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ORIGINAL ARTICLE

A survey of gastrointestinal parasites of goats in a goat market in Kathmandu, Nepal

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Abstract Gastrointestinal (GI) parasites in goats are predominant around the world. They may be the underlying cause of the pathological and immunological consequences leading to significant economic losses of the goat industry. The main aim of the current research was to reveal the prevalence of gastrointestinal parasites, including protozoa, trematodes, and nematodes in the fecal samples of goats in the goat market in Kathmandu, Nepal. A total of 400 goat fecal specimens were purposively collected from the goat market, and they were processed by direct wet mount, sedimentation, floatation techniques, and acid-fast staining, and were observed under the compound microscope. Out of a total 400 fecal samples, 349 (87.25%) were found to be infected with GI parasites among which *Eimeria* (80.75%), Strongyle (59.25%), *Trichuris* (29.75%), *Strongyloides* (28.75%), *Moniezia* (21.75%), *Entamoeba* (20%), *Fasciola* (10.25%), *Balantidium* (7.75%), *Cryptosporidium* (4%), *Capillaria* (1.75%), *Trichomonas* (1.25%), *Ascaris* (1.0%), *Cyclospora* (1%), *Blastocystis* (0.75%), *Giardia* (0.5%), and *Paramphistomum* (0.5%) were reported. GI parasites like protozoa and helminthes were widely distributed in the goats brought for the meat purposes in Kathmandu Valley, showing a high proportion of the goats were having higher risks of morbidity and mortality around the country.

Keywords Gastrointestinal parasites · Goat · Nepal · *Cryptosporidium* · *Eimeria* · *Trichuris*

Introduction

It is usually believed that since the beginning of the civilization, goat farming has been widespread in Nepal, especially in the rural parts, including low altitude to high altitude without any landscape biases. Goat farming has been alternative to crop production for the small as well as marginal farmers. The population of goats in 2007/2008 was 8,135,880 that increased by 1.37 times in 2016/2017, indicating the goat farming an integral part of the people within the country (MOAD 2018). Although their numbers in the Kathmandu Valley comprising the Kathmandu, Bhaktapur, and Lalitpur districts were low, the Valley seems to be one of the best consumers of goats. The goat markets of the Valley obtain goats mostly from outsides like other districts at different landscapes of the country. Interestingly, it has been estimated that the smallholder farmer earns a net income of 100–300US$ per annum after selling goats for meat (https://www.jica.go.jp/nepal/english/office/others/c8h0vm0000bjww96-att/tm_8.pdf, accessed on: March 20, 2019), however, it depends on the breeds that may include local races like Terai, Khari, Sinhal, and Chyangra, foreign goat breed like Boer, Barbari, Sannen, Beetal, Sirohi, Jamunapari, and hybrid strains like Boer cross, Jamunapari cross or Khapari, Barbari cross or Khabari, and Sannen cross.

It is true that, from many years, the goat markets have supplied the goat meat demands of the Valley transporting goats from different parts of Nepal. However, poor husbandry practices by farmers, the genetic inferiority of local breeds, and the poor condition of animal health have

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resulted in the lack of productivity (MOAD 2014), and thus, the domestic supply is not being able to meet the increasing demand. That is why supply from India and China, especially in the religious and festive events, has been common. Therefore, it has been a subject of interest how the poor health of these goats has been resulted by microorganisms like parasites, viruses, bacteria, and fungi. Among these etiologic agents, parasites are usually neglected, although they may lead to colossal morbidity and mortality of the goats resulting in the substantial economic losses (Babják et al. 2017; Chartier and Paraud 2012; Chikweto et al. 2018; Das et al. 2017; Dixit et al. 2017; Donkin and Boyazoglu 2004; Fakae 1990; Godara et al. 2014; Hashemnia et al. 2015; Kaur et al. 2019; Koudela and Boková 1998; O'Handley and Olson 2006; Wang et al. 2014). For example, postmortem reports of goats in a hilly region of the country were published byNAST in the Nepal Journal of Science and Technology, and had listed a total of 27 etiologies including parasitosis like monieziasis, hydatid cyst, and strongylosis (Khakural 2003). The author also quantified about 64% of major parasitic diseases treated by the experts in a few areas illustrating a critical problem of parasites in Nepal (Khakural 2003). The laboratory inside NAST already reported the eggs of *Trichostrongylus*, *Fasciola*, and the oocysts of *Eimeria* and *Isospora* in the feces and stomach with *Paramphistomum*, the abomasum with *Haemonchus*, liver with *Fasciola*, and the intestinal tract with the tapeworms in the goats in Nepal (Ghimire 2018).

Interestingly, it is widely believed that male goats at the goat markets might be free from any pathogenic agents, primarily due to their healthy and robust physical appearances. However, a pilot survey conducted by our laboratory found massive infection of goats with protozoa and helminthes (Ghimire 2018), indicating these agents are predominantly found in asymptomatic hosts. Thus, it is essential to understand the presence of various endoparasites that are released via the GI tract of the goats. The current study has tried to find out the prevalence of different protozoan and helminthic parasites in the fecal samples of the goats in the goat-market of the Kathmandu valley, Nepal.

Materials and methods

Sample collection, preservation, and transportation

From August 1, 2018, to February 6, 2019, the fresh fecal sample was collected from the Kalanki Goat Market, Kathmandu. The sample was put in sterile vials containing 2.5% potassium dichromate solution and was transported to the Animal Research Laboratory, NAST, Lalitpur.

Laboratory processing and examination

The laboratory methods of processing and examination of parasites explained in the literature (Zajac et al. 2012; Dryden et al. 2005; Basnett et al. 2018) were slightly modified by our laboratory (Animal Research Laboratory). Thus, the collected samples were stored in sterile vials with 2.5% potassium dichromate solution and were brought to the laboratory, where they were put at 4 °C in the refrigerator. About 5 g of the sample was taken in the mortar, and 10 mL saline (0.9% NaCl) was added on it. The mixture was filtered with the help of tea strainer. The filtrate was examined by the following three techniques separately:

Direct wet mount technique

To detect the trophozoites, cysts, oocysts, eggs, and larval stages of the endoparasites, the filtrate of stool sample was directly observed at 2.5% potassium dichromate, 0.9% saline solution, and Lugol's Iodine.

Saturated salt floatation technique

About one mL of filtrate and 13 mL of NaCl (45%w/v) were kept in the centrifuge tubes. The mixture was centrifuged (1200 revolution per minute, rpm × 5 min), and the centrifuge tubes with mixtures were kept in the test tube stands. The concentrated solution of NaCl was added entirely to the brim forming convex surface at the top at the tube. The tube was covered by the coverslip so that the solution touched the coverslip. After 15–20 min, coverslips were removed and kept on glass slides. The slides were examined under the microscope.

Sedimentation technique

One mL of filtrate and 13 mL of 0.9% NaCl were mixed in a 15 mL centrifuge tube the mixture was centrifuged (1200 rpm × 5 min). The supernatant liquid was discarded, and the settled solution was used for the experiment. Two drops of the solution were kept on a glass slide containing Lugol's iodine, and parasitic stages were examined on the microscope.

Acid-fast staining

The coccidian-positive (2–5 g) sample was centrifuged first in 0.85% NaCl, and then in 10 mL 10% Formalin and 5 mL ethyl acetate in a 15 mL centrifuge tube (1200 rpm × 5 min). A portion of sediment was used to prepare in the slide and allowed to dry at room temperature. Then, it was fixed with absolute methanol for 2 min, and stained with Carbol Fuchsin for 10 min, and then washed with distilled water. Finally, destaining by Acid Alcohol for 2 min was followed by washing with distilled water and restaining with Malachite Green (3 min) was developed by rinsing with distilled water. Using immersion oil, the dried slide was observed at 1000× magnification.

Parasite identification

All the sample were observed under light microscope (Optika Microscopes Italy, B-383PLi) at a total magnification of 40×, 100×, 400×, and 1000× and images (1280 pixels × 720 pixels) were taken using SXView 2.2.0.172 Beta (Nov 6, 2014) Copyright (C) 2013–2014 and sizes were measured using ImageJ 1.51 k (National Institute of Health, USA). Parasitic stages like cysts, oocysts, trophozoites, eggs, and larvae were analyzed and identified according to the literature (https://parasitology.cvm.ncsu.edu/m_keys/ruminant/parasite/strongyle.html, accessed on March 15, 2019) (Zajac et al. 2012; Taylor et al. 2016; Koudela and Boková 1998; Chartier and Paraud 2012).

Data analysis

Data were analyzed using Prism 5 for Windows, Version 5.00, March 7, 2007. Prevalence was calculated, dividing total numbers of infected by total numbers of collected feces and multiplying it by 100. The analysis of variance (ANOVA) and Student's t-tests were used to analyze the association between the pattern of coinfection and the stool consistency at different levels of significance (e.g., $p < 0.01$–0.001).

Results

In the current study, a total of 349 (87.25%) out of 400 fecal samples were found to be infected with GI parasites. The prevalence of each parasite was as follows: *Eimeria* (80.75%), Strongyle (59.25%), *Trichuris* (29.75%), *Strongyloides* (28.75%), *Moniezia* (21.75%), *Entamoeba* (20%), *Fasciola* (10.25%), *Balantidium* (7.75%), *Cryptosporidium* (4%), *Capillaria* (1.75%), *Trichomonas* (1.25%), *Ascaris* (1.0%), *Cyclospora* (1%), *Blastocystis* (0.75%), *Giardia* (0.5%), and *Paramphistomum* (0.5%) (Figs. 1, 2). It was interesting to note that single infection was absent in this study and importantly, the % of any double, triple, quadruple, quintuple, sextuple, and septuple species of parasites was 34.38%, 40.4%, 14.9%, 4.3%, 4.87%, and 1.15% respectively (Fig. 3).

We also analyzed whether stool consistency (solid, semisolid, liquid) was associated with the presence of variously co-infected parasites. Both stool consistency ($p = 0.0003$) and co-infection ($p = 0.027$) significantly affected the results. Bonferroni posttests found that there was a statistically significant difference between solid versus semisolid stools containing *Eimeria*, *Moniezia*, and Strongyle ($t = 4.499$, $p < 0.01$), between solid and liquid stools containing *Eimeria* + *Moniezia* + Strongyle ($t = 4.74$, $p < 0.001$) and *Eimeria* and Strongyle ($t = 4.13$, $p < 0.01$) (data not shown).

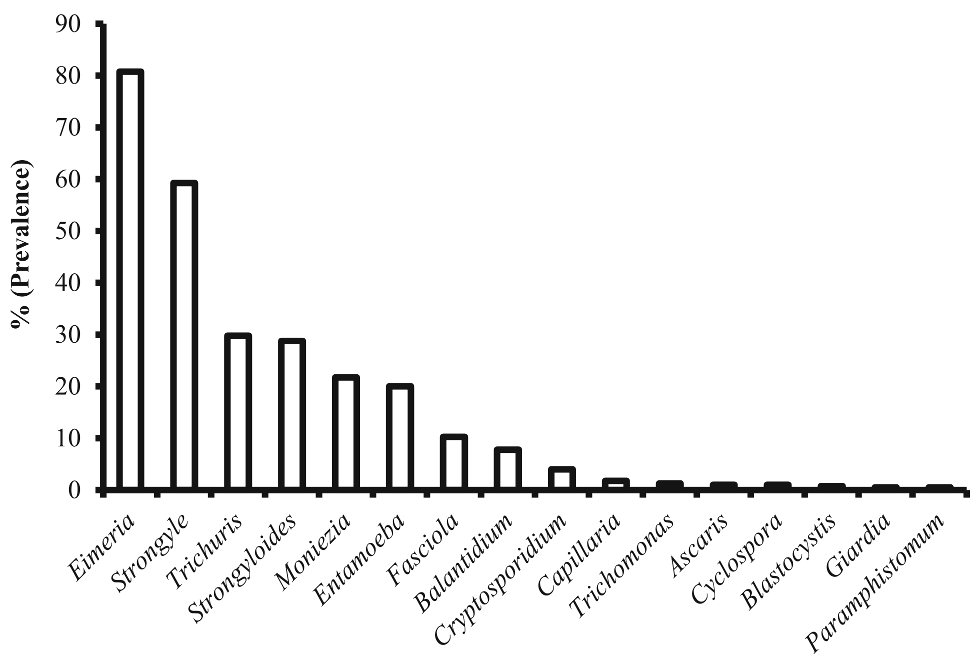

Fig. 1 Percent prevalence of particular parasite in fecal sample of goats in Goat Market, Kalanki, Kathmandu Valley, Nepal. Prevalence was calculated by using formula n*100/N, where n = number of positive sample and the N = total number of stool examined

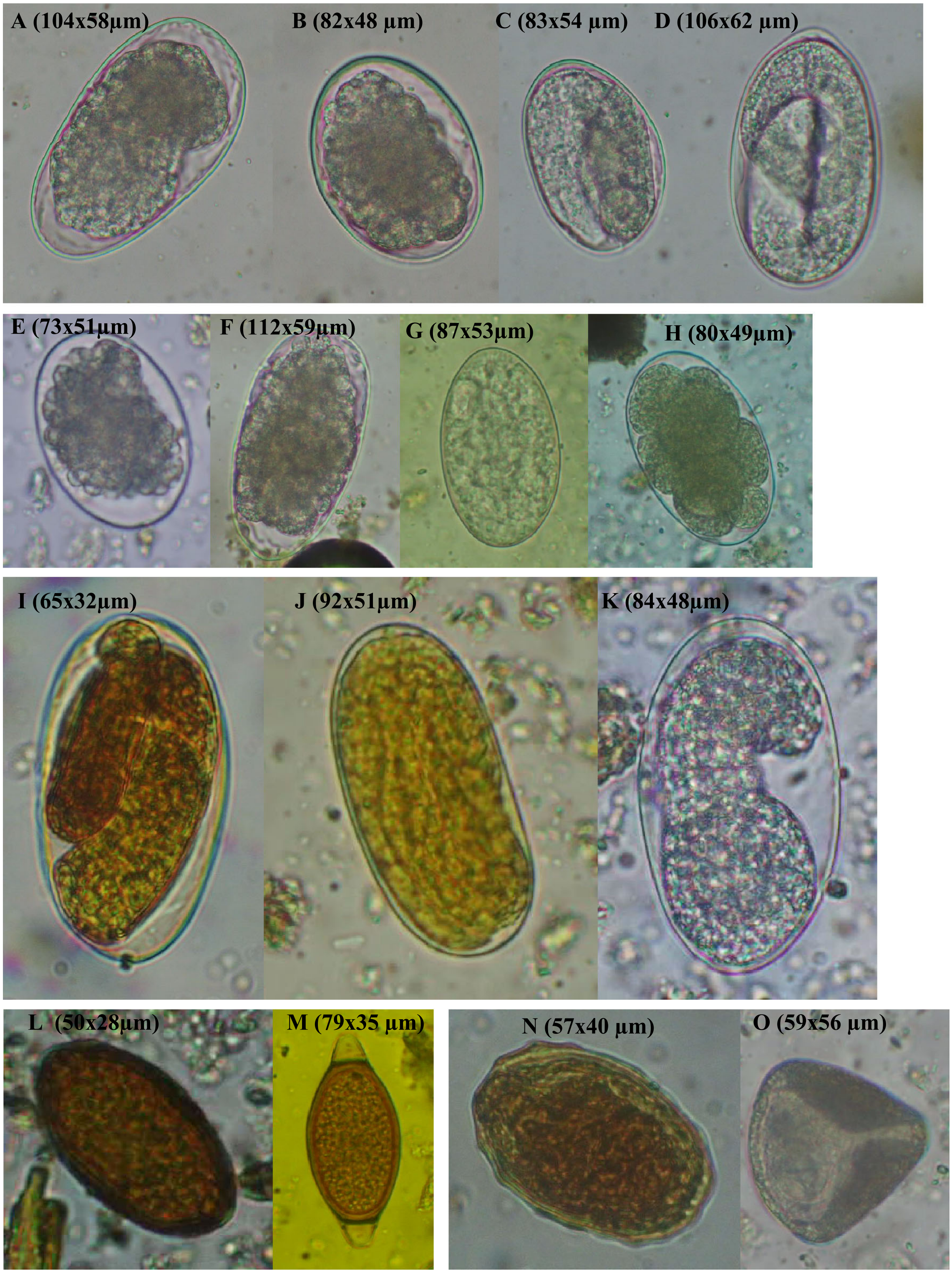

Fig. 2 Various types of eggs of Strongyle parasite (×400). **a**, **b** Blastomeres, **c**, **d** growing larvae, **e**, **f**, **g**, **h** Various types of eggs with Blastomeres of Strongyle parasites (×400). **i**, **j**, **k** Various types of eggs of *Strongyloides* spp. with growing larval forms (×400). Egg of *Capillaria* sp. (**l**), *Trichuris* sp. (**m**), an Ascarid (**n**), *Moniezia expansa* (**o**) (×400). Cyst of *Giardia* sp. (×1000) (**p**), oocyst of *Eimeria* sp. (×400) (**q**), and egg of *Fasciola* sp. (×400) (**r**). Trophozoite of *Balantidium* sp. (**s**), cysts of *Entamoeba* sp. (**t**), and cyst of *Blastocystis* (**u**) after Gram's Iodine staining at ×400 magnifications, and oocyst of *Cryptosporidium* (**v**) after modified acid-fast staining (×1000). Oocyst of *Cyclospora* at the direct wet mount (**w**) (×400) and after modified acid-fast staining (×1000)

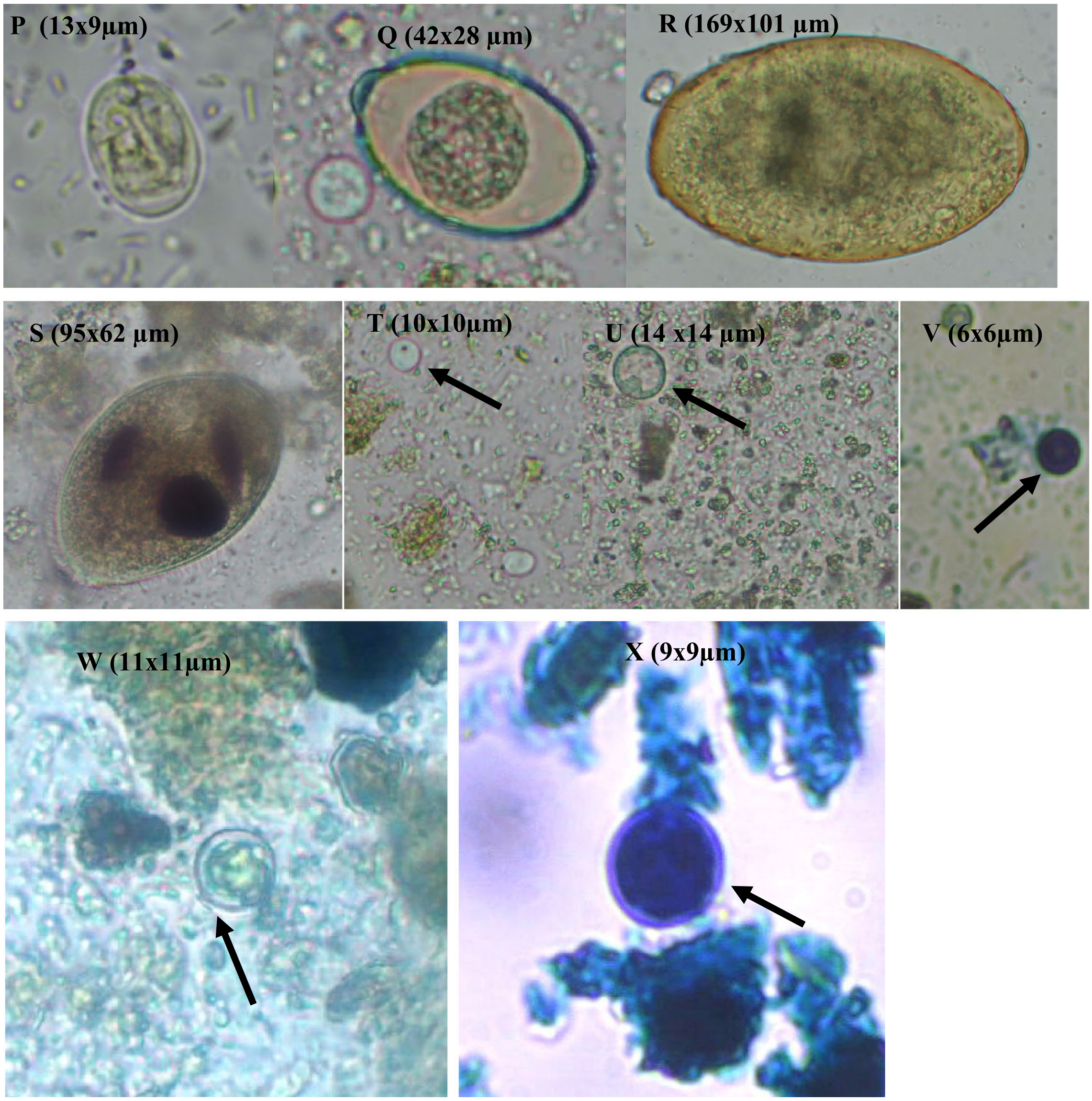

Fig. 2 continued

Discussions

The study of GI parasites of the goat in goat market in the Kathmandu Valley is believed to be exciting research mainly because the Valley obtains various types of goats transported from all over the country reared in different environments, and therefore the goats may accompany with different parasites. The current study has tried to explore the prevalence of GI parasites in those goats. In this study, the recorded prevalence of 87.25% was lower than reported from the West region of Cameroon (90.4%) (Ntonifor et al. 2013), Slovakia (95.9%) (Babják et al. 2017), West Indies (98%) (Chikweto et al. 2018), and Iraq (91.5%) (Hassan and Barzinji 2018), and higher than those from various States of India (50.51–86.05%) (Pathak and Pal 2008; Gul and Tak 2016; Tariq et al. 2010; Choubisa and Jaroli 2013; Verma et al. 2018; Dappawar et al. 2018), from Poland (80.6%) (Gorski et al. 2004), and from Central Oromia, Ethiopia (81.5%) (Kumsa et al. 2011). The different results might be due to the difference in deworming and management practice, season, age and sex of the host, and altitude and climatic conditions (Daniel et al. 2014; Gul and Tak 2016). Interestingly, 100% sample showing concurrent infection with more than two parasites, including up to septuple infection, indicating a considerable density of the parasites in those goats. Mixed infections are critical

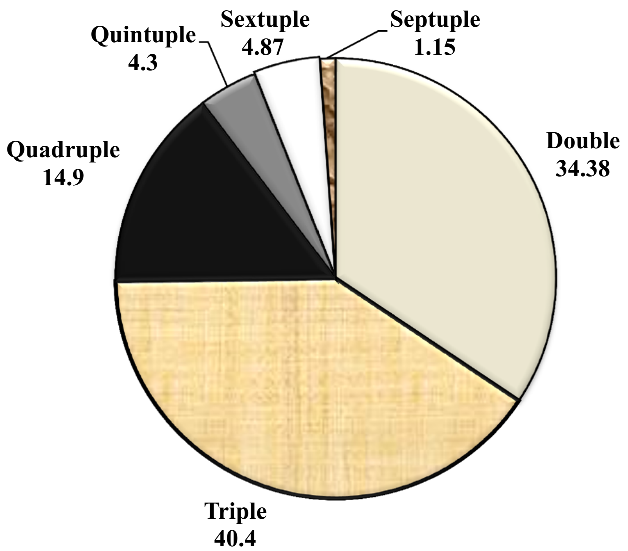

Fig. 3 Percent of co-infection pattern in the feces of goats in Goat Market, Kalanki, Kathmandu Valley. Double = two, triple = three, quadruple = four, quintuple = five, sextuple = six, and septuple = seven different parasites

as they consequently lead to the death of hosts. For example, severe malnutrition leading to pulmonary edema following the death of the Black Bengal goat kid has been reported as a consequence of the mixed infection of *Moniezia* and *Trichuris* (Maity et al. 2018).

This study found *Eimeria* as the most common species of GI tract in which it leads to goat coccidiosis, the most common enteric disease of goats (Agyei et al. 2004; Hassan and Barzinji 2018; Kaur et al. 2019). This coccidian parasite may induce high mortality rates, and decrease in the production of milk, hair, or meat (Koudela and Boková 1998; Donkin and Boyazoglu 2004); however, the disease may be subclinical in feature (Mohamaden et al. 2018). Its current prevalence rate was lower than reported by previous studies from western Ukraine (100%) (Balicka-Ramisz et al. 2012), from Southern Portugal (98.6%) (Silva et al. 2014), from Punjab state of India (96.7%) (Kaur et al. 2019), from Kuala Terengganu, Setiu, and Besut (89.2%) (Mat Yusof and Md Isa 2016), from Czech Republic (92.2%) (Koudela and Boková 1998), and higher than those from India (5.94–71.45%) (Verma et al. 2018; Sharma et al. 2009; Dappawar et al. 2018), from northern Jordan (54%) (Abo-Shehada and Abo-Farieha 2003), from Poland (76%) (Balicka-Ramisz et al. 2012), from Iraq (42.2% in adults and 19.2% in kids) (Hassan and Barzinji 2018), and from Egypt (60%) (Mohamaden et al. 2018) indicating a critical eimeriosis exists in the Nepalese goats.

Another coccidian parasite, *Cryptosporidium* sp. was currently reported to be a prevalence rate (4.0%) that was higher than reported from China (3.48%) (Wang et al. 2014), and from Chandigarh, Punjab, and Haryana in India (0.5%) (Utaaker et al. 2017), and lower than reported from Pakistan (20%) (Elmadawy and Diab 2017), from China (11.4%) (Mi et al. 2014), from Brazil (4.8%) (Bomfim et al. 2005), and from Greece (7.1%) (Tzanidakis et al. 2014). Although *C. xiaoi* and a zoonotic species, *C. ubiquitum* (Wang et al. 2014; Mi et al. 2014; Tzanidakis et al. 2014; Kaupke et al. 2017), are associated with slightly older age groups of goats, adult animals develop immunity and in general, *Cryptosporidium* spp. are recognized to primarily infect goat kids (Utaaker et al. 2017; Robertson et al. 2014). Thus, the low prevalence may be probably since most samples were derived from adult animals. However, few factors like age and sanitary condition of the stalls and stalls made of wood slats and raised from the ground are possible determinants of cryptosporidiosis (Bomfim et al. 2005).

We have recorded various morphologic forms of *Entamoeba* spp. similar to those listed in goats by others (Noble and Noble 1952; Hoare 2009; Hooshyar et al. 2015) indicating this sarcodina is common in goats. Our record of prevalence rate of *Entamoeba* spp. was higher than reported from Western Australia (6.4%) (Al-Habsi et al. 2017) and Brazil (1.8%) (Radavelli et al. 2014). We also reported *Balantidium* with a prevalence rate of 7.75% that was higher than reported from Egypt (7.1%) (Elmadawy and Diab 2017), from Kenya (3%) (Kanyari et al. 2009), from Tanzania (4.8%) (Mhoma et al. 2011), and from Pakistan (3.46%) (Jamil et al. 2015). Although goats are not natural hosts of this ciliate (Hassell et al. 2013), it may lead to inflammatory reactions in the large intestines (Elmadawy and Diab 2017). Both protozoa are zoonotically important for human (Slifko et al. 2000).

The current study found *P. hominis* (1.25%), *Blastocystis* (0.75%), and *Giardia* (0.5%) indicating although found in goats, these species are critical for human health (Li et al. 2018; Kamaruddin et al. 2014; Dimasuay and Rivera 2013; Tzanidakis et al. 2014; Utaaker et al. 2017). Although *P. hominis* was reported from solid, semisolid, and diarrheic stools, the latter can provide anaerobic environment fostering opportunistic overgrowth of this flagellate (Li et al. 2016). Its low prevalence might be associated with the typical dry and solid structure of goat fecal samples that lack a favorable environment for the growth and reproduction. The current prevalence rate of *Giardia* sp. was lower than reported from China (4.8%) (Chen et al. 2019), from Egypt (5%) (Elmadawy and Diab 2017), from Brazil (22.6%) (Radavelli et al. 2014), from Greece (40.4%) (Tzanidakis et al. 2014), and from Nigeria (45.7%) (Akinkuotu et al. 2019). Several factors like age and the sanitary condition of the stalls might determine the *Giardia* infection (Bomfim et al. 2005). *Giardia* infection, though asymptomatic, may cause diarrhea and ill-thrift leading economic losses and reduced welfare of the flock

(Utaaker et al. 2017; O'Handley and Olson 2006) and can pose a significant risk of zoonosis (Akinkuotu et al. 2019).

Notably, we have firstly reported the presence of *Cyclospora*-like oocysts in the stool with a 1% prevalence rate. The previous study in Tamil Nadu in India found that the range of prevalence was 0–33.3% (average prevalence 1.85%) indicating a slightly lower prevalence rate in our samples. This discrimination might be because the authors used PCR assays, and we depended only on a direct wet mount and modified acid-fast techniques. *Cyclospora* spp. have been conflictingly reported from dogs, chicken, monkeys (Chu et al. 2004) and red panda (Lama et al. 2015) from Nepal and cattle from China (Li et al. 2007), captive primates from Europe (Marangi et al. 2015) and monkeys from Ethiopia (Eberhard et al. 1999) and China (Li et al. 2015) although previous studies suggested that there were no oocysts similar to *Cyclospora* in domestic animals and birds in Nepal (Ghimire et al. 2010) and Haiti (Mark et al. 1999). Thus, in the absence of the epidemiologic proofs, further studies of this coccidian in goats to act as natural reservoir hosts should be investigated.

Regarding nematodes, we did not perform larval cultures for a complete diagnosis, and thus, we named 'strongyle' that includes the strongyle-type of eggs of *Haemonchus, Ostertagia, Trichostrongylus, Teladorsagia*, *Cooperia*, *Bunostomum*, and *Oesophagostomum*. We found its prevalence (59.25%), and interestingly in different states of India, the range of prevalence was 26.9–85.1% (Dixit et al. 2017; Singh et al. 2015; Verma et al. 2018; Dappawar et al. 2018). These parasites show a dilution effect of GI infections between domestic livestock species, and that is why their prevalence is negatively related to the goats' densities (Sun et al. 2018). It has been reported that 7.4% of deaths in goats were caused by strongyle infestation, suggesting a critical role of these nematodes in Nepal (Khakural 2003). Among strongyles detected in sheep (66%) and goats (89%), *Haemonchus contortus* has been proved to be a causal agent leading 29% mortality suggesting strongyles are critical for the livestock health (Chikweto et al. 2018). *Trichuris* sp. was comprised of about 29.75% prevalence which was higher than reported from India (3.24–20.8%) (Sorathiya et al. 2017; Das et al. 2017; Dappawar et al. 2018; Singh et al. 2015; Shakya et al. 2017). *Strongyloides* egg contains larva that comprised 28.75% prevalence rate which was higher than reported from India (0.79–11.9%) (Sorathiya et al. 2017; Singh et al. 2015; Dappawar et al. 2018; Dixit et al. 2017) and from Iraq (7.7% in kids and 8.9% in adults) (Hassan and Barzinji 2018). *Capillaria* spp., though look like *Trichuris*, are critical in goats and share a wide range of herbivores including man (Rehbein and Haupt 1994; Fakae 1990; Odermatt et al. 2010). Notably, our reports of *Ascaris* spp. in 1% of the sample may suggest that these nematodes are successfully matured and established in the goats (Rajamohan et al. 1970; Martin 1926). The high prevalence of these nematodes might be attributed to their usual favorable transmission via ingestion of infective egg or larval stages and or transmission via udder milk and larval penetration.

Cestode like *Moniezia* was prevalent (21.75%) in goats, and it was higher (3.0–18.7%) than reported from India (Das et al. 2017; Bihaqi et al. 2017; Choubisa and Jaroli 2013; Singh et al. 2015; Verma et al. 2018). It is transmitted via the ingestion of pasture mite infected with larva. Although this parasite is regarded to be non-pathogenic, postmortem reports of 13.95% death cases of goats were linked to monieziasis in Nepal (Khakural 2003), and these cases might reflect the consequences of coinfection (Maity et al. 2018). *Fasciola* and *Paramphistomum* are the trematodes reported in this study. The prevalence rate (10.25%) of *Fasciola* said was higher than reported from India (0.32 to 4.06%) (Dixit et al. 2017; Singh et al. 2015; Sorathiya et al. 2017), and lower than reported from Nepal (21.28–35%) (Singh and Sah 1994; Shrestha 1996). The prevalence rate (0.5%) of *Paramphistomum* was lower than reported from India (4.9–13.6%) (Godara et al. 2014; Maitra et al. 2014). They are highly pathogenic with the potentiality to cause anemia, hemorrhage, loss of weight, weakness, enhanced mortality, and decreased production (Singh et al. 1984; Godara et al. 2014; Hashemnia et al. 2015). These trematodes are transmitted via consumption of water or food containing metacercarial stages, and in Nepalese contexts, fascioliosis is prevalent in the stall-fed buffaloes compared to those in the grazing populations due to the presence of metacercaria in the lower parts of the straw (Joshi and Mahato 2013).

Conclusions

In conclusion, male goats brought to the Kathmandu valleys from different parts of the country are heavily infected with GI parasites that can have high morbidity and mortality. Although few of these parasites may not lead to pathologic consequences, many of them are associated with both veterinary and public health significance, thus, posing a significant challenge to both humans and animals of a particular area. This situation might be worsening when the infected goats are transported from a place to crowd areas for meat purposes. Critical health problems might exist for the presence of *Cryptosporidium, Trichostrongylus, Entamoeba, Giardia*, and *Balantidium* in the environment. Thus, based on our results, we recommend government and non-governmental organization to conduct an awareness program related to zoonosis, chemotherapeutics, and management practices involving rotational grazing and preventive options for farmers and goat business persons connected to the meat industry. Although there may be substantial data of parasitic infestation in goats, we believe that this observation is supportive in planning chemotherapeutic and prophylactic strategies for goats around the country.

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Author's contribution TRG planned, worked in the laboratory, analyzed the data, and wrote the manuscript. NB worked in the field and laboratory and analyzed the data. Both authors read the paper and finalized it.

Compliance with ethical standard

Conflict of interest The authors declare that they have no conflict of interest. The work is a part of the research proposal of Red Book (075/076, Planning Division, NAST).

Animal rights The authors declare that the study was conducted on naturally-infected goats which had been brought to the goat market from all over Nepal. No experimental infection was established during this research work. Goats were not directly involved in the study.

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