

Gastro-intestinal Parasites of Sheep (*Ovis aries*, Linnaeus, 1758) in Laxmipur VDC, Dang, Nepal

G. Rana¹ and J. R. Subedi^{1,*}

¹Central Department of Zoology TU, Kirtipur, Kathmandu

*Corresponding author: Janak Raj Subedi, janzoology@gmail.com

ABSTRACT

Sheep are being raised by the farmers of the Dang district for an economic purpose, but parasitic diseases remain a significant problem to their health. The study was conducted to determine the prevalence and seasonal occurrence of gastro-intestinal parasites of sheep in the Laxmipur Village Development Committee of Dang district. A total of 110 fecal samples were collected from April to September 2017, preserved in 2.5% potassium dichromate, and microscopically examined using concentration techniques. The study revealed overall 80% prevalence of gastro intestinal parasites including total 15 genera belonging to 5 classes. The gastro-intestinal parasites found in sheep were *Eimeria* sp., *Entamoeba* sp., *Haemonchus* sp., *Strongyloides* sp., *Strongyle* sp., *Trichostrongylus* sp., *Oesophagostomum* sp., *Bunostomum* sp., *Trichuris* sp., *Nematodirus* sp., *Chabertia* sp., *Ascaris* sp., *Fasciola* sp., *Paramphistomum* sp. and *Moniezia* sp., Parasitic infection during rainy season (92.93%) was found significantly higher compared to summer season (67.27) ($\chi^2=9.6023$; $p<0.05$). Most number of sheep (80.68%) were found infected with more than two parasites, among them the combination of *Haemonchus* sp., *Strongyloides* sp. and *Strongyle* sp. were most common. The results suggest that major nematodes belonging to genera *Haemonchus*, *Strongyloides* and *Strongyle* were prevalent in the study areas. The agro-climatic conditions like overstocking of the animals, grazing of young and adult animals together with poorly drained land provide an ideal condition for the transmission of the endoparasites to build up clinical infestation of the host.

Keywords: Concentration, Concurrency, Gastro-intestinal parasites, *Haemonchus* sp., Seasonal occurrence, Sheep

INTRODUCTION

Ruminants are characterized by the presence of a four - compartment stomach, among these sheep (*Ovis aries*) are globally distributed, widely adapted to different climates, mainly arid and semi-arid areas of the world. Sheep are economically important for meat, manure, milk, hide and skin, wool, horns, bones, medicine etc. Gastro-intestinal parasitic infection is one of the greatest limiting factors in the production of sheep worldwide (Perry and Randolph, 1999; Torres-Acosta and Hoste, 2008; Calvete *et al.*, 2014; Mendoza-de *et al.*, 1998). A total of 8,00,658 sheep population was reported in Nepal in 2016, including 34,091 from

Dang. (SIONA, 2016). The temperature, moisture, rainfall, sharing of grazing area of other ruminants etc. enhance the occurrence of the parasite in Dang. Similarly, the farmers are suffered from the unavailability of veterinarians. Gastrointestinal parasitism is a primary cause of losses in sheep production in dang. Parasitic infections cause a serious threat and limit the productivity of livestock due to the associated morbidity, mortality, and cost of treatment and control measures (Nwosu *et al.*, 2007; Raza *et al.*, 2010; Lashari and Tasawar, 2011). The diseases are usually transmitted by the ingestion of the infective eggs/oocyst or larvae or by its penetration through the skin (Ibrahim *et al.*, 2008). The prevalence of gastrointestinal helminths is related to the agro-climatic conditions like quantity, and quality of pasture, temperature, humidity and grazing behavior of the host (Pal and Qayyum, 1993). The degree of nematode infection varies according to the host immune response to these worms (Greer, 2008). Commonly occurring helminth parasites are *Fasciola* sp., *Haemonchus* sp., *Ostertagia* sp., *Strongylus* sp., *Chabertia* sp., *Trichostrongylus* sp., *Cooperia* sp. (Abouzeid *et al.*, 2010), *Strongyloides* sp., *Trichuris* sp., *Oesophagostomum* sp. (Yaro *et al.*, 2015), *Bunostomum* sp., *Cotylophora* sp., *Dicrocoelium* sp., *Echinococcus* sp., *Moniezia* sp., *Nematodirus* sp., *Paramphistomum* sp. (Raza *et al.*, 2014).

Coccidiosis parasites of small ruminants is a protozoan caused by several species of the genus *Eimeria* located in the different parts of the intestine affects young animals. Coccidian parasites damage the lining of intestine that the animals need to absorb nutrients. Therefore, the most common sign of coccidian infestation is diarrhea (detected by dirty hind ends), and failure to thrive, or weight loss (Villarroel, 2013). Similarly, the most occurring gastrointestinal nematodes infecting sheep are *Haemonchus*, *Strongyloides*, *Strongyle*, *Trichostrongylus* and *Oesophagostomum*. Nematodes adversely affect ruminants, causing hematological, biochemical disturbances, anorexia, weight loss, poor reproductive performance, and even death of sheep (Yaro *et al.*, 2015). The blood - sucking parasite *Haemonchus contortus* which is found in the abomasum of the sheep and goat causes significant blood loss; each worm removes 0.05 ml of blood per day so that sheep with *H. contortus* may lose about 250 ml per day resulting in a decrease in erythrocytes, lymphocytes, hemoglobin, PCV, body weight and wool growth (Urquhart *et al.*, 1987). The present study was undertaken to determine the prevalence and seasonal fluctuation of gastrointestinal parasites in sheep to provide the baseline data for the management of the potential disease.

MATERIALS AND METHODS

Study Area

The study was conducted in Laxmipur Village Development Committee Dang which is located in the Mid-Western Region of Nepal. It is located about 5 km east of the district headquarter (Fig 1. Dang valley is the largest valley in Asia; this district is also known as inner terai. It lies on the geographical coordinates of latitude 27°37' to 28° 39' North and longitude 82°2' to 82°54' East. The maximum and minimum temperatures of this district are around 34°C and 14°C respectively and rainfall averages more than 1300 mm annually.

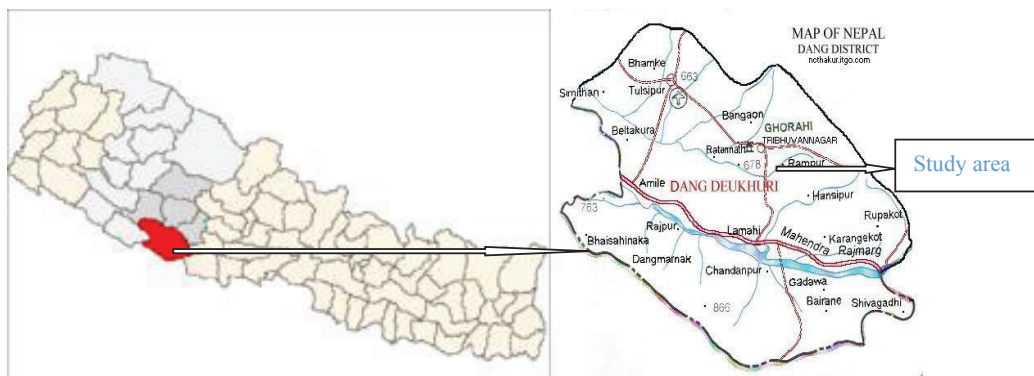


Fig.1 Study area

Collection, preservation, and laboratory examination of sample

A total of 110 faecal samples of sheep were collected purposively from the study area in two seasons (summer and rainy) from April to June and August to September 2017. Fresh faecal samples were collected immediately after voiding, transferred inside the sterile plastic vials, and preserved in 2.5% potassium dichromate. The faecal samples were transported to the Central Department of Zoology TU, Kirtipur Kathmandu and examined by concentration techniques (floatation and sedimentation). Approximately 3 gm of faecal sample was placed in a beaker with 42 ml of water and filtered. The filtrate solution was centrifuged for 5 minutes. The filtrate was saturated with NaCl, and again centrifuged. The top mixture was examined by adding methylene blue and the sediment was stained with an iodine solution to detect eggs, trophozoites and cysts of parasites (Soulsby, 2009).

Identification and data analysis

Identification was determined based on shape and size as described previously (Soulsby, 1982; Indre et al., 2010; Sangma et al., 2012 and Poddar et al., 2017). The data were coded and entered into a Microsoft Excel spreadsheet. Data were used to determine the prevalence of parasites. In addition, the data were tested whether these are equally distributed according to seasons. All data were analyzed in R, version 3.3.1 software packages. The differences in the parasite occurrences on different groups and seasons were compared using Chi-square tests. Data were analyzed using the R Programme (R Core Team 2019). In all cases 95% confidence level (CI) and $p < 0.05$ were considered for statistical significance.

RESULTS

Overall prevalence of gastro-intestinal parasites in sheep

Overall (80%) samples were found to be positive for parasitic egg, cyst, and larvae. A total of 13 helminths and two protozoan parasites were isolated from the faecal samples and identified. The intestinal protozoan parasites were *Eimeria* sp., and *Entamoeba* sp. The

identified helminths were *Haemonchus* sp., *Strongyloides* sp., *Strongyle* sp., *Trichostrongylus* sp., *Bunostomum* sp., *Oesophagostomu* sp., *Trichuris* sp., *Nematodirus* sp., *Chabertia* sp., *Ascaris* sp., *Moniezia* sp., *Fasciola* sp. and *Paramphistomum* sp. For intestinal protozoan parasites *Eimeria* sp. (15%), showed the highest prevalence than *Entamoeba* sp. Similarly, for intestinal helminths parasites *Haemonchus* sp. showed a higher prevalence than other parasites (Table1).

Table.1 Prevalence of gastro-intestinal parasites in sheep.

| S.N. | Class | Parasites Name | Frequency | Prevalence % |
|------|---------------------------|-----------------------------|---------------------|--------------|
| 1 | Sarcodina | <i>Eimeria</i> sp. | 4 | 3.64% |
| 2 | Sporozoa | <i>Entamoeba</i> sp. | 3 | 2.73% |
| 3 | Nematoda | <i>Haemonchus</i> sp. | 37 | 33.64% |
| 4 | | <i>Bunostomum</i> sp. | 11 | 10% |
| 5 | | <i>Oesophagostomum</i> sp. | 15 | 13.64% |
| 6 | | <i>Strongyle</i> sp. | 28 | 25.45% |
| 7 | | <i>Strongyloides</i> sp. | 34 | 30.91% |
| 8 | | <i>Trichostrongylus</i> sp. | 16 | 14.55% |
| 9 | | <i>Nematodirus</i> sp. | 6 | 5.45% |
| 10 | | <i>Trichuris</i> sp. | 9 | 8.18% |
| 11 | | <i>Ascaris</i> sp. | 2 | 1.82% |
| 12 | | <i>Chabertia</i> sp. | 5 | 4.54% |
| 13 | | Trematoda | <i>Fasciola</i> sp. | 12 |
| 14 | <i>Paramphistomum</i> sp. | | 8 | 7.27% |
| 15 | Cestoda | <i>Moniezia</i> sp. | 7 | 6.36% |

Season-wise prevalence and concurrency of the parasite in sheep

The study period was categorized as rainy and summer seasons. The relationship between the two seasons indicated that among 110 faecal samples (55 from rainy and 55 from summer) occurrence of parasitic infection in sheep showed higher infection of gastrointestinal parasites in the rainy season (N=51) than in the summer season (N=37) (Fig

2). The occurrence of gastro-intestinal parasites in sheep at two seasons was varied ($\chi^2=9.6023$; $p<0.05$). Overall *Haemonchus* sp. was found the highest prevalence in both the season; followed by *Strongyle* sp., *Strongyloides* sp., *Trichostrongylus* sp., *Oesophagostomum* sp., *Trichuris* sp., *Fasciola* sp., *Bunostomum* sp., *Paramphistomum* sp., *Chabertia* sp., *Moniezia* sp., *Eimeria* sp., and *Entamoeba* sp. Similarly, *Nematodirus* sp. and *Ascaris* sp. were not found in summer seasons but recorded only in the rainy season (Table 2).

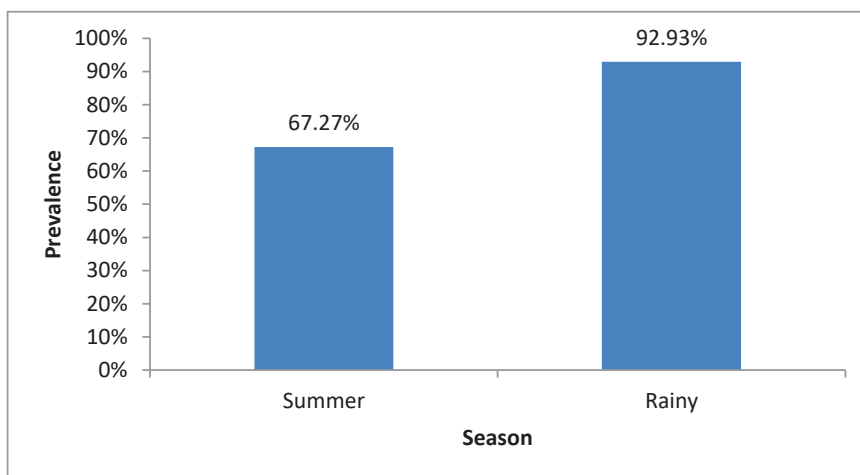


Figure 2. Season-wise prevalence of gastro-intestinal parasite in sheep

Table 2. Overall season-wise prevalence of gastro-intestinal parasite in sheep

| S.N | Parasite Name | Summer season (April) | | Rainy season (August) | | χ^2 | p-value |
|-----|----------------------------|-----------------------|----------------|-----------------------|----------------|----------|---------|
| | | No. of positive | Prevalence (%) | No. of positive | Prevalence (%) | | |
| | <i>Eimeria</i> sp. | 2 | 3.63 | 2 | 3.63 | 0 | 1 |
| | <i>Entamoeba</i> sp. | 1 | 1.82 | 2 | 3.63 | 0 | 1 |
| | <i>Haemonchus</i> sp. | 18 | 32.73 | 19 | 34.55 | 0 | 1 |
| | <i>Bunostomum</i> sp. | 5 | 9.09 | 6 | 10.91 | 1.121 | 1 |
| | <i>Oesophagostomum</i> sp. | 6 | 10.91 | 9 | 16.36 | 0.309 | 0.578 |
| | <i>Strongyle</i> sp. | 11 | 20 | 17 | 30.91 | 1.198 | 0.274 |
| | <i>Strongyloides</i> sp. | 12 | 21.82 | 22 | 20 | 3.448 | 0.06 |
| | <i>Trichostrongylus</i> | 7 | 12.73 | 9 | 16.36 | 0.073 | 0.787 |
| | <i>Nematodirus</i> sp. | 0 | 0 | 6 | 10.91 | 4.407 | 0.036 |
| | <i>Trichuris</i> sp. | 2 | 3.63 | 7 | 12.73 | 1.936 | 0.164 |

| | | | | | | | |
|--|---------------------------|---|------|---|-------|-------|-------|
| | <i>Ascaris</i> sp. | 0 | 0 | 2 | 3.63 | 0.509 | 0.476 |
| | <i>Chabertia</i> sp. | 2 | 3.63 | 3 | 5.45 | 0 | 1 |
| | <i>Fasciola</i> sp. | 5 | 9.09 | 7 | 12.73 | 0.093 | 0.76 |
| | <i>Paramphistomum</i> sp. | 3 | 5.45 | 5 | 9.09 | 0.135 | 0.713 |
| | <i>Moniezia</i> sp. | 3 | 5.45 | 4 | 7.27 | 1.127 | 1 |

Polyparasitism was observed among the examined fecal samples of the sheep. The result indicated that the maximum number of sheep were suffered from mixed parasite infection, either protozoan or helminth parasites showing prevalence of 71 samples for mixed infection and 17 samples were detected to have a single infection (Fig 3). Among the mixed infection the combination of *Haemonchus* sp., *Strongyloides* sp. and *Strongyle* sp. were most common in maximum samples followed by *Trichostrongylus* sp., *Oesophagostomum* sp., *Trichuris* sp., *Fasciola* sp., *Bunostomum* sp., *Nematodirus* sp., *Paramphistomum* sp., *Chabertia* sp., *Moniezia* sp., *Eimeria* sp., *Entamoeba* sp. and *Ascaris* sp. also common parasites in single infection during both seasons. Multiple infections was not observed in the summer season. However, the distribution of single and multiple parasites was not varied among the examined sheep $p>0.05$) (Fig 3).

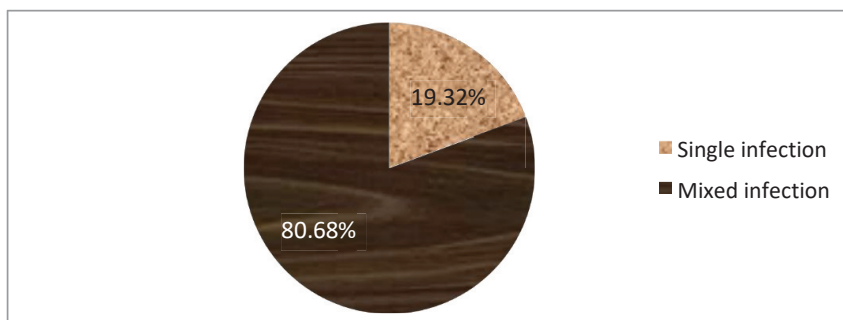


Figure 3. Polyparasitism of gastro-intestinal parasites

DISCUSSION

Plenty of researches has been done on sheep in a global context, but hardly a few in the national context. The study sets to investigate the presence of gastro-intestinal parasites in sheep in Dang. The prevalence of gastro-intestinal parasites is considerably influenced by climatic and geographical factors. Generally, the warm and humid conditions, continuous high rainfall, a decrease of grazing area, contaminated pasture, nutritional factors, and traditional rearing system provide a good condition for many gastro-intestinal parasites to flourish in the study area. In rural areas such as Dang, veterinary services to support farmers technically was not enough and people don't frequently report the need for treatment of that farm animals. In addition, the current climatic fluctuation in seasons such as rainy and summer might also increase the more parasitic prevalence. The climatic conditions such as temperature, moisture, rainfall, humidity, etc. in the grazing area,

faecal pellet, and grasses enhance the high prevalence of gastro-intestinal parasites in the study area. Apart from the above, these factors also increase the rapid life cycle of parasites from eggs to larvae in warm wet conditions (Mekonnen, 2007). Sheep are grazing in different grazing areas according to the season, while moving sheep from one pasture land to another they might be infected by parasites. Generally, the mode of infection is high in moveable livestock than in domesticated animals (Svensson *et al.* 2000 and Rook *et al.*, 2004). Furthermore, a higher prevalence of the nematodes in the present area might be due to the grazing of both young, and adult in poorly drained land, malnutrition, weak immunity and sharing of the same land for grazing by cattle, sheep, and goats (Asif *et al.*, 2008). Nematode infection seems higher in the studied sheep might be due to overcrowding of sheep and cattle, inadequate nutrition, the susceptibility of entry of parasites, the longevity of larval stage in the soil for a long period of time and finally, intermixing of pasture land and exposure of eggs and embryo. Similarly, the age of host dietary level, physiology, exposure of parasites, etc. increase the eggs output of nematodes (Miller *et al.*, 2012).

A total of 110 faecal samples of sheep was collected from Dang and examined by sedimentation and concentration methods. The majority of samples (80%) were found to be positive for both protozoan and helminth parasites. This prevalence rate of sheep was almost similar results of Getachew *et al.* (2016) from Ethiopia, Africa, Mavrot *et al.* (2015) from Europe and Koinari *et al.* (2013) from Papua New Guinea, Australia who reported 86.6%, 86% and 72% respectively with minor differences. Similarly, the present result is also in harmony with the range of 72% to 80% in different South Asian countries (Yadav *et al.*, 2006; Asif *et al.*, 2008 and Sangma *et al.*, 2012). Previous studies among sheep from different area of Nepal has shown the positivity ranging from 78% to 87% Khakural *et al.*, 2005 and Acharya, 2017) though prevalence rates were seen to vary according to locality. The prevalence of parasites in these sheep suggests that parasitic infections are among the main health problems in small ruminants globally (Mbuh *et al.*, 2008; Kantzoura *et al.*, 2012). The prevalence of parasites in sheep observed in the present study is lower than the study carried out by (Ibrahim *et al.*, 2014; Pedreira *et al.*, 2006 and Bansal *et al.*, 2015). The higher prevalence observed in different parts of countries could be due to overstocking, poor nutrition, poor management practice of the animals (lack of sanitation) and frequent exposure to the communal grazing lands that have been contaminated. Whereas, in comparison to other studies conducted in different countries present study recorded higher prevalence in sheep which range from 7% to 66% (Sultan *et al.*, 2016; Kantzoura *et al.*, 2012; Poddar *et al.*, 2017; Mohammedameen, 2016; Vijayalakshmi, 2015 and Lemma and Aber 2013). Lower prevalence in those countries could be due to good management practice, existence of unfavorable climatic or environmental factors that could support prolonged survival and development of the infective larval stage of parasites. The high infection rates of GIT helminths in sheep in the study area could be due to the presence of favorable environmental conditions for the existence and development of the gastro-intestinal parasites, foraging behavior of sheep (Tefera *et al.*, 2009; Bansal *et al.*, 2015).

A total of 13 helminths and two protozoan parasites were isolated from the faecal samples and identified from the study area. Among them *Eimeria* sp. and *Entamoeba* sp. from the protozoan and *Haemonchus* sp., *Strongyloides* sp., *Strongyle* sp., *Trichostrongylus* sp., *Oesophagostomum* sp., *Trichuris* sp., *Nematodirus* sp., *Chabertia* sp., *Ascaris* sp., *Moniezia*

sp., *Fasciola* sp., *Bunostomum* sp. and *Paramphistomum* sp. were identified as helminths parasites. In the current study, *Haemonchus* sp. (33.64%) showed the highest prevalence in helminths parasites followed by others which is almost similar to the work of Almalaik *et al.* (2008), Tariq *et al.* (2008), Tefera *et al.* (2009) and Khajuria *et al.* (2013) who also reported *Haemonchus* sp. as predominant parasite by 53.4%, 59.6%, 67.5% and 61.18% from Tulus Area, Sudan, Kashmir valley, India, Bedelle, South-Western Ethiopia and Jammu, India respectively although the prevalence rate is higher than present finding. The higher prevalence of *Haemonchus* sp. could be due to poor management practices, grazing, traditional rearing system, resistance of parasites, nutritional factors, etc. The prevalence rate of *Ascaris* sp. (1.82%) in the present study is less than the report of Ibukun and Oludunsin (2015) who reported 9.4% from Nigeria.

Among the protozoan parasites the prevalence rate of *Eimeria* sp. is almost similar with the report of Yadav *et al.* (2006) who reported 6.73%, from R.S. Pura, Bishnah and Samba tehsils of Jammu district, India, less than the report of Bhat *et al.* (2012), Koinari *et al.* (2013), Ibrahim *et al.* (2014) and Sultan *et al.* (2016) who reported 9.8%, 17.3%, 11.7% and 16.52% from Kashmir valley of India, Papua New Guinea, Jimma town, Western Ethiopia and Nile Delta, Egypt respectively and less than the report of Gizachew *et al.* (2014) who reported 30.9% from Bako town, Western Ethiopia. From these results, it was seen that cooler and temperate climate is suitable for the development of *Eimeria* sp. than warm and tropical climates. Only a few studies have been done regarding protozoan parasites. Furthermore, *Entamoeba* sp. (2.73%) is another protozoan parasite identified in study area.

Regarding the concurrency of gastro intestinal parasites both singles and mixed infections were recorded. Out of positive samples, (80.68%) samples for mixed infection and (19.32%) samples were detected to have single infection. Among the mixed infection the combination of *Haemonchus* sp., *Strongyloides* sp. and *Strongyle* sp. were most common in maximum samples followed by other parasites. This finding is in harmony with reports of previous studies of Raza *et al.* (2014) and Getachew *et al.* (2016) conducted in Cholistan desert of Pakistan and Areka, Ethiopia who also detected mixed infection in their studies. In present study, mixed infection was detected in 80.68% samples which is higher than the finding of previous studies conducted by Kenea *et al.* (2015) and Getachew *et al.* (2016) who reported 17.7% and 26% from, Kaffa and Bench Maji zones, Southwest Ethiopia and Areka, Ethiopia respectively and almost similar with the study conducted in Patiala and its adjoining areas (Kaur and Kaur, 2008) who reported mixed infection in 85.71% samples. The variance in mixed infection in different studies could be due to a lack of sheep de-worming, less use of anti-parasitic drugs, dietary deficiency, poor management system, lack of knowledge for sanitation and climatic and geographical variation.

Gastro-intestinal parasitic infection in a sheep in the rainy season was found 92.93%, whereas in summer season, 67.27% with a significance difference ($\chi^2=9.6023$; $p<0.05$). The highest prevalence of parasites in rainy season is in consent to the research carried by Bansal *et al.* (2015) and Varadharajan and Vijayalakshmi (2015) in Mhow, Indore and Coastal areas of Tamil Nadu. On the contrary few workers have also observed and reported a high incidence of these parasitic infections in summer (Tariq *et al.*, 2008), autumn (Khalafalla *et al.*, 2011) and spring (Mir *et al.*, 2013) season which is not in agreement with the present

study. During rainfall the number of infective larval stage of nematodes is increased, that's why the animal becomes more susceptible to infection whereas, the infection is generally less during the dry season (Kantzoura *et al.*, 2012). The development and distribution of gastro-intestinal parasitic helminths will show the maximum prevalence during the rainy season, and lower incidence during summer (Dappawar *et al.*, 2018). The high level of gastro-intestinal helminth parasite infections in sheep is because of lack of management and parasite treatment program, mainly on traditional farms. In traditional sheep rearing enclosure sanitation is not possible for accurate attention, so the transmission of different gastro-intestinal parasites occurs.

CONCLUSION AND RECOMMENDATIONS

The present study demonstrates that the sheep in Dang were affected by various gastro-intestinal parasites. Good husbandry practices, veterinary health program and appropriate prevention, treatment with broad spectrum anthelmintic and control strategy actions should be adopted by both stockowners and government to minimize the gastro-intestinal parasites of sheep.

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