Original paper

Prevalence and risk factors of gastrointestinal parasites in the Chepangs in Nepal

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ABSTRACT. Gastrointestinal (GI) infection is predominant globally, especially in people with low socio-economic status with existing illiteracy, ignorance, poor housing and lifestyle, and the surrounding environment. It has been implicated as a significant public health concern in the rural parts of many developing countries like Nepal. This study aimed to determine the diversity and prevalence of GI parasites in the highly marginalized indigenous Chepang communities in central Nepal. One hundred fresh stool samples of Chepangs were collected and preserved in 2.5% potassium dichromate solution. The samples were analyzed by direct wet mount, sedimentation, flotation, and acid-fast techniques and examined under the microscope at the total magnifications of 100×, 400×, and 1000×. We detected 97% prevalence rates with eight protozoan and six helminth parasites. Considering the infection's concurrency, mixed pattern of infection was found to be higher than single pattern. Mixed infections from two to seven parasitic species were differently recorded in different samples. We also found that people's eating habits were associated with GI parasitism (p=0.0034). "One Health" Approach/Principle accompanied by the detailed molecular and epidemiologic studies of parasitic transmission is required to identify the causal evidence critical in controlling and preventing parasitic infections.

Keywords: *Balantidium*, indigenous Chepangs, *Cryptosporidium*, *Cyclospora*, gastrointestinal parasites

Introduction

Gastrointestinal (GI) infections include amoebosis, balantidiosis, cryptosporidiosis, giardiosis, and helminthoses caused by various protozoan and helminth parasites. They are the underlying causes of malabsorption, wasting, diarrhea, anemia, impaired work performance, and weak growth leading to significant morbidity and mortality [1–4]. They are predominant worldwide, especially in developing and underdeveloped countries, where the occurrence reaches 90–95% [5,6]. Even, recent data suggests that more than 1.5 billion people are infected with soil-transmitted helminths globally [7].

In this context, low socio-economic status of the people might be determining factor [8]. For example, existence of illiteracy, unawareness, poor housing and lifestyle, defective drinking water system, and the poor environmental conditions may contribute to the acquisition of diverse parasites resulting subsequently into the high illness and diseases [9–12]. Our previous study indicated that the prevalence of soil-transmitted helminthoses (STH) in a community is largely determined by individual characteristics or hygiene behavior [13]. Also, illiteracy [14,15], knowledge of intestinal parasites [15], and occupations [15,16] have been reported to increase the risk of STH infection in many indigenous populations in Nepal.

"Chepangs", first mentioned by British resident Brian Houghton Hodgson in Nepal, are highly marginalized indigenous people with existing socio-economic discrepancies like poverty, illiteracy, and

other issues [17,18]. They spend a traditional life of hunting, gathering wild foods, fishing, and depend entirely upon agricultural and forest products for their livelihood [19]. Thus, they usually suffer from an inadequacy of nutritious food for their positive physical health [20,21]. Besides, they are even unable to afford necessary primary health care facilities and access to safe drinking water indicating the probability of a high risk of GI parasitic infection in them. Parasitological survey among various ethnic and indigenous group of people had been an interesting topic of study within the country and few studies have already been conducted among different groups like Tharu, Musahar, Kumal, Jalari, Dalit, Terai/Madhesi, Muslims, Tibeto-Burmans, Indo-Aryans, and Chepangs [13,15,22–27]. Yet, data are still scarce for indigenous Chepangs who have possessed a unique bat hunting and consumption practices. As bats are evinced to carry coronaviruses and few zoonotic parasites, consuming bats by these people and the consequences in their health is a matter of research for the scientists. In our experiences of working with the Chepangs and bats in the Shaktikhor area in Chitwan, we found that socio-economic determinants were keys to their health as well as livelihood [28,29], which may also impact on the parasitic acquirement and transmission. A single study confined to a single location different from the current landscape has been conducted before [27], however, this study might not necessarily explain or represent the existing GI parasitism in this highly dispersed population. Therefore, in this study, we have determined the diversity and prevalence of protozoan and helminth parasites and few risk factors associated with GI parasitism in the indigenous Chepang communities in the remote Shaktikhor area, in central Nepal.

Materials and Methods

Study area

The current study was carried out in the Shaktikhor area (251–1003 meters above mean sea level) that lies in ward numbers 8, 9, and 10 of Kalika Municipality, Chitwan, Nepal (Fig. 1). The total population of these wards is 6059 in 1144 households (Current Municipality Profile). Its climate ranges from tropical to sub-tropical (average temperature of 29.3°C in summer and
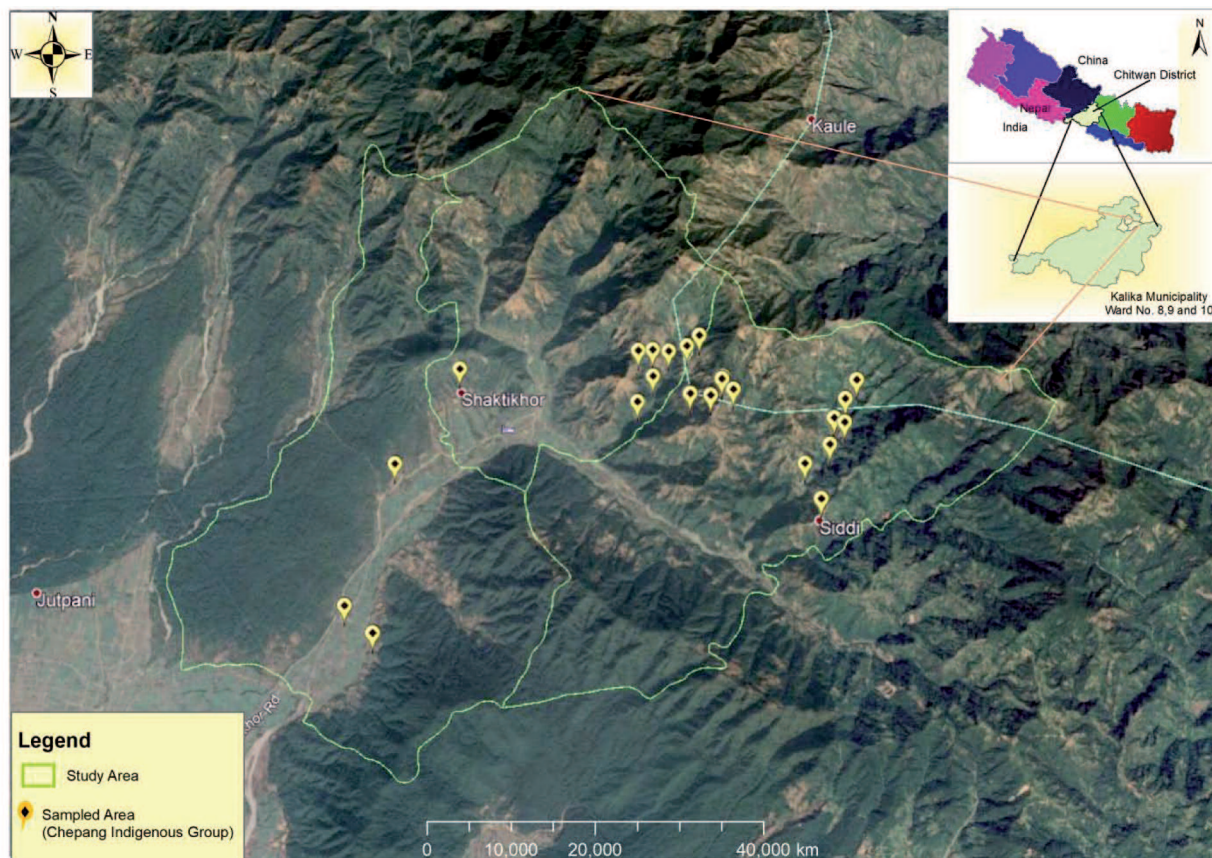

Figure 1. Geographic Information System (GIS) map of the study areas showing ward numbers 8, 9, and 10 of Kalika Municipality, Chitwan in central Nepal

Table 1. Parasitic species, their concurrency, and prevalence in the indigenous Chepangs in the central Nepal

Parasitic infections	Overall prevalence n (%)	p-values (Chi-square test, $p<0.05$)
Protozoan infections		
Entamoeba histolytica	47 (47%)	$p<0.0001$
Entamoeba coli	28 (28%)	
Iodamoeba buetschlii	27 (27%)	
Cryptosporidium sp.	25 (25%)	
Cyclospora cayetanensis	17 (17%)	
Blastocystis hominis	13 (13%)	
Giardia lamblia	8 (8%)	
Balantidium coli	2 (2%)	
Total protozoan infection	78 (78%)	ns
Total helminthic infection	67 (67%)	
Helminthic infections		
Ascaris lumbricoides	41 (41%)	$p<0.0001$
Hookworm	26 (26%)	
Trichostrongylus	16 (16%)	
Strongyloides stercoralis	13 (13%)	
Hymenolepis nana	4 (4%)	
Trichuris trichiura	2 (2%)	
Multi-parasite infections		
Single infection	23 (23%)	$p=0.0012$
Duplet Infection	25 (25%)	
Triplet Infection	22 (22%)	
Quadruplet Infection	15 (15%)	
Pentuplet Infection	5 (5%)	
Hexuplet Infection	4 (4%)	
Septuplet Infection	3 (3%)	

9.4°C in winter) with an average rainfall of 1993 mm annually [30]. The significant populations inhabiting this area are Chepangs, Tamangs, Magars, Brahmins, Chhetries, Gurungs, Dhamis, and Newars. Most of them practice traditional farming and animal husbandry as a part of their subsistence.

Sample collection, preservation, and transportation
From July 2018 to February 2019, 100 fresh stool samples of indigenous Chepangs aged 10–82 years (yrs) were collected via a purposive sampling technique non-invasively. Initially, the samples were macroscopically examined for blood, mucus, segments of worms, and then preserved in 2.5% weight/volume (w/v) potassium dichromate solution in a 20 ml sterile vial. These samples were then transported to the Animal Research Laboratory (ARL) of the Nepal Academy of Science and Technology (NAST), Lalitpur, Nepal and were stored in the refrigerator at 4°C temperatures.

Laboratory processing and examination
The preserved faecal samples were examined

Table 2. GI infection by demographic, socioeconomic, occupational and behavioral characteristics among indigenous Chepangs of Nepal

Demographic characteristics		Total examined (N=100)			
	Subgroups	Total persons (N)	Infected persons (n)	Prevalence % (100n/N) %	*p*-values, *p*<0.05 (Chi-square test)
Gender	Male	43	43	100	ns
	Female	57	54	94.7	
Age groups	10–19	15	15	96.2	ns
	20–39	37	36	97.3	
	40–59	22	22	100	
	60–82	18	17	94.4	
Occupation type	Laborers	5	5	100	ns
	Farmers	24	24	100	
	Farmers + House workers	46	44	95.7	
	Students	18	17	94.4	
	Carpenters	2	2	100	
	Witch doctors (Jhakri)	1	1	100	
	Teachers	1	1	100	
	Security guards	2	2	100	
Source of drinking water	Tube-well	15	13	86.7	ns
	Well	8	8	100	
	River/Spring	12	12	100	
	Kuwa/Mul	36	36	100	
	Traditional system reservoir	29	28	96.6	
Consumption of drinking of water	With treatment	12	10	83.3	ns
	Without treatment	88	87	98.8	
Use of slippers/sandals and shoes	Always	55	52	94.5ns	
	Never	8	8	100	
	Occasional	37	37	100	
Defecation type	Open defecation	24	24	100	ns
	Temporary latrine	50	49	98	
	Permanent latrine	23	21	91.3	
Hand washing before meal and after defecation	Water Only	38	38	100	ns
	Water + Mud	30	30	100	
	Water + Ash	23	22	95.6	
	Water + Soap	19	17	89.4	
Feeding habit	Non-vegetarians	94	94	100	0.0034
	Vegetarians	6	3	50	
Bat feeding habit	Bat consumers	50	50	100	ns
	Bat non-consumers	50	47	94	
Consumption of drugs	Between 6 months to present	7	5	71.4	ns
	One years ago	23	22	95.6	
	Never/unknown history	72	72	100	
Type of house	Muddy house	67	65	97.01	ns
	Concrete house	33	32	96.9	
Family size	Less than 5 members	48	45	93.7ns	
	More than 5 members	52	52	100	

ns: not significant

microscopically applying four techniques (briefed below) as previously described [29,31–35].

Direct wet mount technique

About 2 grams of the faecal sample were stirred/mixed carefully. A single drop of each sample was put on the glass slide with or without Gram's iodine stain. Then, by covering the sample with a coverslip, it was observed under a microscope at a total magnification of 100× and 400×.

Saturated salt floatation technique

About 2 grams of the faecal sample were thoroughly mixed in a 12 ml of 0.9% w/v sodium chloride (NaCl) following filtration via a strainer into a 15 ml centrifuge tube. The filtrate was proceeded to centrifuge (1200 revolutions per minute, rpm×5 minutes). The supernatant was then discarded, and the tube was filled with 45% w/v NaCl. The sample was centrifuged (1200 rpm×5 minutes). Then, the tube was completely and slowly filled with saturated NaCl and left undisturbed for 10 minutes, covering its mouth with a coverslip. Finally, the coverslip was carefully removed and kept on a glass slide for microscopic examination at a total magnification 100× and 400× with or without Gram's and Lugol's iodine.

Formalin-ethyl acetate (FEA) sedimentation

About 2 grams of the faecal sample were thoroughly mixed in 12 ml of 0.9% w/v NaCl in a 15 ml centrifuge tube. The sample was centrifuged (1200 rpm×5 minutes) and the supernatant was discarded. Then, 10 ml of 10% formalin and 3 ml of ethyl acetate was added in the tube for subsequent centrifugation (1200 rpm×5 minutes). Finally, the supernatant was discarded, and the sediments were examined under a microscope at a total magnification of 100× and 400× with or without Gram's iodine.

Acid-fast staining

The sediments obtained following the formal-ether method were used to prepare thin smears. The smears were dried at room temperature, fixed in absolute methanol for 2 minutes, and then stained with carbol fuchsin for 15 minutes. The smears were then serially and gently washed with distilled water, acid alcohol, and distilled water for 2 minutes. The smears were again re-stained with malachite green for a minute and rinsed with distilled water. Finally,

using immersion oil, the dry smears were observed under a microscope at a total magnification 1000×.

Parasite identification

All the above techniques were used to find the possible parasitic stages. However, coccidia were confirmed by the acid-fast staining methods. All the samples were observed under the optical microscope (Optika Microscopes Italy, B-383PLi). The microscopic images were taken using SXView 2.2.0.172 Beta (Nov 6, 2014) Copyright (C) 2013–2014 and morphometric analysis of various stages of parasites such as cysts, trophozoites, oocysts, and eggs was performed using ImageJ 1.51 k (National Institute of Health, USA). Identification was based on the images provided by the Center for Disease Control and Prevention, GA (www.cdc.gov/parasites/). Also various stages of few parasites were morphologically compared with those previously obtained from the bat stool [29].

Data analysis

The collected data were encrypted and entered into Microsoft Excel 2007 spreadsheet. Data were analyzed using Fisher's exact test (Two-sided) and Pearson's Chi-squared (χ^2) test with Prism 5 for Windows (Version 5.00, and March 7, 2007 software). Significance was analyzed among different protozoan species, among different helminth species, between total protozoan and total helminthic species, or among different intensity of concurrencies. Association of GI parasitic infections with respect to demographic, socioeconomic, occupational, and behavioral characteristics among the studied populations as well as bat consumers and bat non-consumers were also analyzed. In all cases, 95% confidence interval (CI) with $p<0.05$ were considered for the statistically significant difference.

Ethics approval and consent to participate

The required permission for the collection of the faecal samples was issued by the Nepal Health Research Council (NHRC) Ethical Review Board (Permission number: 463/2018), District Public Health Office (DPHO), Chitwan and Kalika Municipality, Chitwan (Permission number: 55/2018). Prior to the survey, the study's detailed purpose and procedures were explained verbally to the participants in the Nepali language. All participants signed written informed consent for both the questionnaire and sample collection. No
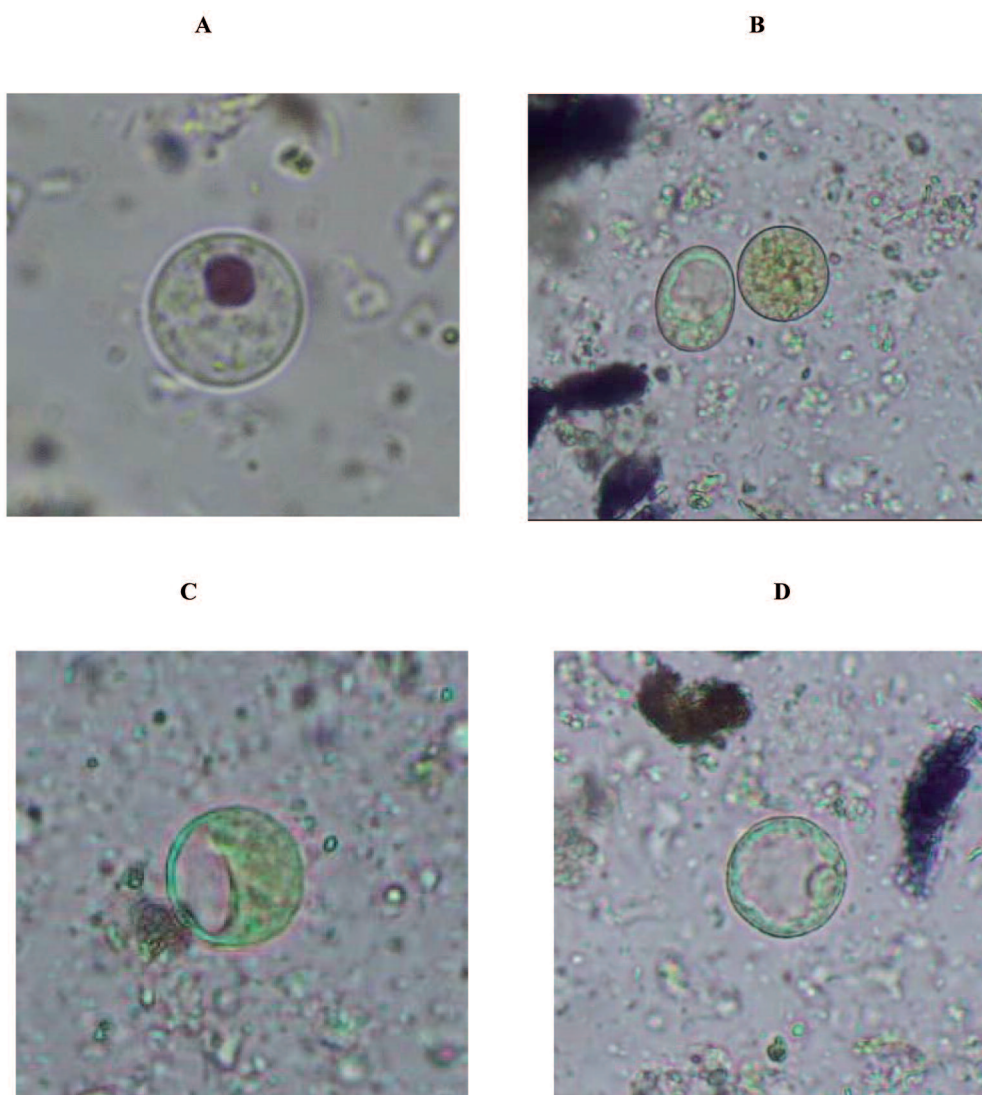
Figure 2A-D. A: *Entamoeba histolytica* (12×12 μm), 1000×, at Gram's iodine stain, B: *Iodamoeba buetschlii* (Oval, 21×15 μm) and *E. coli* (Round, 19×19 μm), 400×, at Gram's iodine stain, C: Cyst of *Iodamoeba buetschlii* (17×16 μm), 400×, at Gram's iodine stain, D: *Blastocystis hominis* (18×18 μm), 400×, at Gram's iodine stain.

experimental infection was established during this research work.

Results

In the current study, a total of 97 (97%) out of 100 faecal samples of indigenous Chepangs were found to be infected with GI parasites. Regarding diversity, 14 different species of GI parasites were identified (Figs 2,3) (Tab. 1). Among these parasites, there were eight protozoa like *Balantidium coli*, *Blastocystis hominis*, *Cryptospo-ridium* sp., *Cyclospora cayetanensis*, *Entamoeba coli*, *Entamoeba histolytica*, *Giardia lamblia*, and *Iodamoeba buetschlii* with significant differences in their prevalence (p<0.0001). In the same way, there were six species of helminths like *Ascaris lumbricoides*, hookworm, *Hymenolepis nana*, *Strongyloides stercoralis*, *Trichostrongylus*, and *Trichuris trichiura* with significant differences in their prevalence (p<0.0001). However, the total prevalence of protozoan and helminthic species (78% versus 67%) was statistically insignificant (p=ns) (Tab. 1). Considering the infection's concurrency, mixed pattern of infection was found to be higher than single pattern. The prevalence rate of infections in a different pattern of single and multiple infections was statistically significant (p= 0.0012) (Tab. 1).

Table 2 shows the bivariate (unadjusted) association between overall GI infection with demographic, socioeconomic, occupational, and behavioral characteristics among indigenous
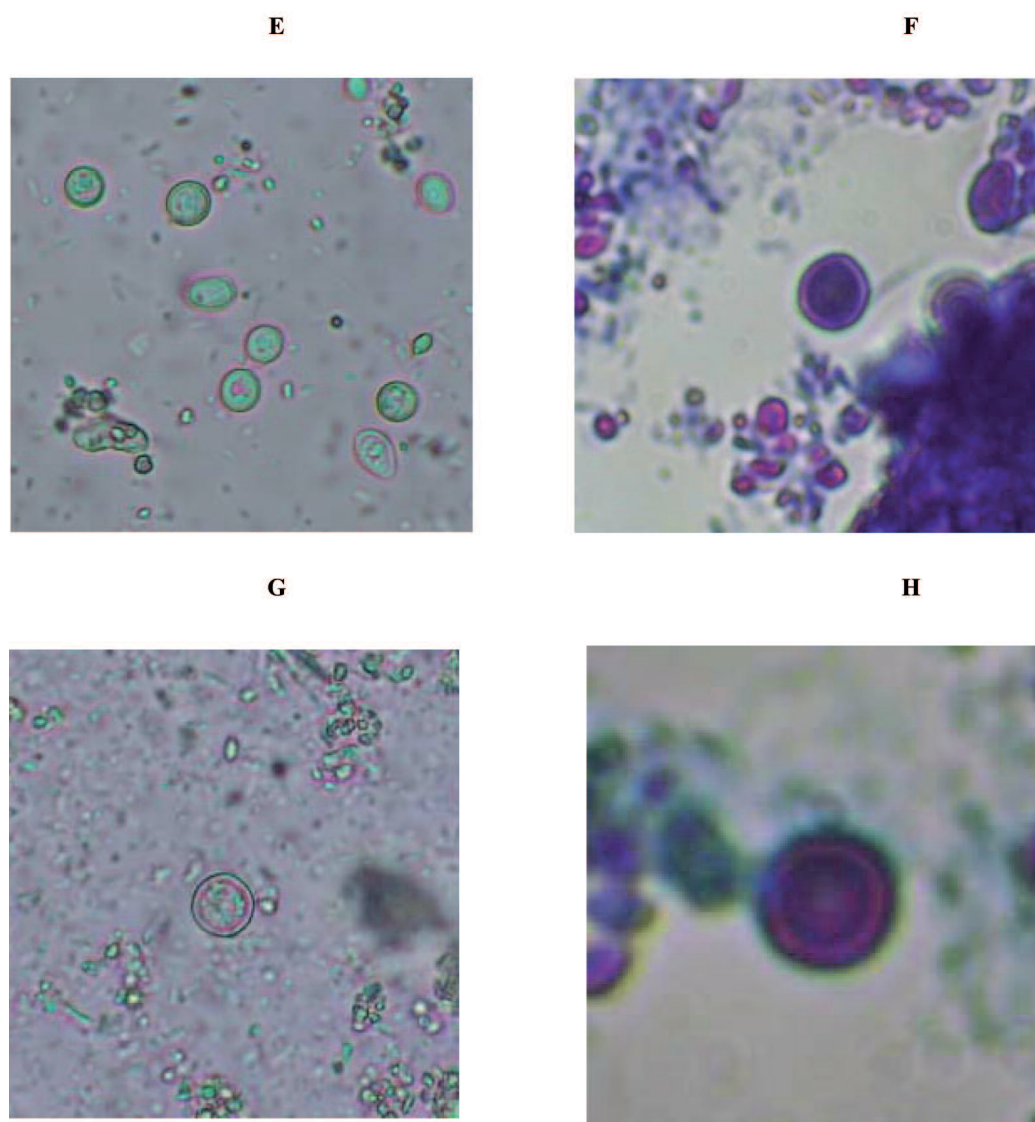
Figure 2E-H. E: Oocysts of *Cryptosporidium* spp. (4×4 μm), 400×, after FEA concentration, F: Oocyst of *Cryptosporidium* sp. (5×4 μm), 1000× under immersion oil, after acid-fast staining, G: Oocyst of *Cyclospora cayetanensis* (9×9 μm), 400×, direct wet mount, H: Oocyst of *Cyclospora cayetanensis* (7×7 μm), 1000× under immersion oil, after acid-fast staining.

Chepangs of Nepal (Tab. 2). Out of 13 different factors evaluated, the prevalence rate of GI parasites was statistically different in the people with different feeding habits (p=0.0034). Yet, unbalanced sample size because of very small number of vegetarians (n=6 vs 94) with high (97%) overall prevalence should be considered for careful interpretation of the finding.

To determine the roles of bat consumption in parasitic prevalence and diversity, the prevalence rates of different parasitic infections in the stools of bat consumers and non-consumers were analyzed (Tab. 3). Interestingly, bat consumers had 14 different parasitic species in their stool, whereas bat non-consumers had only nine species. Similarly, bat consumers had statistically higher rates of protozoa (p=0.0099) and nematodes (p=0.0103) compared to those in bat non-consumers. Higher rates of prevalence of *E. histolytica* (p<0.0001)*, E. coli* (p=0.0177)*, C. cayetanensis* (p=0.0002)*, A. lumbricoides* (p<0.0143)*,* hookworm (p<0.0001), and *Trichostrongylus* (p<0.0001) were also found among the bat consumers compared to bat non-consumers, while parasites like *B. hominis*, *B. coli*, *S. stercoralis*, *H. nana*, and *T. trichiura* were reported only in bat consumers indicating their significance in these hosts. In addition, analysis of parasitic stages in the faecal samples of bat consumers and previously described bat population [29] indicated that *E. histolytica*, *S. stercoralis* and *H. nana* were morphologically similar (Tab. 4).

In bat consumers, very low numbers of samples

Table 3. Patterns of GI parasites in the stool samples of bat consumers (N=50) and bat non-consumers (N=50)

Variable	Bat consumer	Bat non-consumer	p-values (Fisher's exact test, Two sided, $p<0.05$)
Numbers of GI species	14	9	
Entamoeba histolytica	31 (62%)	16 (32%)	<0.0001
E. coli	18 (36%)	10 (20%)	0.0177
Iodamoeba buetschlii	14 (28%)	13 (26%)	ns
Giardia lamblia	6 (12%)	2 (4%)	ns
Cryptosporidium sp.	13 (26%)	12 (24%)	ns
Cyclospora cayetanensis	13 (26%)	4 (8%)	0.0002
Blastocystis hominis	13 (26%)	0 (0%)	–
Balantidium coli	2 (4%)	0 (0%)	–
Ascaris lumbricoides	16 (32%)	25 (50%)	0.0143
Hookworm	22 (44%)	4 (8%)	<0.000
Strongyloides stercoralis	13 (26%)	0 (0%)	–
Trichostrongylus	14 (28 %)	2 (4%)	<0.0001
Hymenolepis nana	4 (8%)	0 (0%)	–
Trichuris trichiura	2 (4%)	0 (0%)	–
Protozoa	43 (86%)	35 (70%)	0.0099
Cestodes	4 (8%)	0 (0%)	–
Nematodes	38 (76%)	29 (58%)	0.0103

were positive for single infection compared to that in bat non-consumers ($p<0.0001$) (Tab. 5). However, very high numbers of samples were positive for four different parasites (quadruple) compared to that in bat non-consumers ($p<0.0001$). Bat consumers had one to seven parasitic concomitance, however, non-consumers had only one to four parasitic concomitance and most of the samples of non-consumers possessed one to three species (Fig. 4).

Discussion

The current study indicates the prevalence, diversity, and associated risk factors for GI infections among indigenous Chepangs in central Nepal. The overall parasitic prevalence (97%) in the current study was slightly higher than the findings from other indigenous groups from various parts of Nepal (22.1–63.9%, N=114–296) [13,22,24–26]. It was notable that compared to previous reports, the current study recorded the greater diversity of the GI parasites with the detection of *B. coli* and *Trichostrongylus* in our indigenous population. Similarly, comparing our result with global

indigenous population, the current prevalence rate was also higher than reported from Brazil (50–94.6%, N=112–409) [10,36–40], Argentina (87.8–95%, N=178–303) [41,42], Australia (66.7%, N=87) [43], China (68.3%, N=411) [44], Malaysia (24.6–91.3%, N=80–1273) [45–49], Philippines (34.1%, N=572) [50], Venezuela (92.5%, N=160) [51], and Colombia (84%, N=572) [52]. Compared to the results in these countries, we have found a higher diversity of GI parasites with the higher prevalence rate of *Cryptosporidium* sp., *C. cayetanensis*, *B. coli*, and *Trichostrongylus*. The difference in these results might be because of different sampling geographies and their climatic conditions, different socio-economic conditions, and behavioral practices by various indigenous groups, and the different laboratory techniques used in the faecal analysis. We have carried out a sampling from the remote and underdeveloped parts where the studied indigenous peoples are poor, illiterate, and far from developmental activities. Besides, the sampling period was monsoon and early spring, and we have used direct-wet mount, sedimentation, flotation, and acid-fast staining techniques in each fecal sample. All these factors might have favored the higher

Table 4. Ranges of average length and breadth (l×b) of the selected parasitic stages and their characters observed in different stool samples (n = number of specimens of the particular species analyzed)

Parasites	Bat consumers' stool	Bat stool	Notes
Entamoeba histolytica	(12–16 μm × 11–15 μm), n=10, spherical or oval, round, dark, central nucleus in cysts	(11–16 μm × 11–15 μm), n=10, oval or spherical, indistinct nucleus in cysts	Morphologically both similar and different forms
Ascaris lumbricoides	(56–65 μm × 40–50 μm), n=10, round or ovoidal shape with thick shell either corticated or non–corticated	(54–58 μm × 36–40 μm), n=10, ovoidal, with thin corticated shell	Morphologically different forms
Strongyloides stercoralis	(56–66 μm × 32–41 μm), n=10, oval, transparent, and thin–shelled, with a larva ready to hatch	(62–87 μm × 36–47 μm), n=10, oval to abround, transparent with thin–shelled, with a larva ready to hatch	Morphologically both similar and different forms
Hymenolepis nana	(45–56 μm × 35–45 μm), n=10, oval, embryonated egg shells with two distinct membranes with six-hooked oncosphere, color light purple to brown	(42–66 μm × 39–62 μm), n=10, oval–shaped, embryonated egg shells with two distinct membranes with six-hooked oncosphere, light purple to dark brown	Morphologically both similar and different forms
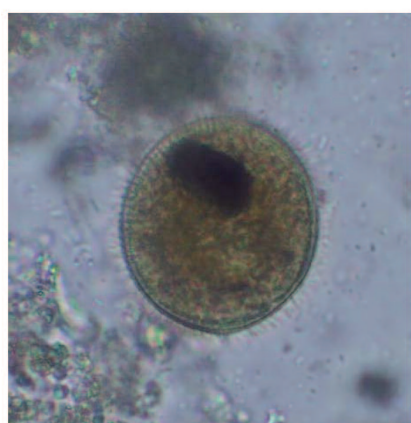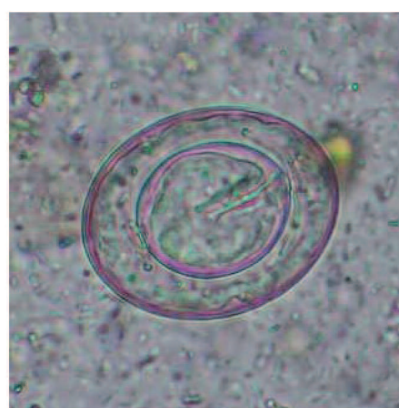
Figure 2I-L. I: Cyst of *Balantidium coli* (49×44 μm) 400×, at Gram's iodine stain, J: Trophozoite of *Balantidium coli* (87×59 μm), 400×, at normal saline (0.85% w/v), K: Cyst of *Giardia lamblia* (12×8 μm), 400×, at Gram's iodine stain, L: Egg of *Hymenolepis nana* (45×35 μm), 400×, at Gram's iodine stain.
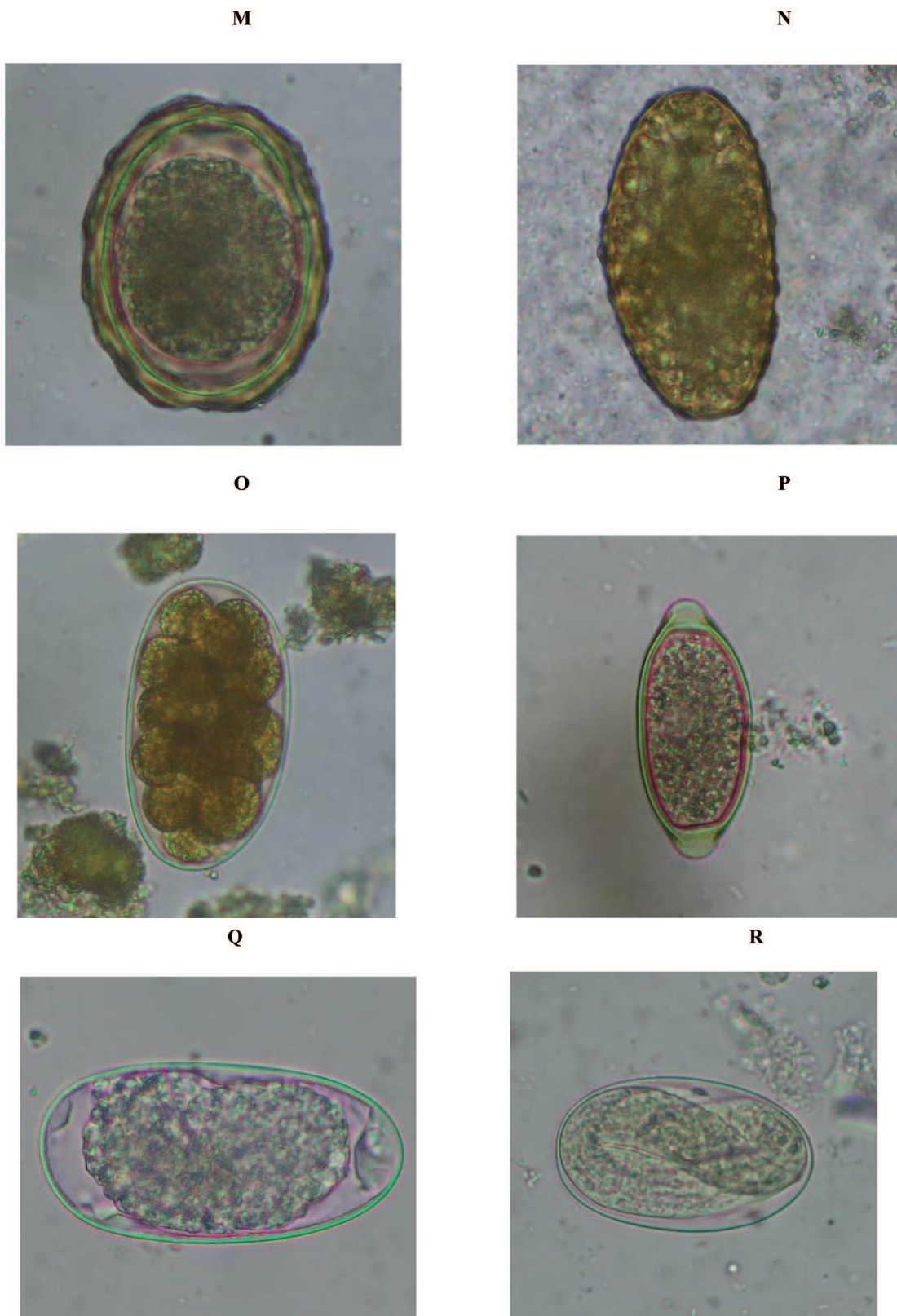
Figure 2M-R. M: Fertilized egg of *Ascaris lumbricoides* (63×50 μm), 400×, after saturated salt (45% NaCl w/v) flotation technique, N: Unfertilized egg of *Ascaris lumbricoides* (84×43 μm), 400×, FEA concentration at Gram's iodine stain, O**:** Egg of hookworm (67×39 μm), 400×, saturated salt (45% NaCl w/v) at Lugol's iodine stain, P: Egg of *Trichuris trichiura* (52×23 μm), 400×, after saturated salt (45% NaCl w/v) flotation technique, Q: Egg of *Trichostrongylus* (77×36 μm), 400×, after saturated salt flotation technique, R: Egg of *Strongyloides stercoralis* (65×40 μm), 400×, at Gram's iodine stain.
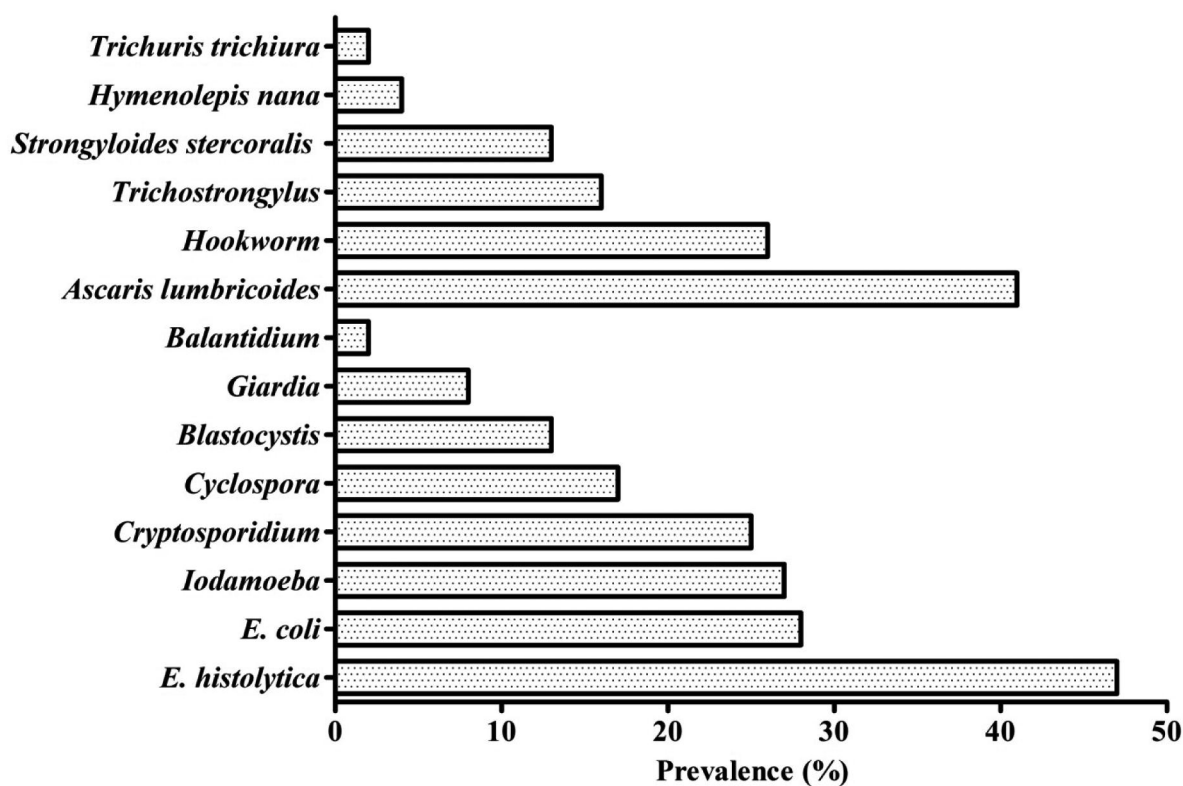
Figure 3. Prevalence (%) rate of various GI parasites in the stool (N=100)

parasitic positivity in our study.

Regarding protozoa, the prevalence of *E. histolytica* was the highest (47%). This finding was lower than the results from indigenous communities of Brazil (48.9%) [37] and higher than the findings from Nepal (11–20.7%) [22,25,26], Malaysia (8–33.7%) [4,47,49,53,54], Kenya (25%) [55], Brazil (6.5–31.6%) [10,36,39], Venezuela (10.8–17.8%) [56]. These data suggest that similar to global indigenous groups, *E. histolytica* is important in the current study (Chepang) population. Besides, large intestinal commensal, *E. coli* was also reported in 28% of the samples. This prevalence rate was in accordance with the findings from Malaysia (28.1%) [46], lower than the findings from Kenya (54%) [55], Venezuela (33.8–43.2%) [56], Malaysia (40.6%) [54], Brazil (32.58–38.3%) [36,38], and higher than that reported from Nepal (3.4–17%) [22,25,57]. It was notable that we reported *I. buetschlii*, a large intestinal commensal, in 27% of the faeces. This rate was lower than the findings from Kenya (31%) [55] and higher than the results from Brazil (2.58–3.1%) [36,38,39,57] and Malaysia (2.8%) [46].

Regarding the flagellate, the prevalence rate of *G. lamblia* was 8% that was similar to the result from Brazil (8–8.6%) [36,38,57], Malaysia (8.6%) [47], lower than the reports from Nepal (17–33.1%) [22,25,26], Australia (15.2–36.6%) [58,59], Argentina (33.3%) [41], Malaysia (9.5–25.7%) [4,45,46,54,60], Brazil (18.2–32%) [37,39,61], Venezuela (13.8–28.4%) [51,56], and slightly higher than reported from Malaysia (3.8%) [49].

The current report of 25% prevalence rate of *Cryptosporidium* sp. was higher than the report from Venezuela (0.6–10.2%), Brazil (3.66–8.26%), Malaysia (2.3–4.2%) [47,49]. Similarly, 17% of the current population was positive with *C. cayetanensis*. The rate was higher than the reports from Nepal (8–8.3%) [25,26], Venezuela (11.9%) [51], and Brazil (0.9%) [10]. These coccidian parasites significantly affect the gastrointestinal health of immunocompetent and immunocompromised patients. Both parasites are foodborne, waterborne, and soilborne [2,31,62–66]. Unlike *Cyclospora*, person-to-person and zoonotic transmission of *Cryptosporidium* are possible [31,63,67–72]. Therefore, the presence of an infected member in overcrowded situations, mud-built houses, usual open defecation practices, and close contact with domestic animals like goats, pigs, cattle, chicken, dogs, and cats might have
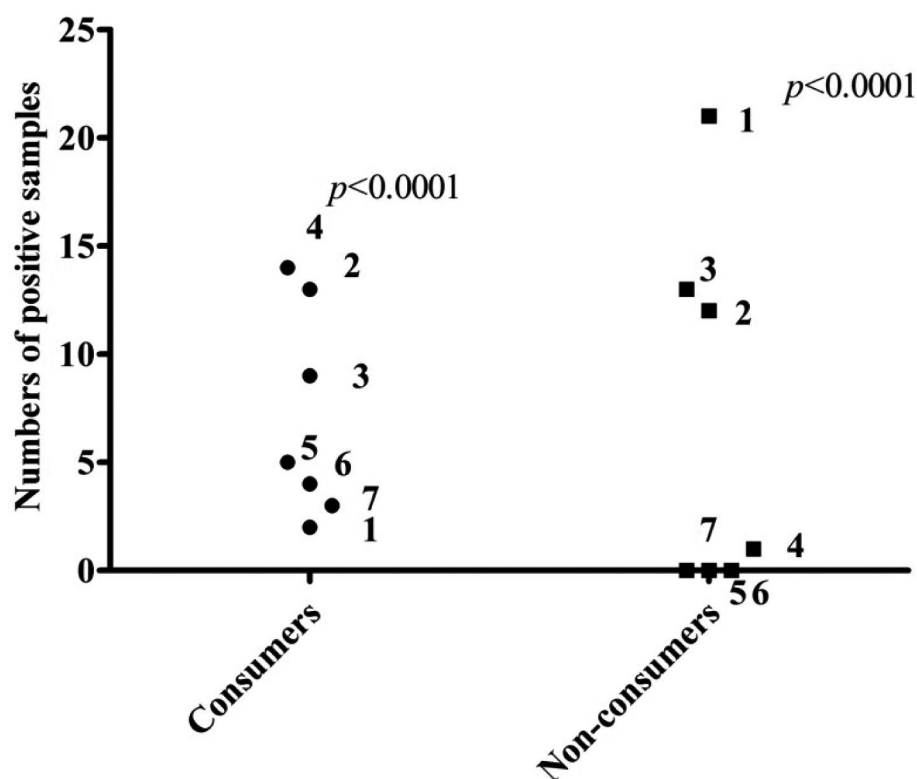
Figure 4. Concurrency of GI parasitic infections in bat consumers and bat non-consumers. The probability (*p*) values were calculated comparing the numbers of positive samples between two similar concurrencies. Each symbol indicates the number of parasite.

contributed to the acquisition of one or both of these coccidia in the study area.

B. hominis is a protozoan parasite with an ambiguous taxonomic position and is an etiologic agent of extreme GI illness. Its prevalence rate was 13% of the study population. This rate was similar to that reported from Brazil (13.9%) [38], lower than those reported from Malaysia (91.4%) [54], Brazil (21–58.9%) [36,39,47,73], and higher than those reported from China-Myanmar border (4.5–9.3%) [74], Brazil (7.4%) [10], Nepal (3–5%) [22,26] indicating its global impact in the indigenous populations.

Interestingly, *B. coli*, the only ciliate parasite in the human GI tract, was reported in 2% of the Chepangs. This rate was slightly higher than reported from Venezuela (0.8–1%) [56], and Malaysia (0.3%) [46]. Although the current rate is low, its presence suggests that *B. coli* is critical for zoonosis and is usually predominant in pig-rearing areas [75,76], for example, in the same areas in our reports in which we have reported the high prevalence rate of *B. coli* in the pigs [34].

In the current study, we reported the eggs of *H. nana*, a cestode, at the prevalence rate of 4% which

was similar to that reported from Nepal (4.8%) [25], lower than those from Brazil (5.32–31.2%) [10,36,38,39,57,73], Australia (20.4%) Victoria (7.4%) [77], and Venezuela (6.9%) [56], and higher than that reported from Nepal (3%) [22,26] and Kenya (2%) [55].

It was interesting that *A. lumbricoides* was the dominant nematode detected with the prevalence rate of 41%. The rate was slightly lower than the findings from Brazil (64.84%) [38], Victoria (54.7%) [77], Malaysia (53.9–43.3%) [47,49], Venezuela (47.34%) [56], higher than the findings from Venezuela (38.8%) [51], Malaysia (13.4–39%) [4,45,48,53], Philippines (26.5%) [50], and Nepal (6.9–26.3%) [13,22–26]. The higher prevalence rate in the study areas indicated the possibility of cross-transmission of *Ascaris* from pigs as it is common in pig rearing areas [78–80], for example, this species was predominantly present in the pigs reared by the current studied populations [34].

Hookworm is another nematode that had a prevalence rate of 26% which was lower than recorded from Australia (76%) [59], Argentina (60.7%) [41], Brazil (58%) [39], Nepal (30.87%) [23], and higher than those reported from China

Table 5. Concurrency of GI parasites in the stool samples of bat consumers (N=50) and bat non-consumers (N=50)

Numbers of parasitic species	Bat consumers	Bat non-consumers	*p*-values (Fisher's exact test, Two sided, *p*<0.05)
One	2 (4%)	21 (42%)	<0.0001
Two	13 (26%)	12 (24%)	ns
Three	9 (18%)	13 (26%)	ns
Four	14 (28%)	1 (2%)	<0.0001
Five	5 (10%)	0 (0%)	–
Six	4 (8%)	0 (0%)	–
Seven	3 (6%)	0 (0%)	–

(22.5%) [44], Venezuela (17.09%) [56], Philippines (13.6%) [50], Nepal (1.5–10%) [13,22,25,26], Malaysia (9.1%) [4]. Similarly, *S. stercoralis* was present with the prevalence rate of 13% that was lower than the report from Argentina (41.9%) [41] and Brazil (24.1%) [39] and higher than the report from Brazil (3.8–11.7%) [36,57], Venezuela (6.3–11.5%) [56], and Nepal (2.68–5%) [24,26]. It was notable that *Trichostrongylus*, a common strongyle of herbivores, was also reported in the present study. Its 16% prevalence rate was higher than the report from Kenya (8%) [55]. Its presence indicates that Chepangs may have acquired the parasites via cross-transmission from domestic animals like goats and cattle, which they rear close to their surroundings. This type of transmission occurs in a cyclic pattern between animals and humans in usual contact [81,82].

T. trichiura, the intestinal nematode, was reported in 2% Chepangs. The current prevalence rate was lower than the findings from Malaysia (35.7–72.1%) [48,49,60], Nepal (6–8.3%) [25,26], Brazil (4.68–5.4%) [38,83] and higher than those reported from Brazil (0.5%) [37]. The presence of this nematode is crucial because of its enormous potentiality to infect the huge human population.

While various parasites were detected in the current population, most individuals were infected with multiple (duplet to quadruplet) infections similar to many other research findings [45,84,85]. Multi-parasitism may favor greater virulence and higher exploitation of host defense mechanisms [86–88]. Hence, further studies related to immunopathology on these indigenous groups should be performed.

It is widely believed that parasitism is determined by the socio-economic characteristics and behavioral practices of people [8,10,11,89].

Because of the small sample size (lack of statistical power), most of the demographic, socioeconomic, occupational, and behavioral variables remained insignificant. However, the trend of overall GI infection was higher among most indigenous Chepang individuals who lived in mud-built houses with large family size accompanied by overcrowding. Also, high prevalence rates of the GI parasites were observed in the farmers, who worked in fields, in the people with the habit of open defecation, in the people who never or occasionally wore shoes/sandals, in the people who drank water from unsafe sources without treatment, and in the people who did not practice hygienic hand-washing practices. It indicates that these factors are crucial in susceptibility and transmission of GI parasites. Most GI parasites get transmitted via mouth while consuming contaminated food or water or via skin, while walking barefoot, and notably, the above-mentioned behavioral factors are favorable [88–92]. Open defecation in the surrounding environment especially in the fields, near water sources, on roads increases the risk of exposure of parasites to the mechanical vectors like houseflies and cockroaches that transmit the parasitic stages [93–95]. Besides, soil, air, water, foods, and domestic animals act as reservoirs for many parasites in these complex landscapes [2,15,31,62–64,76,96–99]. Similarly, awareness and drug-administration have also a role in parasitism, for example, all people who had never taken drugs were infected with parasites. People who used drugs had only less than 100% prevalence suggesting a positive role of antiparasitic medications in controlling parasites. Regarding awareness, the knowledge of intestinal parasites has been already shown to significantly play the transmission of different GI parasites among Jalari and Kumal indigenous people in a remote area in

the hilly area in Nepal [15].

We reported, with a significant difference in the prevalence rate, a 50% rate of GI parasites in vegetarians compared to a 100% prevalence in the non-vegetarians who used to consume chicken, mutton, buff, and pork. However, due to the low sample size of vegetarians, it is not easy to hypothesize the role of feeding behavior in parasite transmission.

In another context, most local people usually consumed bat meat even without removing its viscera [28]. Interestingly, nine species of parasites were detected in bat non-consumers, and 42% of these people had only single parasitic infection. In contrast, bat-consumers were infected with 14 diverse parasites, and 96% of them had concomitant infections indicating high intensity of the GI parasitism in these people. Few parasites like *Hymenolepis* sp. might be zoonotically transmitted from bats to humans because this cestode was only detected in the bat-consumers, and it was previously reported in the faecal sample of bats from the same areas [100,101]. Also, although not defined by molecular methods in this and previous study [29], *E. histolytica* was reported from a King Horseshoe Bat (*Rhinolophus rex*) from China [102] indicating a zoonosis may exist in the transmission of this ameba among closely associated human population with bats. Therefore, it suggests that consuming raw or improperly cooked bat meat might be a risk factor of protozoonosis and helminthosis in humans. However, studies following "One Health" Concept involving the research on humans, bats, and environmental factors like water sources, soil, food, and climatic conditions would give answers to the possible cross-transmission of parasites. In addition, detailed molecular and epidemiologic studies will help identify the causal evidence of parasitism in humans via different feeding habits.

Several limitations of this study should be considered. The foremost methodological limitation of the study is the smear assessments, which might not be enough to accommodate day-to-day and within-stool variations in the presence of GI parasites. Also, GI parasite prevalence may not represent the severity of infection as we could not evaluate GI infection density. A second limitation is the small sample size (n=100) available in the sub-group analysis, which may affect the risk of type II error. Thirdly, the possible effects of sampling bias caused by convenient/non-random selection of participants might limit the findings' generalizability.

Since participants were selected on a first-come-first-serve basis, there could be a chance that people with health concerns would be more likely to be included. Finally, given the study's cross-sectional nature, we are unable to identify the precise reasons behind the linkages we observe through this study.

In conclusion, this study can serve as baseline data for evaluating and planning effective mechanisms to control and prevent GI parasitic diseases. This study shows that the Chepang community has a high prevalence, greater diversity, and concomitant patterns of parasitic species. In these contexts, many socio-economic and behavioral factors in the community have directly or indirectly play a role by enhancing parasites' transmission. Notably, many of the species reported in this study are zoonotically important and can be transmitted among domestic animals and bats and within humans in the local areas. However, further detailed molecular and epidemiologic studies of parasitic transmission must identify the causal evidence, especially by following the One Health Approach principle. Also, an effective deworming and awareness program regarding parasites must be integrated with many developmental programs of Chepangs, especially for the control and prevention of such infections among the indigenous communities around the country.

Acknowledgements

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