, produces long-term effects, leads to deficiency of vital nutrients, and hampers growth and development. This prospective study aimed to assess the prevalence of gastrointestinal parasitosis in stool samples among the people of Uttarakhand. To identify intestinal parasites, 1528 samples were examined for consistency and the presence of any parasitic particles using the visual, direct wet mount, and concentration methods. The SPSS 21 was used to conduct statistical analysis. Differences with a p-value of less than 0.05 were considered significant. The overall prevalence of intestinal parasitic infection rate was 32.85%. The prevalence of the parasites was in the following order - Giardia lamblia (29.48%), Entamoeba histolytica (20.91%), Ascaris lumbricoides (18.52%), Hymenolepis nana (7.96%), Trichuris trichiura (7.56%), Taenia sp. (7.17%),Strongyloidesstercoralis (5.97%), Ancylostoma duodenale (2.19%), and Enterobius vermicularis (0.19%).

The chi-square test ($\chi 2$ – 30 and P-value = 0.224) was found to be insignificant while Levene's test was significant (F = 10.08, P-value = 0.008) of A. duodenale in both genders. Parasitosis of high levels was detected among the children. Health education, personal hygiene, and safe drinking water would reduce infection. Designing specific control measures requires proper identification. The present study may prove helpful for developing such measures for Uttarakhand in general and Dehradun in particular.

Keywords: Prevalence, Enteroparasitosis, Epidemiology, Ascaris lumbricoids, Dehradun, Hookworm

1. Introduction

There are diverse and alarming effects of intestinal parasitic infections worldwide. Parasitic infections in the intestine have harmful effects on the endurance, hunger, growth and development, physical health, school attendance, and cognitive capability of school-going children.[1] Gastrointestinal parasites cause mortality and morbidity, particularly in children. Diseases caused by intestinal parasites are an excellent indicator of the quality of life in a socioeconomical area. According to the World Health Organization (WHO) estimates, 870 million children show high prevalence. Africa, Asia, and South America are the most affected. More than 173 million people in developing countries, primarily in Africa, have *A. lumbricoides* infections, whereas 198 million and 162 million people, respectively, have hookworm and *T. trichiura* infections. In Ethiopia, 25.3 million of the 81 million residents who live in endemic regions are school-attending children.[2]

India alone contributes nearly 25% globally. Intestinal parasitic diseases affect preschool and school-going children.[3,4] They cause slow physical and mental growth and affect overall achievement. They may lead to deficiencies of iron, protein, and other vital nutrients, and thus hamper growth. Some studies showed that children harboring heavy populations of gastrointestinal parasites lose an average of 3.75 IQ points for each gastrointestinal parasitic infection.[5] Other associated morbidities include anemia, chronic dyspepsia, diarrhea, intestinal bleeding, loss of appetite, malabsorption of nutrients, protein-calorie malnutrition, vitamin A deficiency, vomiting etc.[6] Conditions that may need surgical treatment include intestinal obstruction or rectal prolapse. Parasitic infections may complicate pregnancies and birth.[7-8] Serious anemia from blood and gastrointestinal parasitic infections in pregnant women can endanger the lives of both the mother and the fetus. Intestinal parasites can greatly reduce nutrients received from the mother leading to low birth weight.[6-10]

In India, as in other developing nations, intestinal parasite infections constitute a significant public health issue. *A. lumbricoides* was the most prevalent helminthic parasite detected (0.65%, 6.58%), followed by *Giardia lamblia* (10.7%, 14.3%, 37%), *H. nana* (1.51%, 2.9%, 3.77%), and other parasites, according to studies conducted on children and old age groups in rural and urban locations in and around Dehradun. Epidemiological information on the presence and spread of intestinal parasites among low socioeconomic area residents is insufficient.[11-15] This pattern motivated us to examine the prevalence of intestinal parasites among residents in and around Dehradun according to their age, sex, diet, health, and hygiene practices.

The motive of the study was to investigate the current status of the prevalence of gastrointestinal parasites in all age groups in Uttarakhand in general and Dehradun and its adjoining areas in particular.

2. Material and Methods

2.1. Study area: This study was conducted in six main socioeconomic areas in Uttarakhand such as Dehradun, Raipur, Mussoorie, Premnagar, Sahaspur, and Vikashnagar regions (Figure 1). The samples were taken from the homes of the participants as well as from the health care centers.

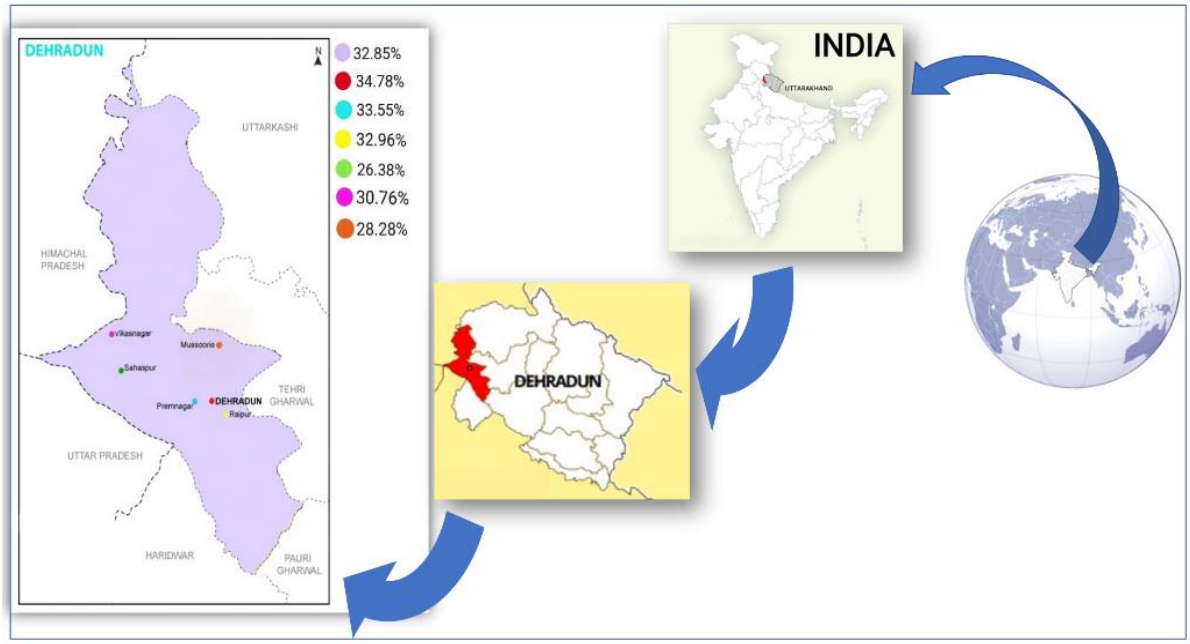

Figure 1: Map of Uttarakhand showing the areas where the study was conducted.

2.2. Study Design: The local community having the information for every resident in the area, cooperated in the study, which was carried out between January 2017 and January 2018. House to house visits were a feature of the fieldwork, which promoted engagement from each

person. Each participant provided verbal informed permission before the trial. Information on name, sex, age, education, and family ties was gathered. Besides collecting fresh stool samples, the subjects were questioned about their socioeconomic situation, health, access to toilets and clean water, the nutritional status of their children, available local therapies, and prior parasite infections.

2.3. Collection of Samples: For the detection and diagnosis of intestinal parasites, proper sample collection is crucial. Each volunteer who accepted to take part in the study received a tiny plastic bottle with a screw cap and a wooden scoop. They were instructed to fill the bottle halfway and to properly dispose the scoop after use. Samples were collected and brought to the laboratory for processing the following day. Each container had a sample that was appropriately labelled with the appropriate sample number, date, and location. A total of 1528 samples were collected, with samples coming from the age groups of 0–10 years, 10–20 years, 20–30 years, 30–40 years, and 40–50 years. Of those, 51.59% of the samples came from men and 48.41% from women. Before collecting faeces, the standard safety procedures were followed. Consentees were instructed to avoid combining stool samples with urine and to avoid administering oil, greasy emulsions, barium, or bismuth salts prior to stool analysis.

2.4. Preservation of Samples: After the samples were transferred to the laboratory, various staining procedures, including saline and iodine wet mount, were carried out. The remaining material underwent concentration procedures like sedimentation and flotation while being stored in 10% formalin. Faecal specimen preservation is necessary to preserve protozoal morphology and to stop the growth of helminthic eggs and larvae.Microscopic Examination: Binocular microscope observations under 10x and observations under 40x were used to confirm the identification of intestinal parasites.[15]

2.5. Saline and Iodine Wet Mount: On a glass slide, with an applicator stick, 2 mg of faeces sample was collected and combined with a drop of normal saline (0.9%). Materials were removed from well inside the sample to look for parasite eggs if it was a formed stool. A cover slip was placed over the preparation, which was then examined under a microscope. Using a wooden stick, 2 mg of stool sample was collected for the fabrication of the iodine wet mount. A drop of diluted Lugol's iodine was then added. It was examined under a microscope while being protected by a coverslip.[14-15]

2.6. Modified Ziehl-Neelsen Stain: 5-7 drops of carbol fuchsin were flooded for 2-3 minutes after the smear on the slide had been fixed with methanol for 10 minutes. It was then treated for 30 seconds with 5% sulfuric acid to remove the colour. The smear was then counter-

stained for a minute with methylene blue. The smear was then rinsed, drained, air-dried, and examined at 10x, 40x, and with oil immersion (100x).[15]

2.7. Floatation Techniques:In order to prepare a suspension, 1 ml of faeces sample was combined with a few drops of salt solution. The container was filled with more salt solution. The floated debris that was unclean was eliminated. The glass container was filled to the top until a convex meniscus developed, after which it was set on a level surface. Carefully, a glass slide was placed on the top of the container in such a way that the fluid just touched its center. After allowing the preparation to stand for 20–30 minutes, the glass slide was swiftly raised, smoothly turned over to prevent liquid spilling, and viewed under a microscope.[14]

2.8. Zinc Sulphate Centrifugal Floatation*:* 1gm of faeces was combined with ten 10ml of lukewarm, distilled water to make a fine stool suspension. Through a wire gauge, the large particles were strained out. After being collected in a tube, the filtrate was centrifuged for one minute at a speed of 2500 rpm. After draining the supernatant liquid, the sediment received distilled water. When the supernatant fluid turned clear, it was drained out after being centrifuged and shook well two or three times. 33% zinc sulphate was added to the sediment in amounts of 3–4ml. The sediment was stirred, and then zinc sulphate solution was added to fill the tube up to the top and centrifuged again for at least 1 min at 2500 rpm. The surface film was then removed by a loop on to a glass slide, covered by a cover slip, and observed under the microscope.[14-15]

2.9. Formal-Ether Concentration: 1gm of stool was used to emulsify 7ml of 10% formalin for ten minutes. The filtrate was then collected in a centrifuge tube after being strained through a wire gauge. It was mixed with 3 ml of ether and briskly shaken for one minute. It was then centrifuged for 2 minutes at 2000 rpm before being allowed to settle. A stick was used to loosen the debris; fatty debris was then removed from the test tube's upper portion, and the liquid supernatant was decanted, leaving one or two drops behind. After shaking, the deposit was collected onto a glass slide, gently covered with a cover slip, and examined under a microscope. This process was suitable for both protozoal cysts and helminths eggs.[16]

2.10. Analysis of results: The results were analyzed by Statistical Software - IBM SPSS Statistics V21.0 and cross-checked by STATA software. The chi-square test was used to compare a group with value and to assess the significant difference. T-test was used to determine significant differences between the means of two variances or groups. F is the test statistic of Levene's test. Differences and associations were considered statistically significant at P values < 0.05.

3. **Results and Discussion**

For the present analysis of parasitic infection, a total of 1528 sampleswascollected. Of these, 502 (32.85%) samples were positive. Of the positive, 51.59% were males and 48.41% were females. The percentage of infections was in the following order - Dehradun (34.78%), Prem Nagar (33.55%), Raipur (32.96%), Vikashnagar (30.76%), Mussoorie (28.28%), and Sahaspur (26.38%). The parasites were *A. duodenale* (2.19%), *A. lumbricoides* (18.52%), *E. histolytica* (20.91%), *G. lamblia* (29.48%), *H. nana* (7.96%), *S. stercoralis* (5.97%), *T. trichiura* (7.56%), *Taenia* sp. (7.17%) and *E. vermicularis* (0.19%) (Figure 3,8 and Table 2).

The highest prevalence was of *G. lamblia* in the age group of 0-10 years and the lowest was of *T. trichiura*. No statistical significance was observed in both males and females ($\chi 2 = 42$, 40 and P = 0.227, 0.225 respectively). Further distribution and prevalence of parasitic infection in different age groups are mentioned in Table1.

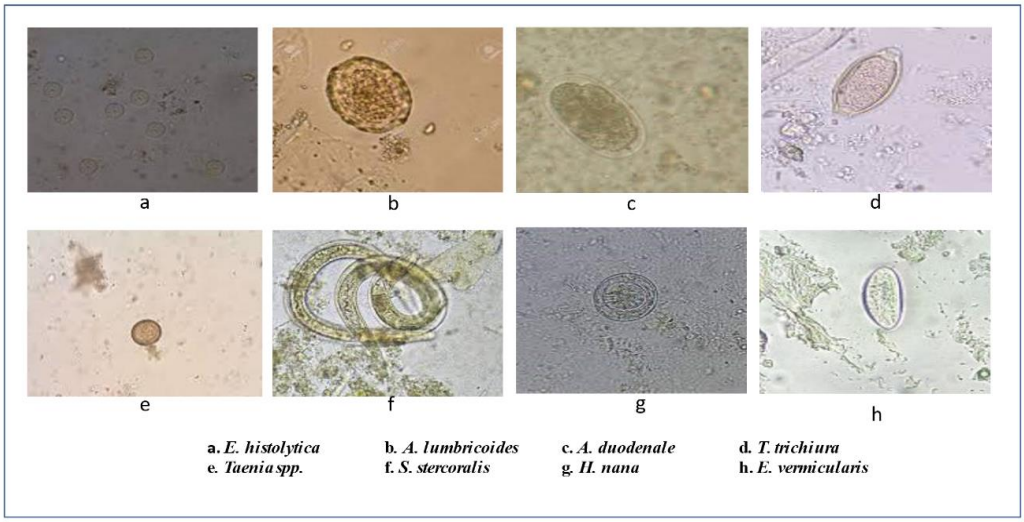

Figure 2:Parasites isolated from stool samples. ***(a) E. histolytica (b) A lumbricoides*** **- decorticatedegg,** ***(c) A. duodanale, (d) T trichiura (e) Taenia sp. (c) S. stercolaris (g) H. nana, (h) E. vermicularis***

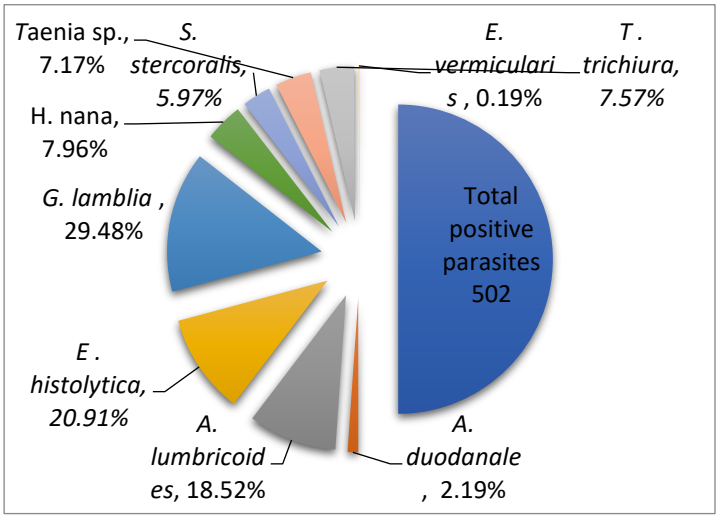

Figure 3: Distribution of common gastrointestinal parasites

The highest prevalence was of *G. lamblia* in the age group of 0-10 years and the lowest was of *T. trichiura*. No statistical significance was observed in both males and females (χ^2 = 42, 40 and *P* = 0.227, 0.225 respectively). *G. lamblia* infection was the highest (9.68%: 4.58% males, 5.10% females) followed by *E. histolytica* (6.87%: 3.46% males, 3.40% females). *A. lumbricoides* followed with 6.08% infections (3.07% males, 3.01% females). The rest followed the order - *H. nana* (2.61%: 1.04% males, 1.57% females), *T. trichiura* (2.48%: 1.24% each males and females), *S. stercoralis* (1.96%: 0.78% males, 1.17% females, *Taenia* sp. (2.35%: 2.15% males, 0.19% females), *A. duodenale* (0.71%: 0.52%, 0.19% females) and *E. vermicularis* infected only one male (Figure 7, Table 1).The highest occurrence of *A. duodenale* in age groups 31-40 years was 2 (1.72%) in males and 2 (0.84%) in females in 11-20 years group. The Levene's test was significant (F = 10.08, p-value = 0.008) in both sexes.

In males, the distribution of *A. lumbricoides* was highest in 41-50 years age group (8 or 11.11%), and in females, it was in 11-20 years group (19 or 8.02%), and the lowest in males was in 51-60 years group (1 or 2.50%) and in females, it was observed in 41-50 years (1 or 11.46%). Levene's test was not significant.

The highest distribution of *E. histolytica* in males was in the age group of>60 years (15.79%) and the lowest was found in the 21-30 years group (2.69%). In females, the highest distribution was in the age group of 41-50 years (11.43%), and the lowest was in 0-10 years group (2.91%). Levene's test was insignificant.

The highest distribution of *G. lamblia* in males was in 0-10 years group (13.68%) and the lowest was in 51-60 years group (1.72%) and in females, the highest and lowest were in the 0-10 years (13.59%), and 51-60 years group (2.94%) respectively. Levene's test was not significant.

The highest distribution of *H. nana* in both males (5.56%) and females (11.43%) was in the same age group of 41-50 years. However, the lowest distribution in males (1.72%) was in the age group of 31-40 years and in females (1.94%), it was in the 0-10 years. Levene's test was significant (F = 8.403, p-value = 0.013) (Table 1).

Age		Male		Female		Levene's Test	
		Positive	**Prev. (%)**	**Positive**	**Prev. (%)**	**F**	**p- value**
0--10		2	1.37	2	1.94	8.403	0.013
11--20		2	0.94	8	3.38		
21--30		4	2.15	10	5.05		
31--40		2	1.72	0	0.00		
41--50		4	5.56	4	11.43		
51--60		2	5.00	0	0.00		
>61		0	0.00	0	0.00		

Table 1: Age and sex-wise prevalence of *H. nana*

The distribution of *T. trichiura* was highest in females (21-30 years) and it was lowest (0.84%) in the 31-40 years group. In males, the highest distribution (4.30%) was in the 21-30 years group and the lowest (0.86%) was in the 0-10 years group. Levene's test was insignificant.

The highest distribution of *Taenia* sp. both in females (5.71%) and in males (10%) was found in the 41-50 years group (Figure 7).

The lowest distribution in females (0.84%) was in the group of 31-40 years while in males, it (0.68%) was in the group of 0-10 years. Levene's test was found significant (F = 13.221 and p-value = 0.003) (Figure 7).

Only one male (0.68%) in the 0-10 years group was infected with *E. vermicularis*. It wasn't recorded in females. Levene's test was significant (F = 5.76, p-value = 0.034) (Figure 7).

The prevalence of positive samples was as follows -January (1.11%), February (0.79%), March (2.62%), April (3.40%), May (4.97%), June (9.62%), July (0.20%), August (2.62%), September (0.65%), October (1.18%), November (4.71%) and December (0.98%) (Figure 3). Difference in the pattern of monthly prevalence was statistically insignificant (X2 = 120 and p-value = 0.242) (Figure 4).

Areas	**Total sample**	**Total posi. case**	***A. duodenale***		***A. lumbricoides***		***E. histolytica***		***G. lamblia***		***H. nana***		***S. stercoralis***		***T. trichiura***		***Taenia* sp**		***E. vermicularis***		**t value**	**p value**
Uttarakhand	1528	502	positive	Prev (%)	positive	Prev (%)	positive	Prev (%)	positive	Prev (%)	positive	Prev (%)	positive	Prev (%)	positive	Prev (%)	positive	Prev (%)	Positive	Prev (%)		
Dehradun	759	264	6	0.79	49	18.56	52	19.7	81	30.68	22	8.33	16	6.06	19	7.2	18	6 .82	1	0.38	0.989	0.413
Mussoorie	99	28	0	0	3	10.71	6	21.43	6	21.43	1	3.57	4	14.29	4	14.29	4	14.29	0	0	1.011	0.386
Prem Nagar	149	50	1	0.67	11	22	12	24	13	26	3	6	3	6	4	8	3	6	0	0	0.897	0.436
Raipur	273	90	2	0.73	14	15.56	21	23.33	25	27.78	7	7.78	6	6.67	8	8.89	7	7.78	0	0	0.861	0.418
Sahaspur	144	38	1	0.69	7	18.42	9	23.68	13	34.21	3	7.89	1	2.63	2	5.26	2	5.26	0	0	1.011	0.386
Vikash Nagar	104	32	1	0.96	9	28.13	5	15.63	10	31.25	4	12.5	0	0	1	3.13	2	6.25	0	0	1	0.391

Table2: Prevalence of gastrointestinal parasites in different areas of Uttarakhand

Seasons	**Total samples**	**Posi. samples**	***A. lumbricoides***		***E. histolytica***		***G. lamblia***		***H. nana***		***T. trichiura***		***S. stercoralis***		***Taenia* sp.**		***A. duodenale***		***E. vermicularis***	
			Positive	Prev (%)	Positive	Prev (%)	Positive	Prev (%)	Positive	Prev (%)	Positive	Prev (%)	Positive	Prev (%)	Positive	Prev (%)	Positive	Prev (%)	Positive	Prev (%)
Winter	443	168	33	7.45	33	7.45	42	9.48	16	3.61	12	2.71	11	2.48	15	3.39	6	1.35	1	0.56
Summer	627	200	39	6.22	43	6.86	68	10.85	11	1.75	13	2.07	12	1.91	11	1.75	3	0.48	0	0
Rainy	280	90	14	5	23	8.21	23	8.21	6	2.14	10	3.57	5	1.79	7	2.5	2	0.71	0	0
Autumn	178	44	7	3.93	6	3.37	15	8.43	7	3.93	3	1.69	2	1.12	3	1.69	0	0	0	0
$\chi 2$ (df)	12																			
p-Value	0.213																			

Table3: Seasonal prevalence of gastrointestinal parasites in Uttarakhand

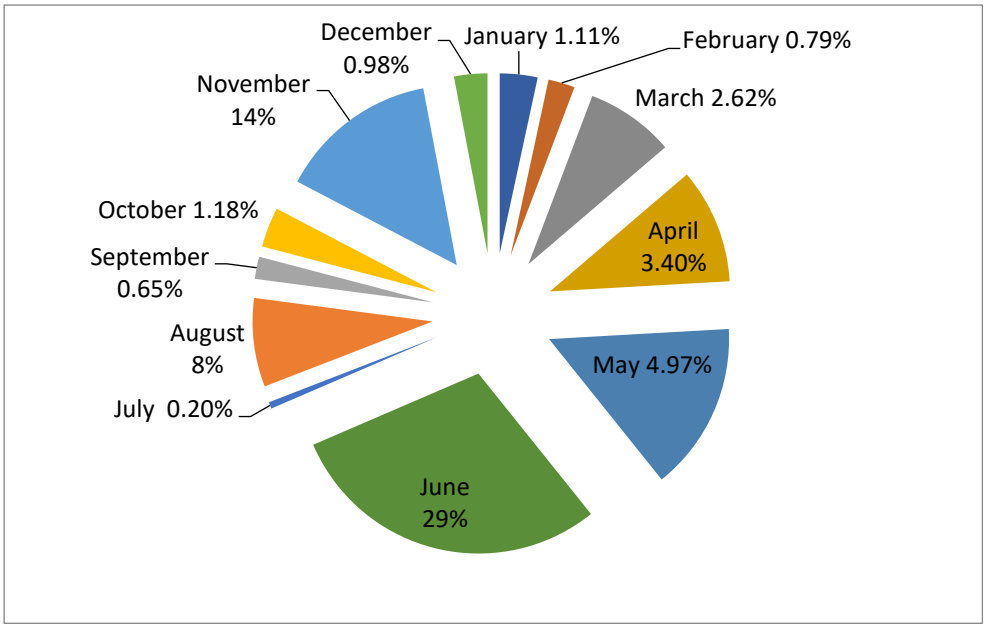

Figure 4: month-wise prevalence of parasitic infections

The highest prevalence of *A. duodenale* (1.35%) was observed in the winter season and the lowest (0.71%) was in the rainy season, while for *A. lumbricoides*, the highest (7.45%) was in winter and the lowest was (3.93%) in autumn. The highest prevalence of *E. histolytica* (8.21%) was in the rainy season and the lowest (3.37%) was in autumn. *G. lamblia* was recorded highest (10.85%) in summer and the lowest (8.21%) was in the rainy season. *H. nana* was recorded highest (3.93%) in autumn and lowest (1.75%) was in summer. *T. trichiura* was recorded highest (3.57%) in the rainy season and the lowest (1.69%) was in autumn. Prevalence of *S. stercoralis* was highest (2.48%) in winter and lowest (1.12%) was in autumn and for *Taenia* sp., the highest prevalence (3.39%) was in winter and the lowest (1.69%) was in autumn. Only one case of *E. vermicularis* was recorded (0.56%) in winter. The prevalence insignificantly differed in all the seasons (X2 = 12, p-value = 0.213) (Table 3).

Various parasites identified among the studied population were protozoa and helminths - 49.60% protozoa and 50.39% helminths. Among the total positive patients, 51.59% were males and 48.40% were females. The prevalence of protozoa in males and females was 8.05% and 8.51% respectively. The prevalence of helminths in males and females was 8.90% and 7.40% respectively. Difference in the distribution of protozoa and helminths was statistically insignificant (t = 0.533 and p-value = 0.688) (Table 4).

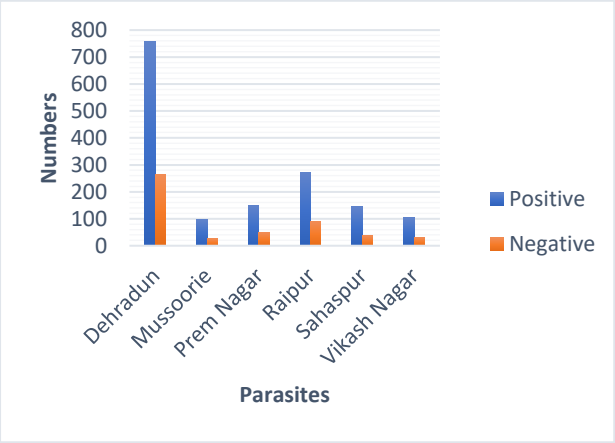

Figure 5: Distribution of samples in low socioeconomic areas from Uttarakhand

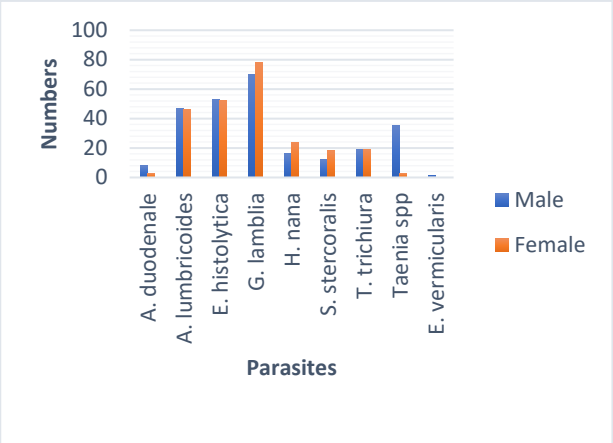

Figure 6: Distribution of parasites among males and females

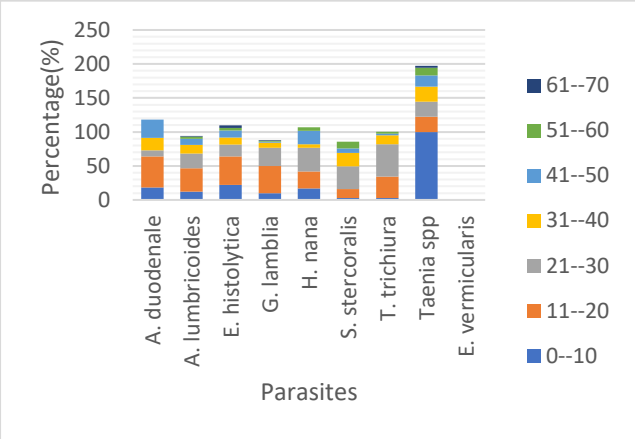

Figure 7: Distribution of parasites among different age groups from low socioeconomic areas of Uttarakhand

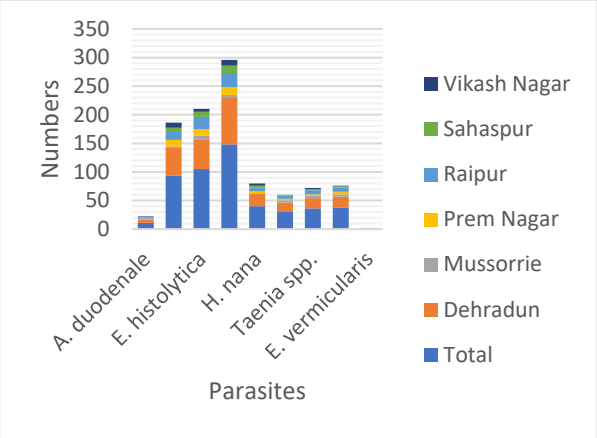

Figure 8: Distribution of parasites among different areas of Uttarakhand

			Male		**Female**			
Categories	Total no. of samples	Total number of positive samples	No. of positive samples	Prev (%)	No. of positive samples	Prev (%)	t	p- value
Protozoa	1528	253	123	8.05	130	8.51	0.533	0.688
Helminths		249	136	8.9	113	7.4		

Table 1: Distribution of protozoa and helminths in Uttarakhand

The present study revealed that infections with protozoa and helminths were very common in the studied area. The *G. lamblia* was the most common parasitic infection whereas *E. vermicularis* is the least. The prevalence of gastrointestinal parasitic infections is quite different in Indian cities.[15],[17-18] The prevalence of GI parasites varies in Dehradun from 24-60%.[11,12,13,21,22] Mathuria *et al.* reported the prevalence of intestinal parasites in Moradabad as 15.86%, a lower prevalence compared to that of Uttarakhand.[18] Tripathi *et al*. reported the prevalence of intestinal parasitic infection in Bhopal as 40.7%, a greater prevalence compared to that of Uttarakhand.[19]

An insignificant difference in prevalence was seen between males and females. However, some of the parasites were more prevalent in males (*Taenia* sp. And *A. duodenale*), while some were more prevalent in females (*H. nana and G. lamblia*). Prevalence may be affected by lifestyle and surrounding conditions. Age factor plays a crucial role in the prevalence of GI parasitic infections. The 10-20 years age group was the most common age group for the infections and also vary report to report. Several studies from different states of the country have reported the prevalence of GI infections.[15,17-23]

Asurvey reported the prevalence of parasitic infections in hilly reason of Uttarakhand that the prevalence was very low as compared to our study.[21]Nyundo*et al.* reported a prevalence of 75.21% in males and 24.79% in females from Tanzania, and another high prevalence of 55.2% in males and 44.8% in females was reported from Nigeria.[22, 23] The prevalence was lower in the neighbor country Nepal (19%) and higher in Pakistan (82%) with multiple parasitic infections[24-26]

The age group is crucial in the prevalence of GI parasitic infections. Children are the most common group. Singh *et al.* observed in Dehradun that the highest prevalence (22.64%) was in age group of 30-39 years which was different from our study. This may be because of the individuals involved in different work. The present study found a prevalence higher than that of Moradabad.[18]

Temperature and the surrounding environment affect the prevalence of parasitic infections. Temperature variation favors an increase or decrease in parasitic infection.[25-27]The prevalence in the hot season was higher than those in cold and dry days. The prevalence of *G. lamblia, A. lumbricoides,* and *E. histolytica* was observed higher in May, June, July, August, and September. This can be correlated to the consumption of contaminated food and water. Earlier studies have shown the overall prevalence of parasitic infections to be different in

different seasons.[27-28]The study from Nepal was from a comparatively lower temperature and from Pakistan was from a comparatively higher temperature.[24-26] So, surrounding environment especially weather plays important role in prevalence and distribution of infections.

Kotian *et al.* reported a lower prevalence of parasites in Uttarakhand than the current findings.[21] The higher or lower prevalence is dependent upon the time and duration of the study. Yasmeen & Singh reported the prevalence of protozoa and helminths in Moradabad as 29.41%, and 70.50% respectively.[30-33]Their findings were higher than that of the current findings of Uttarakhand. The study from Nepal also observed that the prevalence of protozoa was higher than that of helminthes.[24,33]

5. **Conclusion**

In Uttarakhand, India, intestinal parasite infections are a serious public health issue of school and university students. According to our study, students have a much greater prevalence of infections than other groups. Moreover, protozoa were discovered to be more common than helminths, which may be related to drinking water. Parasitic infections have a strong correlation with the socioeconomic status, line of work, age, and ethnicity of the parents.

The data presented can be used to gain a better understanding of risk factors for gastrointestinal parasites of this region. It will enable us to suggest a more evidence-based, comprehensive approach towards education for the prevention and eradication of these gastrointestinal parasitic infections. It may provide a guide map for the control of parasitic infections. Furthermore, the lack of such studies from several other parts of the country requires urgent attention. An exhaustive knowledge of the burden of the disease will be helpful in allocating resources, funding, and designing survey strategies for the control and monitoring of infections in any given area. Further research incorporating cutting-edge microscopic and molecular techniques would be beneficial for a correct diagnosis and the application of efficient prophylaxis.

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Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of the manuscript.

Authors' Contribution

Ashok K Sah, Farhana Zahir, and Mohammad Mahamood conceptualized the manuscript, Ritu Panda, Rohit Rathore, Kajal Arora, Jesbin Johnsongathered the data with regard to this work. Suresh Jaiswal and Ritam Koley analyzed these data, Ankur Vashishtha, Pankaj Issar and Ashok K Sah necessary inputs were given towards the designing of the manuscript. Ashok K Sah, Farhana Zahir, and Mohammad Mahamoodsupervised and executed the entire study, critically revised and edited and communicated the manuscript. All authors discussed the methodology and results and contributed to the final manuscript.

Data availability

All datasets generated or analyzed during this study are included in the manuscript.

Ethics Statement

The authors are responsible for all parts of the work, including ensuring that any questions about the accuracy or integrity of any portion of the work are thoroughly examined and resolved. All study protocols were authorized by Glocal University's Ethics Committee and CMO (Chief Medical Officer). (Approval ID. 2017-3585). All study participants agreed to the use of their samples for research purposes and provided written informed consent.

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