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Original paper

Minimum risk of intestinal parasitic infection to the visitors of Ghandruk, Kaski, Nepal: a pilot survey

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ABSTRACT. Ghandruk is one of the famous destinations of both international and domestic tourists situated in Kaski, Nepal. Travel-related diseases are an important aspect that one should consider before making a travel plan. Among diseases, zoonotically important ones make serious worries among visitors. In order to assess the existence of the zoonotically important parasitic disease in Ghandruk, a pilot survey was carried out by examining representative stool samples (n=51) of local residence, domestic animals, pet animals in Ghandruk. Samples were examined using direct smear as well as concentration methods. A questionnaire survey was conducted to see the associated risk factors among residents and their livestock of Ghandruk. None of the faecal samples from residents (n=14) found positive for any kind of intestinal parasites (IPs), while samples from most of the livestock: chicken (86%, 6/7), pigeons (75%, 3/4), cow (66%, 2/3), mule (60%, 3/5), and dog (60%, 3/5) showed heavy infection, except goat and buffalo indicated no infection. *Eimeria* spp., *Ascardia* spp. and cestodes spp. were the most prevalent IPs in livestock. Periodic deworming, walking outdoor with sandals/shoes, frequent use of soap and water for handwashing as reported by most of the residents (>80%) could be the main reason behind zero prevalence of IPs in them. The heavy infection among livestock may be incriminated to the contaminated vicinity and free-range livestock and poultry which were noticed in contact with river, sludge, and toilets during our field observation. Conclusively, it indicates that the residents of Ghandruk are conscious about their health, but have not paid satisfactory attention to the hygiene of their domestic animals including livestock, poultry and even pet. Though the observed parasites in livestock are of minimal zoonotic importance regarding safety of visitors, it is deemed necessary for at least to apply some preventive measures to mitigate the burden of parasites in their animals.

Keywords: trekking destination, Ghandruk, indigenous Gurung community, parasites, livestock

Introduction

Intestinal helminth and protozoan parasites are most prevalent parasitic infection in developing countries whereas protozoan parasites are common in developed countries, and more than three billion (mostly children) are estimated to have infection of intestinal parasites (IPs) around the world [1]. In tropical and sub-tropical regions of developing world, where adequate water and sanitation facilities are lacking, the most prevalent soil transmitted helminths (STH) are *Ascaris lumbricoides*, *Trichuris trichiuria*, *Ancyclostoma duodenale* and *Necator americanicus* [2]. Similarly, *Giardia intestinalis*, *Entamoeba histolytica*, *Cyclospora cayetanenesis* and *Cryptosporidium* sp. are most common intestinal protozoan parasites. There are 500 million cases of *Entamoeba histolytica*, 800–1000 million of ascariosis, 200 million of giardiosis, 700–900 million of hookworm and 500 million of trichurosis [3]. Intestinal parasitic infection (IPI) is called as the disease of the poorest. Despite the advancement in sanitation infrastructure and hygiene status, IPI remains a considerable public health problem, especially in developing countries [4]. Several risk factors have been suggested for parasitic infection including low-household income [5], poor practices of personal hygiene like fingernail trimming, hand washing habit with soap before eating or after defecation or barefoot walking [5,6–8], environmental sanitation [9], poor educational background [10], over-crowded living conditions and limited access to safe water supply [5,11], eating contaminated raw vegetables, consumption of poor quality drinking water [12], and soil eating behavior [5,13]. Further, Parajuli et al. [7] reported elevated risk of helminthosis among females compared to male in Tarai and nationally representative samples from Nepal. Rather, IPs were found to be more common in urban and suburban areas rather [14]. Additionally, a majority of indigenous communities of Nepal rely on the river or underground pond water for drinking purpose due to lack of safe drinking water through piped infrastructures [12]. Under the deworming program, Family Welfare Division, Department of Health Services, Government of Nepal have been distributing anthelminthic drugs along with vitamin A supplementation biannually (April and October each year) free of cost [15]. However, IPI still has a high prevalence of 66.6% reported by Sharma et al. [16], 45.5% by Maharjan et al. [17], 54.0% by Gyawali et al. [12], and 48.9% by Gyawali [18] from various parts of Nepal. Intestinal parasitic infection is one of the top ten infectious diseases in Nepal [19]. The rate of IPI can be up to 100% in isolated communities. There had been large numbers of studies conducted regarding distribution of helminth infection in different communities in Nepal [20]. However, different lifestyle, living pattern, livelihood etc. can differ risk factors and parasitic prevalence.

Visitors used to take chloroquine while traveling to terai belt as prophylaxis for malaria. Foreign visitors use vaccination and drugs against disease prevalent while traveling. Health information system of government is yet to be systematic in term of disease of zoonotic though precautions need to be taken while travelling to disease risk areas. Hence, Ghandruk being one of the top 10 trekking destination in Nepal, health risk from different zoonotically important parasites of domestic animal, pet animals and their distribution is important.

Domestic birds (chickens, duck, turkey, pigeon) have significant role in national economy and social economic condition all over the world [21]. Domestic birds are the most common and widespread domestic animals, with a total population of about 26 million [22]. In Asia, the number of chickens is approximately 15 million duck 1 million and turkey 15 thousand [22]. Infections of intestinal parasite in domesticated birds affect their growth and productivity.

Among domestic birds, chickens and sometimes ducks are being widely spread at almost every family in rural parts of Nepal. It is extensively reared for valuable source of protein and cash income [23] mostly among indigenous communities. Most of the domestic birds are managed under backyard production system and undergo poor handling scheme. Backyard production system involves low productivity with less input. Though, domestic bird rearing is a fast-growing sector in Nepal making country self-dependent, however various challenges (shortage of quality feed, poor husbandry practices, prevalence, and wide distribution of infectious and noninfectious diseases) are yet to be addressed. Parasites are among the infectious agents that cause an alarming problem to the industry, posing adverse economic effects. Intestinal parasitism leads to significant economic loss in poultry [24]. Worm infection causes malnutrition, decreased feed conversion ratio, weight loss, lowered egg production and death of young birds thereby resulting considerable damage and great economic loss to the poultry industry [25].

Parasitic diseases caused by IPs constitute a major impediment to livestock production [26]. It is one of the major health problems severely limiting the productivity of dairy animals and lead to economic loss [27]. In Ghandruk, especially local people reared domestic animals such as donkey, buffalo, cow and goat for the milk, meat and manure. Some farmers were found to rear buffaloes systematically in separate shed with proper sanitation whereas some people release livestock to graze in the jungle which may increase the opportunity to encounter with intermediate host of helminths.

Our study area, Ghandruk, the second largest Gurung community, is famous for its unique lifestyle, living pattern and livelihood. This village is now regarded as one of the best trekking route and tourist destination of Nepal. This popularity and heavy tourist flow might have been causing changes in livelihood and lifestyle of the local inhabitants. Here, every household rear domestic animal like

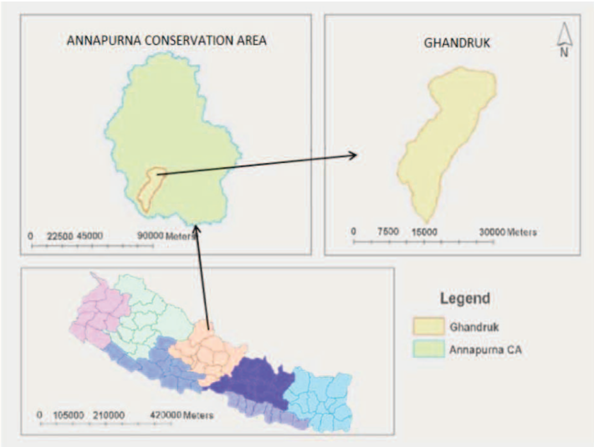

Figure 1. Map of Ghandruk village

chicken, duck, turkey, pigeon, cow, buffalo, goat, mules, etc. for meat, milk, eggs and cat and dog as hobby. However, in most of the places the rearing practice seems unsystematic in terms of maintenance of hygienic condition. They keep large number of animals in the same coop/shed. This confinement is one of the chances of transmission of parasite. During daytime, most of the domesticated animals are released to feed free range on the external environment near the drainage, river and forest as well as elsewhere of house, kitchen and even in toilets. Such sharing of same source for feeding and habitat also enhances the parasitic transmission. Hence, we want to evaluate the prevalence of IPs among residents and livestock and explore if such environmental, socio-demographic condition together with subsistence transition has any association with the status of gastrointestinal parasites of that area.

Therefore, this study is performed to find out the prevalence of IPs in local people and livestock which will at least provide a baseline information and/or any alarming situation, and accordingly, will

Figure 2. Photographic view of Ghandruk area

Figure 3. Study team during questionnaire survey with residents

provide future prospect and necessity of similar kind of studies. Results from this study will also help make the people aware of their status of health and hygiene.

Materials and Methods

Study area

Ghandruk village is situated in Kaski District of the Gandaki province of Nepal (Fig. 1) endowed with exquisite beauty (Fig. 2). Situated at a distance of 32 km from north-west of Pokhara, it lies at an elevation of 2012 masl mostly inhabited by the Gurung communities (56%) followed by Dalit (30%), Khas-Arya and others (14%) [28]. It is an entry point of both Annapurna and Machhapuchre Mountain. Ghandruk is one of the most famous and second largest Gurung settlements in Nepal [29] with 986 households and 5529 population [30]. The first pilot project of Annapurna Conservation Area Project (ACAP) was launched in Ghandruk in 1986.

Sample collection

Study team visited the study area and discussed the plan to visit residents and their livestock with convenient sampling methods (Fig. 3). Each participant was explained about the objective of study and obtained oral informed consent with flexibility to terminate consent any time during the process. All the participants were instructed to scoop a thumb sized stool sample (from its first, middle and last part) of early morning in the provided sterile vial by clean stick and making sure not to contaminate with urine or soil. For livestock, study team themselves collected samples following the protocol as briefed earlier to avoid cross contamination. Each collected sample was tagged

Figure 4. Lab processing of samples

with study code and preserved in 2.5% potassium dichromate solution for the preservation of the parasites and brought to the laboratory of Central Department of Zoology, Tribhuvan University for further work.

Microscopic examination

All the sample and needed materials were brought to the working table safely. Both stained and unstained slides were used for the identification of the parasites. The cyst, eggs and larva were identified according to [31] (Fig. 4). All photos were made by members of study team.

Questionnaire survey

Structured questionnaire was constructed after

Table 1. Characteristic and behavioral feature of the study participants

Demographic characteristics	Characteristics	Frequency	Percentage
Sex	Female	7	50
	Male	7	50
Tap water	Available	14	100
	Absent	0	0
Household water filtering and boiling mechanism	Present	10	71.43
	Absent	4	28.57
Latrine facility	Present	14	100
	Absent	0	0
Fingernails of the respondent	Trimmed	12	85.71
	Not trimmed	2	14.29
Age in years	≥15	2	14.29
	16–30	4	28.57
	31–45	2	14.29
	≤46	6	42.86
Shoe wearing habit	Yes	12	85.71
	No	2	14.29
Hand wash after defecation and before meal	Yes	14	100
	No	0	0
Consumption of anthelminthic drugs	Yes	11	78.57
	No	3	21.43

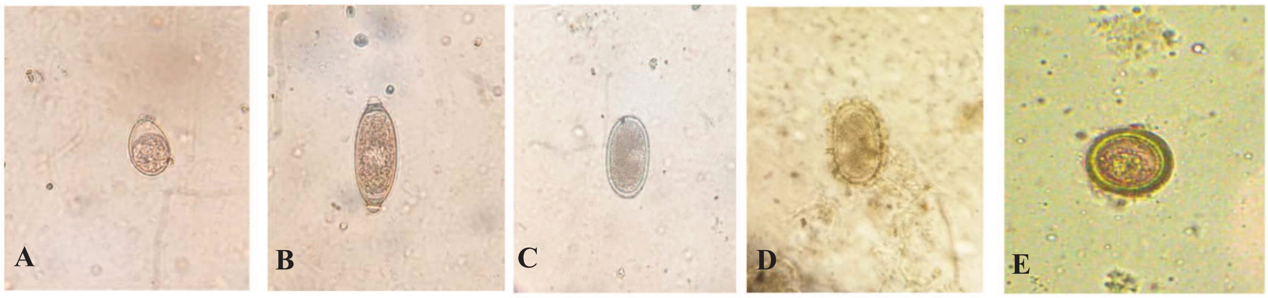

Figure 5. Photographs of gastro-intestinal parasites of chicken and duck (400×)
A – Un-sporulated *Eimeria* spp., B – Egg of *Trichuris* spp., C – Egg of *Heterakis* spp., D – Egg of *Ascaridia* sp., E – Egg of Cestode spp.

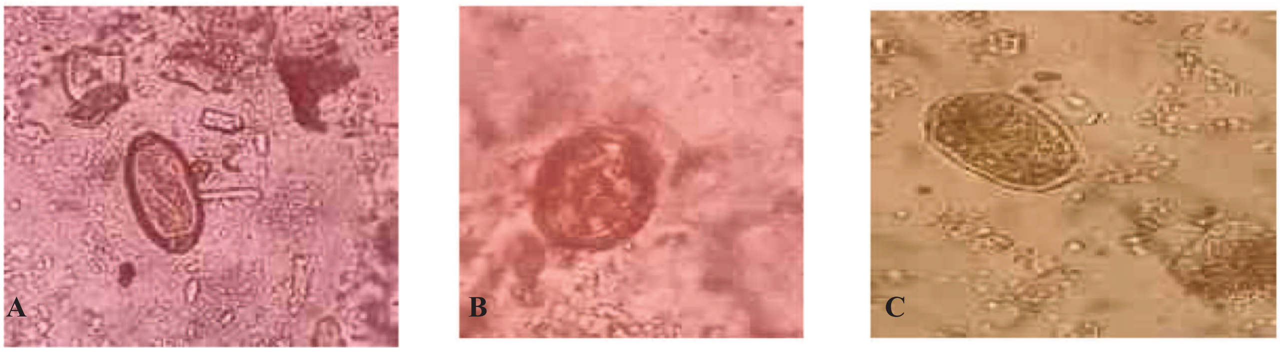

Figure 6. Photographs of gastro-intestinal parasites of of pigeon (400×)
A – Egg of *Capillaria* spp., B – Egg of *Ascaridia* spp., C – *Eimeria* spp.

reading plethora of earlier studies to elicits information on the demographic data (age, gender and education attainment), socioeconomic (occupation), behavioral (personal hygiene such as wearing shoes and hand washing), medical treatment (whether the participant has taken anthelminthic drugs), environmental sanitation and living condition characteristics (type of water supply, latrine system) which was used to assess the potential risk factors for IPI. Similarly, structured questionnaire was customized for every domesticated animal to elicit information regarding their looks, feeding habits, water they drink, if they were free range or not, examination and treatment. Focus group discussion was also conducted regarding environmental contamination, grazing sites, trekking route contamination, etc. Univariate and multivariate analysis was not possible because of limited sample size (n=51). Yet, descriptive data from questionnaire survey and prevalence data are presented for interpretation.

Ethical, legal, and social implications

During the course of study, no animals were harmed. All the research activities were carried out with prior informed consent and in the presence of local stakeholders with their suggestions.

Results

Questionnaire survey revealed that all the participants had tap water facility for drinking with good latrine at their houses, and they had habit of using soap and water for hand washing before taking meal and after using toilet. In addition, majority of participants indicated healthy hygiene behaviors like wearing sandals or shoes while outdoor, and trimmed fingernails regularly. More than 78% participants reported having anthelminthic drugs (Tab. 1). All the activities in the field visit, laboratory processing and identified parasites have been illustrated in supplementary file. Of the total 14 human stool samples (7 each from male and female) examined none of them revealed any parasite cyst/oocysts or larva or ova.

Table 2 shows the prevalence of protozoan and helminth parasites in the faecal samples from domestic birds and livestock (Fig. 5 and 6). Out of 7 chicken faecal samples investigated, *Heterakis* spp. was detected in 3 (43%) samples, *Trichuris* sp. in 2 (29%) samples and *Ascaridia* sp. in 1 (14%) sample among helminths while *Eimeria* sp. in 4 (57%) samples among protozoa. Mix infection was observed in 4 (57%) samples with 86% overall prevalence of IPI. Single faecal samples obtained

Table 2. Prevalence of protozoan and helminth parasites in the faecal samples from domestic birds and livestock (n=37)

Domesticated animals	Helminth	n (%)	Protozoa	n (%)	Mix n (%)	Remarks
Domesticated birds						
Chicken (n=7)	*Heterakis* spp.	3 (43)	*Eimeria* spp.	5 (71)	4 (57)	(1) *Heterakis* spp., *Eimeria* spp., *Trichuris* spp. (2) *Eimeria* spp., *Trichuris* spp. (3) *Heterakis* spp., *Eimeria* spp. (4) *Ascaridia* spp., Cestode spp. *Eimeria* spp. 86% overall prevalence of IPI in chicken
	Trichuris spp.	2 (29)				
	Ascaridia spp.	1 (14)				
	Cestode spp.	1 (14)				
Duck (n=1)	*Ascaridia* spp.	1 (100)				100% overall prevalence of IPI in duck
Pigeon (n=4)	*Capillaria* spp.	1 (25)	*Eimeria* spp.	1 (25)	1 (25)	(1) *Eimeria* spp. and *Ascaridia* spp.
	Ascardia spp.	1 (25)				
					75% overall prevalence of IPI	
Turkey (n=1)						None of stool sample indicated presence of IPI in turkey
Livestock						
Cow (n=3)			*Blastocystis* spp.		1 (33.3)	*Blastocystis* spp., *Isospora* spp.
						67% overall prevalence of IPI
			Isospora spp.	1 (33.3)		
Buffalo (n=5)						None of stool sample indicated presence of IPI
Goat (n=6)						None of stool sample indicated presence of IPI
Mule (n=5)	Hookworm	1 (20)	*Eimeria* spp.	1 (20)	2 (40)	60% overall prevalence of IPI
	Strongyloides spp.	1 (20)				
Pet animals						
Dog (n=5)	Cestode spp.	2 (40)			1 (20)	60% overall prevalence of IPI, 2 sample shows single parasites and 1 (20%) sample shows multiple parasites
	Toxocara canis	1 (20)				
Cat						Could not get stool sample to investigate presence of IPI

IPI: Intestinal parasitic infections

and investigated from duck indicated *Ascaridia* spp. while most of the samples (75%, 3/4) from pigeon were positive for IPI in which one sample recorded mixed infection with *Eimeria* spp. and *Ascaridia* spp. Single sample from turkey did not indicate any IPs. Out of 19 faecal samples examined from livestock, only 5 (25%) samples revealed presence of either helminth or protozoan parasites. None of the samples from goat (n=6) and buffalo (n=5) indicated IPI. Cow and mule recorded mix infection at the rate of 33% and 40% respectively (Fig. 7 and 8). Out of 5 faecal samples examined from dog, 3 (60%) samples were positive for either helminth or protozoan parasites with 1 (20%) having mix

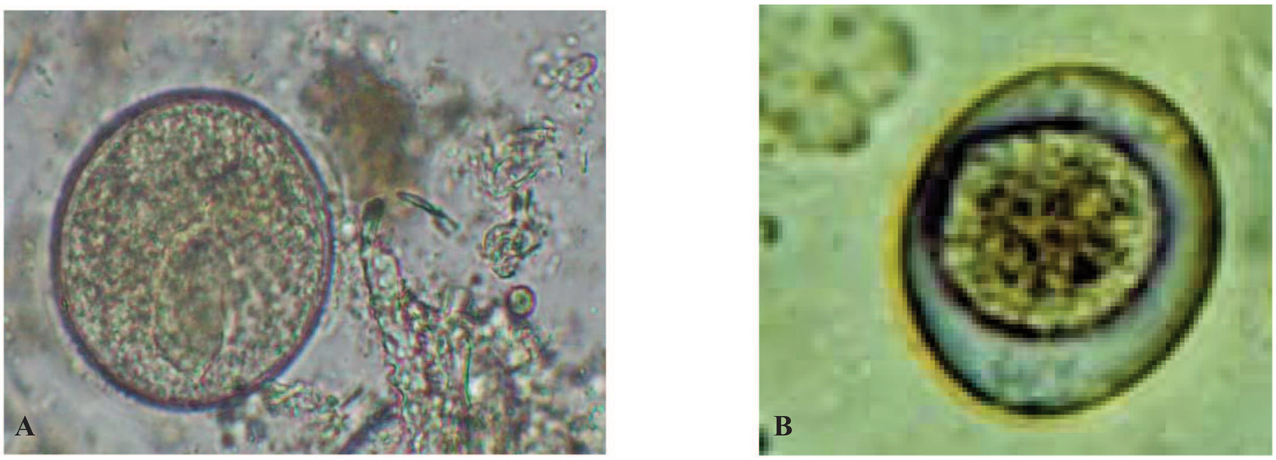

Figure 7. Photographs of gastro-intestinal parasites of cow (40×)
A – *Blastocystis* spp., B – *Isospora* spp.

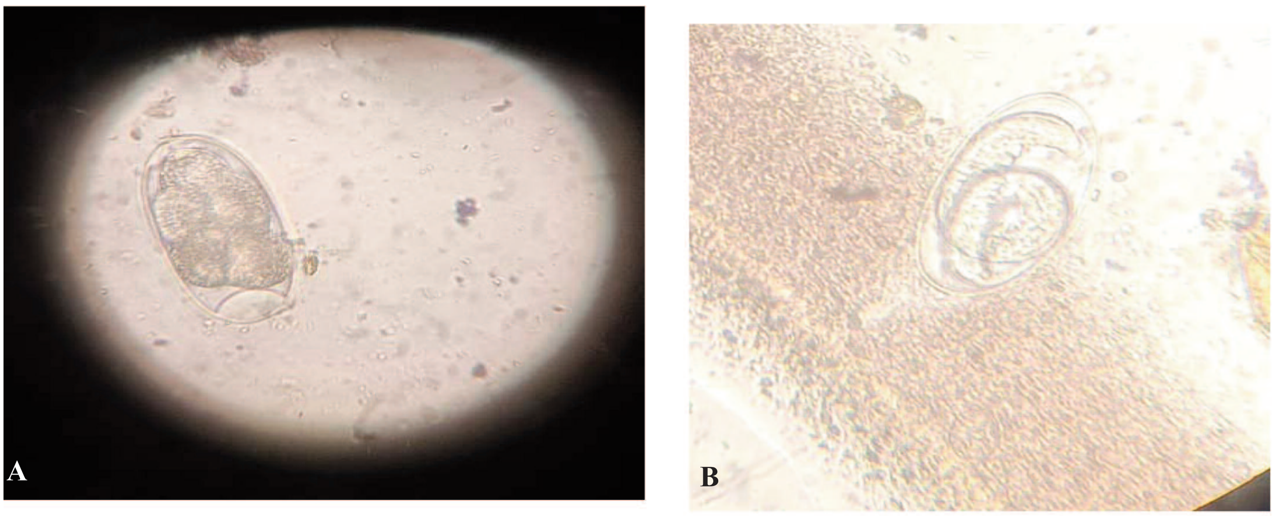

Figure 8. Photographs of gastro-intestinal parasites of cow (40×)
A – Egg of hookworm, B – *Strongyloides* spp.

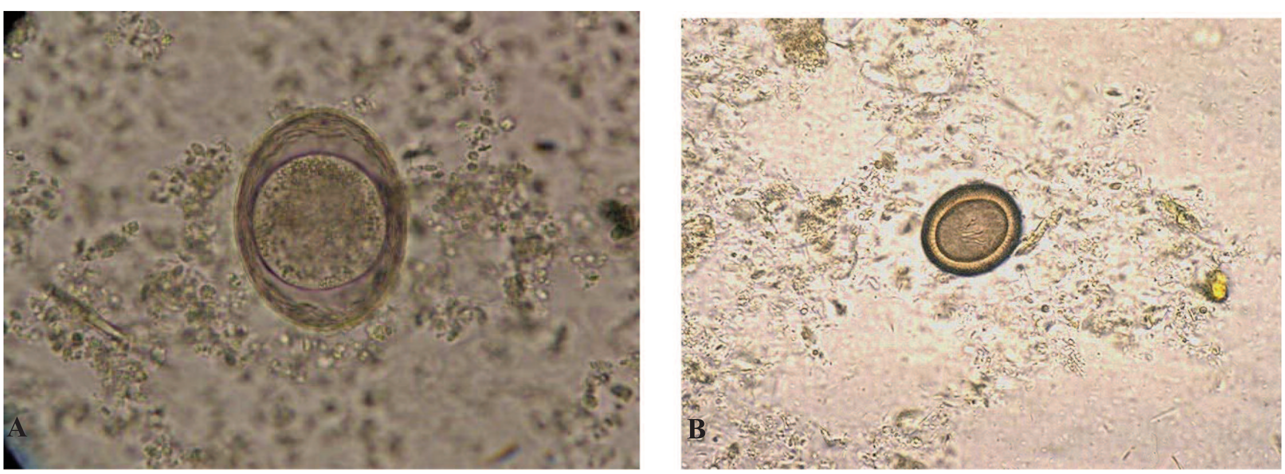

Figure 9. Photographs of gastro-intestinal parasites of dogs (40×)
A – *Toxocara canis* egg, B – Cestode spp. egg

infection (Fig. 9).

Table 3 depicts the summary of results from questionnaires survey regarding rearing practices of domestic birds and livestock in Ghandruk village. In most of the cases, domestic birds coop was cleaned three times a week and reported to eat well and were apparently seen healthy. Owner never got training and never noticed any parasite adults in droppings. So, none of the domestic birds were treated. Mostly chickens were kept in captivity with occasional free range while turkey, pigeon and duck were mostly free ranging. Among livestock, mix rearing was reported with frequent (every day or alternate day) shed cleaning. None of the owner reported training and considered their livestock eating well. As owner rarely noticed adult parasite in dung, their livestock were not treated. Buffaloes were mostly considered healthy while most of the goats were reported as sick. Regarding pet, none of the owner reported training and considered their pet free ranging healthy as eating well and owner never noticed parasite in stool; hence left untreated.

Discussion

Surprisingly, our study indicated zero prevalence of IPs in the resident of Ghandruk despite frequent observation of faecal materials of animals (dogs, livestock and mule) during village tour. Many earlier studies from rural setting comparable to Ghandruk showed higher prevalence. A study from rural area of Kathmandu valley indicated 71.2% infection among children [32]. Yet, few recent studies reported lower prevalence too like that of Mushar (33.3%) [7] and in the Chepang (39.8%) with overall prevalence of IPI of 36.6% [33]. This might be due to the difference in improved living condition in recent years. Further, as seen in our questionnaire survey, all participants reported tap water as source of drinking water with latrine facility and practice of hand wash with soap after defecation and before meal. In addition, majority of participants follow hygiene behaviors like wearing sandals or shoes while outdoor, and trimmed fingernails regularly. Hence, improved hygienic behavior as well as living conditions with regular consumption of anthelminthic drug might have contributed for such zero prevalence of IPs.

Soil associated helminths are highly prevalent in people who move bare foot both indoor and outdoor [7,8]. In our study most (86%) of the participants reported wearing sandals or shoes while outdoor. So, there may be very low chance of infection. This finding is supported by the study done by Estevez et al. [34], in remote villages of Nepal, where skin penetrating parasites were present in 36 out of 40 study samples as they were bare footed or wear thong-type sandals which afford poor protection. A similar study conducted in Ethiopia also showed that regular wearing of shoes had a significant contribution to the low prevalence rate of parasitic infections [35].

It has been observed that, those using soap and water (24.1%) after defecation had significantly lower prevalence of parasitic infection than using only water (63.2%) [36] who also reported that infection was found higher among children having the habit of nail biting (56.5%) and thumbs sucking (56.9%). Since all of this study participants washed their hand with soap before eating and >85% trimmed their fingernails, zero prevalence is reasonable. However, small sample size limits us for any assumption about the community.

Similarly, the use of household water filtering system decreases the odds of protozoa infection by 35% [37]. Systematic review across the globe also confirmed the efficiency of water filtering system for protozoan parasites [38]. Further, Amoah et al. [39] confirmed the efficacy of the water treatment at the household levels to eliminate the eggs or cysts of protozoa from the water. Since all of participants reported tap water with 71% participants using water filtering system, absence of protozoan parasite from studied samples seems convincing. However, small sample size (due to time constraints) does not allow us for generalization as sample is no more representative of the Ghandruk village. Hence, the result needs to be interpreted with care.

Out of 7 faecal samples of chicken collected and investigated from Ghandruk, 86% samples were detected positive. In those samples, total of five species were identified; 4 helminths, one protozoa, and in total four samples had mix infection of nematode and coccidian parasites. Subedi et al. [40] detected the highest prevalence of *Heterakis gallinarum* (22.4%) followed by *Ascaridia galli* (10.4%) in chicken from Lalitpur district. But in our study, we found the highest prevalence of *Eimeria* spp. (57%) followed by *Heterakis* spp. (43%), *Trichuris* spp. (29%) and Cestoda spp. (14%). Higher prevalence in our study in comparison to the earlier one might be because of lack of training and regular checkup or treatment for chicken of Ghandruk as reported during questionnaire survey. Study conducted by Javaregowda et al. [41] in the chicken of Shimoga found *Ascaridia galli* (62.3%), *Heterakis gallinarum* (22.6%). In addition, the study reported 19.67 % of mix infections of both cestode and nematode parasites. In line with the

Table 3. Domestic birds and livestock rearing practices in Ghandruk village (n=37)

	Rearing in		Shed/coop cleaning			Training		Eating well		Apparent health		Parasite in stool		Treatment	
Domesticated animals	Captivity	Free range	Every day	3 times a week	Occasionally	Yes	No	Less	As usual	Sick	Healthy	Yes	No	Yes	No
Domesticated birds															
Chicken (n=7)	4 (66)	2 (33)		6 (100)			6 (100)		6 (100)		6 (100)		6 (100)		6 (100)
Duck (n=1)		1 (100)		1 (100)			1 (100)		1 (100)		1 (100)		1 (100)	1 (100)	
Pigeon (n=4)*															
Turkey (n=1)		1 (100)		1 (100)			1 (100)		1 (100)		1 (100)		1 (100)		1 (100)
Livestock															
Cow (n=3)*															
Buffalo (n=5)	3 (60)	2 (40)	5 (100)				5 (100)	2 (40)	3 (60)	1 (20)	4 (80)	1 (20)	4 (80)	2 (40)	3 (60)
Goat (n=6)		6 (100)		6 (100)				2 (40)	3 (60)	4 (80)	1 (20)	1 (20)	4 (80)	1 (20)	4 (80)
Mule (n=5)*															
Pet animals															
Dog (n=5)		5 (100)		5 (100)			5 (100)		5 (100)		5 (100)		5 (100)		5 (100)
Cat (n=3)		3 (100)							3 (100)		3 (100)		3 (100)		3 (100)

*No questionnaire was applied

study of Javaregowda et al. [41], our study also indicated mix infection of coccidian and nematode parasites. Though villagers emphasized regular cleaning of coops, chicken were reared in group (single chicken coop) resulting high chance of transmission of the parasites among each other. In addition, owners were not trained for any healthy chicken rearing practices. Hence, even though the chicken seems to be apparently healthy, our study indicated that they were heavily infected with multiple IPs. This might be attributed to unhygienic rearing condition together with free range feeding nearby contaminated areas (drainage, nearby human defecation sites, etc.) and chickens were never examined or treated with anthelminthic medicine (Tab. 3).

Single faecal sample collected and investigated indicated prevalence of parasites in duck. While most of the samples (75%, 3/4) from pigeon indicated IPI. As reported in our study, the prevalence of IPs varied a lot according to species of birds. In domestic ducks and geese it was 10–50% in Tanzania [42], 75% in pigeons in Brazil [43], 34% in captive wild birds in India [44], 25–55% in indigenous domestic ducks, 20% in resident wild birds, 40% in migratory birds [45,46], 80% in domestic birds in Manipur, India [47].Yet, the parasite positivity rate in duck was quite high (81.67%) in Chandragiri municipality [48]. Again, because of limited sample size, our study could not be compared with earlier study but can be indicative for further study.

This outcome might be an indication of prevalence of intestinal parasite in duck and pigeon and the availability of infective stages of the worms in the study area. This survey also shows that the ability of the infective stage of the worms to survive outside the host for a long time before, it is picked up by the host. Presence of nematodes and cestodes, and absence of trematodes in duck and pigeon might be due to the absence of suitable molluscan intermediate hosts. The intestinal nematodes have monogenetic life cycle which can complete their life cycle without intermediate hosts [49]. Hence, high prevalence rate of nematodes is reasonable. The prevalence of gastrointestinal parasite in Ghandruk area may be because the duck and pigeon were in continuous contact with river, sludge, toilets where they were in frequent contact with suitable intermediate hosts of parasites and they must have come in regular contact with other animals which results parasite transmission. Chicken and duck were not only found using may common resources but also shared their habitat as well as coop. This might increase the chance for high prevalence rate of parasite. Maybe the chicken and duck were more prone to parasitic infection due to their feeding habit and are reared in unhygienic environment. Furthermore, the climatic conditions, altitude and geographical factor may also vary the prevalence of intestinal parasite. Generally, the warm and humid climate provide suitable condition for development of many intestinal parasites as reported in most parts of South-East Asia [41].

Relatively few faecal samples from livestock indicated parasitic infections. 67% (2/3) samples collected from cow indicated IPI. Similarly, Squireet et al. showed a very high prevalence (95.5%) of parasite infections among cow where 75.1% had multiple parasites in Southern Ghana [50]. However, Gunathilaka et al. [51] showed a bit lower prevalence in cow (11.56%) in Gampaha District of Sri Lanka. Gupta et al. [27] reported 65% prevalence of intestinal infection in cow in Jabalpur, Madhya Pradesh India which almost similar to our study.

Though villagers emphasized regular cleaning of sheds, livestock were mostly reared in group (in a single shed) resulting high chance of transmission of the parasites among each other. In addition, owners were not trained for any healthy rearing practices. Hence, even though the cow were often reported to be healthy, our study indicated that they were heavily infected with multiple intestinal parasites. Heavy infestation may be because of unhygienic rearing condition together with free range feeding nearby contaminated areas (drainage, nearby human defecation sites, etc.) and cows were never examined or treated with anthelminthic medicine. However, goat and buffalo were free from parasitic burden. Ghimire and Bhattarai [52] reported very high prevalence (i.e., 87.25%) of intestinal parasites in goat in Kathmandu. Though goat owners of Ghandruk reported mostly unhealthy and free-range goats with tap water as source of drinking water, opportunities for parasitic transmission can be assumed to be lower. During field observation, goats were found to be reared in separate shed with proper sanitation, proper food, and hygienic environmental condition. Goats usually nibbled from top branches and leaves away from ground which reduce the chances of prevalence of disease. We did not see any opportunity to encounter with intermediate host of cestodes and trematodes.

Earlier study by Sah [53] on intestinal helminth in buffalo of Dhrampur Dhanusha, Nepal revealed 74% and 84.66% positive for the presence of eggs of intestinal helminths for the winter and summer season, respectively. Lower prevalence during winter may explain partly for zero prevalence in our study as we conducted our study during end of winter season [53]. Further, as indicated by our questionnaire survey, hygienic rearing of buffaloes (>80% proper sanitation, proper food and hygienic environmental condition) might have contributed for parasite free buffaloes in the communities as opportunity to encounter with intermediate host of cestodes and trematodes was unlikely. Though none of the samples investigated were positive for parasite egg. Supporting information (hygienic rearing condition) from questionnaire survey to link for zero prevalence was interesting.

Though high prevalence (60%, 3/5) of our study is not comparable with that of Sapkota [54] who reported 45% parasite prevalence from mules of brick kiln of Lalitpur District. Similarly, Tedla and Abichu [55] reported 51.85% prevalence of intestinal strongyles in mules from South-western Ethiopia. As the farm owner never noticed any parasitic infection on mules, they never treated mules with anti-parasitic drugs. However, we observed mule dung was rampant elsewhere in community tracks, and mules feeding on contaminated pasture, with contaminated source of drinking water (as reported); heavy infection is reasonable.

In this study, with 60% prevalence, total 2 species of parasites were detected including 2 helminths (*Toxocara canis*, Cestoda spp.) parasites from the faecal samples of dogs. In line with this report, Ghimire [56] reported high prevalence of IPs (62.08%) with most common *Entamoeba* sp. (35.07%) followed by *Ascaridia* sp. (18.96%), *A. duodenale* (14.22%), *T. trichiura* (10.42%), *Giardia* sp. (6.64%), *T. solium* (1.42%), and *H. nana* (1.42%) in dogs of Kathmandu Metropolitan city (particularly in ward no. 19). Similarly, Satyalet al. [57] reported *Ancylostoma* sp. (52.0%), *Toxocara canis* (41.8%), *Taenia* sp. (15%), *Echinococcus* sp. (9.8%), *Dipylidium caninum* (9.2%) and *Trichuris vulpis* (5.1%) in dogs in Kathmandu, Nepal. However, quite low prevalence (35.7%) was reported by with many species of parasites like hookworm (30.6%), *Trichuris vulpis* (16.0%), *Toxocara canis* (6.6%), *Hymenolepis diminuta* (1.2%), *Spirometra mansoni* (0.6%), and *Dipylidium caninum* (0.2%) in dogs and cats in a refugee camp in Nakhonnayok, Thailand [58].

During the field observation, dogs were observed free ranging nearby contaminated areas (drainage, nearby human defecation sites, etc.) resulting high chance of transmission of the parasites among each other. In addition, owners were not trained for practice of rearing dogs in hygienic manner. Even though the dogs seem to be healthy, our study indicated that the dogs were heavily infected with multiple IPs. Heavy infestation may be because of unhygienic rearing condition together with free range feeding and dogs were never examined or treated with anthelminthic medicine.

Yet the observed parasites in livestock and poultry are species specific and of minimal zoonotic importance. Parasitic infection free local residence as well as prevalence of only nonzoonotic IPs in domestic and pet animals is the good indication of safety of visitors with zoonotically important IPI. However, small sample size of residents and livestock limits us for multivariate inferential statistical analysis, though questionnaire survey data and field observation aided us (to assume) for potential interpretation.

In conclusion, people of Ghandruk village were found to be free of IPI as they practiced healthy hygiene behaviors and used deworming drugs in every six months. Hence, safe water source, availability of latrine facility, household water treatment, hand washing practice with soap before eating, after using latrine, avoiding barefoot outdoor walk and trimming nails regularly may have provided protection from harmful parasitic infestation. The IPI among domestic birds, livestock and pet animals varied significantly. But none of the samples from domesticated animals appears to have GI parasite of zoonotic significance. Hence visitors may be at minimum risk of GI parasites because of consumption of domesticated animals. Different rearing practice seems responsible for such discrepancy in prevalence, and association with IPI in domestic birds, livestock and pets. Prevalence study of STH and intestinal protozoan parasites, and probable underlining factors in human and domestic animals both poultry and livestock need to be carried out in bigger sample size for generalization. However, this research can obviously be used as a pilot study with baseline data for future work.

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