Research Article

Hematological Alterations in Sheep and Goats Infected with *Ostertagia* Species in Mansehra, Khyber Pakhtunkhwa, Pakistan

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Abstract | Current study was conducted at Livestock Experimental Station Jaba Mansehra. Fecal and blood samples were collected from sheep (*n=154*) and goats (*n=46*) and processed at Veterinary Research and Disease investigation center Abbottabad. Qualitative and quantitative examination was performed using fecal flotation method and modified Mc Master Technique for the presence of *Ostertagia* spp. Higher prevalence of *Ostertagia* spp. was observed in sheep i.e. 53 (34.41%) followed by goats 11 (23.91%). In goats, the prevalence was higher in medium age group (2-3 year) followed by young age group (1-12 months), older age group (3-4 year) and lowest in 1-2 years age group. Prevalence was higher in Beetal (72.73%) followed by Kaghani (18.18%) and Angora (9.09%). In sheep, the prevalence was higher in older (above 4 years) and was lowest in younger animals of 2-3 months age; while it was higher in female as compared to male. Prevalenvce was higher in Ramboulet (39.62%) followed by Ramghani (37.73%) and Kaghani (22.64%). Haematology parameters showed that erythrocytes count, Haemoglobin (Hb), hematocrit and Mean Corpuscular Haemoglobin concentration (MCHC) of goats infected with *Ostertagia* spp were significantly (P<0.05) affected while Mean Corpuscular Volume (MCV), MCHC and Total Leucocyte Count (TLC) of young kids was non-significant (P>0.05) as compared to mid age and adult goats. Infestation resulted in significant hematological changes, including anemia, reduced hemoglobin, and altered serum protein profiles, ultimately compromising animal health and productivity. The findings of study will help to mitigate economic losses by timely implementing disease management strategies.

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Introduction

Ostertagiasis is parasitic disease which is caused by *Teladorsagia circumcinata* in small ruminnats resulting in svere weight loss and production. *Ostertagia* adults are slender reddish-brown worms. Adult males are 6–7 mm (0.24–0.28 in) long, adult females 8–11 mm (0.31–0.43 in) long, and the eggs

70–86 µm (0.0028–0.0034 in) long. Identification of adult medium stomach worms is based on the structure of the bursa, genital cone, and spicules in males and on the dimensions of the esophageal valve and the configuration of the synlophe in males and females. Ostertagiosis in the abomasa of sheep can cause high mortality, low meat and milk production, weight loss and reduced fertility (Radostits *et al.*, 2006). In temperate climates, Ostertagiosis is frequent in grazing sheep (Gelberg, 2011) and can lead to reduced gastric acid secretion and increased serum pepsinogen and gastrin (Kataria *et al.*, 2008). Infection rate of Ostertagia in Sheep is affected by host, breed, sex, age, immunity of the host, species and genotype of the parasite, ambient temperature, rainfall, humidity and vegetation (Tasawer *et al.*, 2011).

Small ruminants (Sheep and Goat) are under threat to infestation with gastrointestinal tract (GIT) helminthes lowering their productive and reproductive performance (Ayaz *et al.*, 2013). GIT helmints reduce nutrients absorption and lead to nutrient deficiency, anaemia and retard growth parameters in kids and lambs (Terefe *et al.*, 2012; Hassan *et al.*, 2011). Globally, GIT parasites are the major problems that decrease the fruitful abilities of small ruminants (Sohail *et al.*, 2023; Zvinorova *et al.*, 2016). Hot and humid atmosphere results in accelerated GIT infection (Velusamy *et al.*, 2015). Occurrence of gastrointestinal helminths in sheep and goats has been recorded from 26.5 to 91.7% in distinct areas of Pakistan (Farooq *et al.*, 2012; Tehmina *et al.*, 2014; Raza *et al.*, 2014).

Livestock population of Pakistan is 218.9 million heads out of which small ruminant like sheep and goat contributes 114.4 million heads (52.26%) of total population. Livestock produce 65,745 metric ton of milk whereas small ruminant like goat and sheep contributes 1.61% of total milk production. Similarly, small ruminants contributes 782 metric ton (14.98%) of meat (mutton) as compared to 4478 metric ton of total meat production (Pakistan Economic Survey, 2021-22).

Keeping in view the abobve facts, current study was conducted in District Mansehra of Province Khyber Pakhtunkhwa Pakistan to investigate the occurrence of *Ostertagia* spp., its relationship with age, sex and breed and its impact on hematological parameters of small ruminants reared at alpine pastures of Livestock Experiment Station (LES) Jaba.

Materials and Methods

Study area

Current study was conducted at Livestock Experimental Station (LES) Jaba (34.4407° N, 73.3254° E) in District Mansehra. Faecal and blood samples were collected as per standard protocols and transported to Parasitology and Pathology Laboratories at Veterinary Research and Disease Investigation Center Abbottabad for further analysis.

Animals

Small ruminant (goats and sheep) reared at Livestock Experimental Station Jaba Mansehra were selected for the sampling.

Faecal samples (n=200/10gm) were collected through rectal route and placed in clean polythene bags, labeled properly with breed, age, date of collection etc and shifted to Ice box for further transportation to the labs (Admasu *et al.*, 2014). Blood sample (n=200/3ml) were collected from jugular vein using 5ml sterile disposable syringe in 3ml EDTA containing vacutainer to prevent coagulation and placed in ice box to prevent deterioration (Jatau *et al.*, 2011).

Data collection

Data regarding age, sex and breed of both sheep and goat was recorded to correlate their affect on parasitic load and hematological parameters.

Qualitative and quantitative examination of fecal sample was performed in Parasitology lab using fecal flotation method and Mc Master Technique for the presence of *Ostertagia* spp.

Fecal flotation method

Approximately 30- 40ml of Flotation solution (saturated sodium chloride solution) was mixed with 3gm of fecal sample, centrifuged and left for 10-15 minutes. Supernatant was taken and examined for parasite egg (Badran *et al.*, 2012).

Modified Mc-master technique

The modified McMaster technique was used to obtain egg per gram (EPG). About 2 gram of fecal sample was taken, crushed and mixed with 28ml of saturated salt cum sugar solution (flotation solution) and then

sieved. Centrifuge12 ml of solution at 1500 RPM for 5 minutes. Fill up McMaster slide with supernatant by means of a transfer pipette. Set slid aside for 5 minutes to let parasite eggs to glide on the surface. Examine it under microscope at 10x objective and count the eggs and oocytes in both chamber by rule leave left lower (LLL). Calculate as follows: (Zajac *et al.*, 2012).

$$EPG = \frac{\text{Eggs counted (No's)}}{\text{Volume observed (ml)}} \times \frac{\text{Total volume (ml)}}{\text{weight of feces (gm)}}$$

Statistical analysis

The results collected /obtained were tabulated in Microsoft excel sheets. The effect of age, breed and sex on occurrence of *Ostertagia* spp. and hematological values was calculated by chi-square from the statistic software version 8.1 (Gomez and Gomez, 1984).

Results

A total of 200 fecal samples (Sheep=154, goats=46) were processed for presence of eggs/oocysts of *Ostertagia* spp. Microscopy results showed that in sheep 53 samples (34.41%) were positive for gastrointestinal *Ostertagia* spp. infestation while in goats 11 samples (23.91%) were positive for eggs/oocysts of *Ostertagia* specie. These results revealed that the prevalence percentage of *Ostertagia* spp. was found higher in sheep as compared to goats (Figure 1).

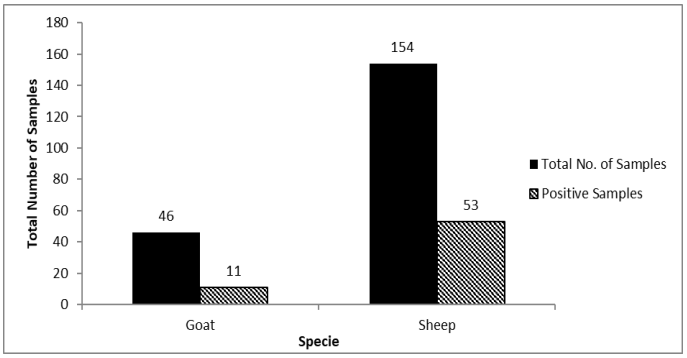

Figure 1: *Prevalence of Ostertagia spp. in small ruminants.*

Age wise prevalence of Ostertagia spp. in goats

The prevalence of *Ostertagia* spp in goats was higher in adult goats of 2-3 years age (36.36%) followed by young goats below one year and mature goats of 3-4 year of age (27.27%) and 18.18% in 1-2 year of age (Figure 2).

Gender wise prevalence of Ostertagia spp. in goats

Effect of gender on occurrence of *Ostertagia* spp. revealed that the prevalence was higher in female goat (54.54%) and lower in males i.e. 45.46% (Figure 3).

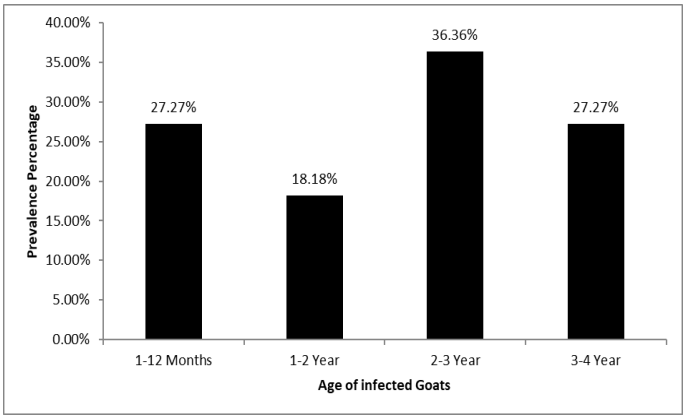

Figure 2: *Age wise prevalence of Ostertagia spp. in goats.*

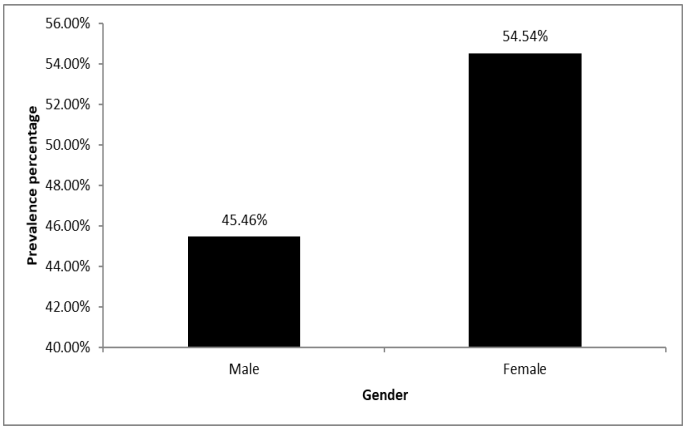

Figure 3: *Gender wise prevalence of Ostertagia spp. in goats.*

Breed wise prevalence of Ostertagia spp. in goat

Fecal samples from 46 goats were processed for *Ostertagia* spp., in which 75.09% (n=35) samples were negative while remaining samples 23.91% (n=11) exhibited infestations in different breeds of goats. Among the positive cases, eggs/oocysts of *Ostertagia* spp was found in Kaghani (18.18%), Angora (9.09%) and Beetal (72.73%). Results revealed that the prevalence of *Ostertagia* spp was found highest in Beetal breed followed by Kaghani and Angora (Figure 4).

Age wise prevalence of Ostertagia spp. in sheep

The results of age wise prevalence of *Ostertagia* spp. revealed that 3.77% samples were positive in 1 to 12 months of sheep, 7.55% were positive for 1-2 years of sheep, 22.64% were positive for 2-3 years of sheep, 24.53% were positive from 3-4 years of sheep and 41.51% were found positive from the sheep of above 4 years sheep. The results of the study revealed that the prevalence percentage was highest in the sheep

of above 4 years of age which decreased gradually in younger age groups. So, the current results conclude that the prevalence percentage of *Ostertagia* spp was increasing with the age of sheep (Figure 5).

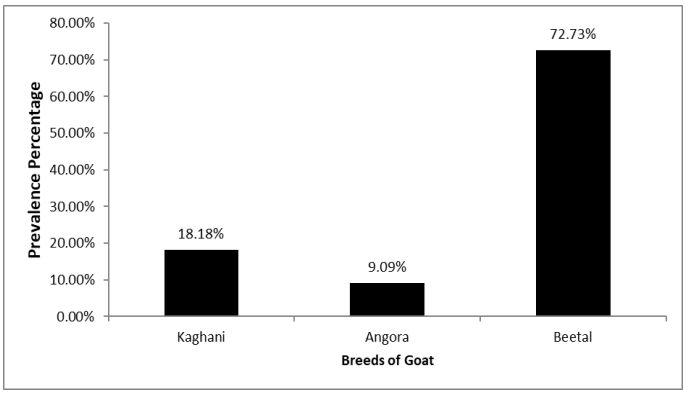

Figure 4: *Breed wise prevalence of Ostertagia spp. in goats.*

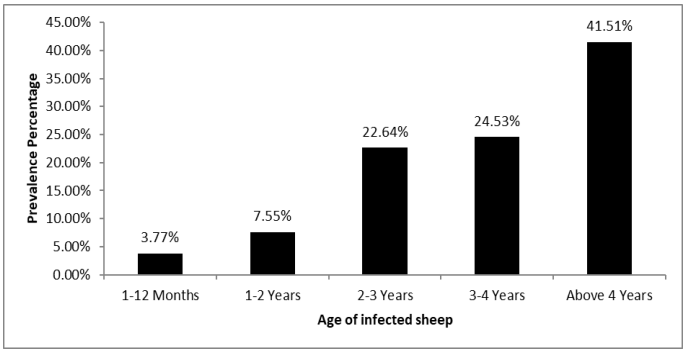

Figure 5: *Age wise prevalence of Ostertagia spp. in sheep.*

Gender wise prevalence of Ostertagia spp. in sheep

Amongst the positive samples for eggs/oocysts of *Ostertagia* spp, it was observed that the prevalence was higher in female sheep (69.81%) as compared to male sheep (30.19%) (Figure 6).

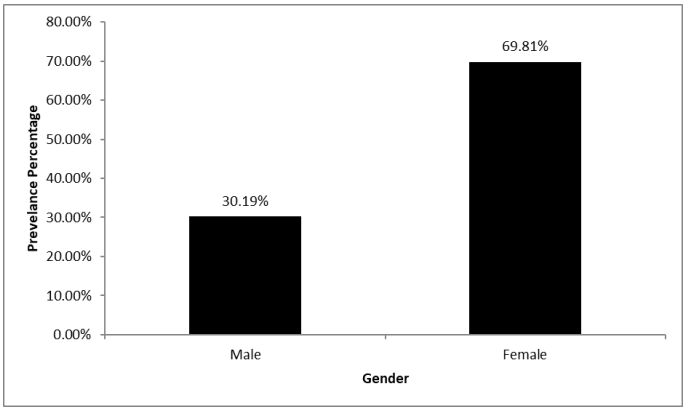

Figure 6: *Gender wise prevalence of Ostertagia spp. in sheep.*

Breed wise prevalence of Ostertagia spp. in sheep

Effect of breed on occurrence of *Ostertagia* spp. in sheep was studied and it was noted that 65.58% samples were negative while remaining samples 34.42% exhibited infestations in different breeds of sheep. Among the positive cases, eggs/oocysts of *Ostertagia* spp were found in Kaghani (22.64%), Ramghani (37.73%) and Ramboullet (39.62%). Results revealed that the prevalence of *Ostertagia*spp was found highest in Ramboullet breed followed by Ramghani and Kaghani (Figure 7).

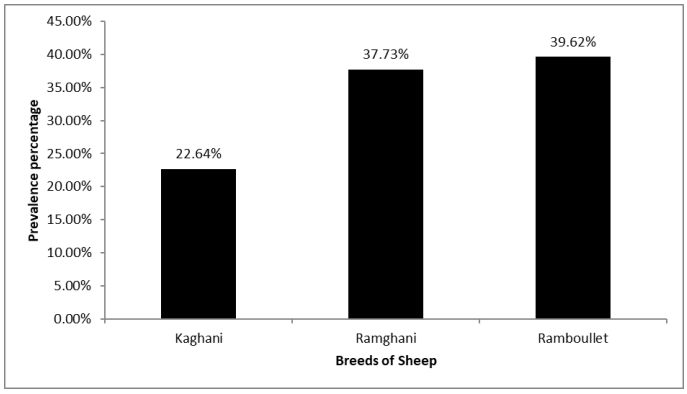

Figure 7: *Breed wise prevalence of Ostertagia spp. in sheep.*

Hematological variations in goats

In the current study, the effect of *Ostertagia* spp on the hematological parameters of goats was studied. For this purpose, certain factors like breeds and age of the goats was also being considered for probability. Breed wise results revealed significant ($P<0.05$) effect on the hematological parameters of goats between the three selected breeds. Age wise data showed that erythrocytes count, Hb, hematocrit and MCH concentrations were significantly ($P<0.05$) differed with age of goats infected with *Ostertagia* spp. while MCV, MCHC and TLC concentration of young kids was non-significantly ($P>0.05$) lowered as compared to mid age and adult goats. From the results it was revealed that the *Ostertagia* spp had a significant effect on hematological profile of the goats (Table 1).

Gender wise effect of the *Ostertagia* spp. on hematological profile shows that there was a significant difference in both male and female goats in all the parameters of the hematological profile of the goats of both genders (Table 2).

Hematological variations in sheep

Breed wise results revealed significant ($P<0.05$) effect on the hematological parameters of sheep. Age wise data showed that there was a significant difference found in all the parameters of the hematology of three selected breeds of sheep along with their age (Table 3).

Table 1: *Effect of breed and age on the hematological parameters in goats.*

Factors	Category	RBC (10^{12}/L)	Hb (g/dl)	HCT (%)	MCH (pg)	MCV (fL)	MCHC (g/dl)	TLC (10^9/L)
Breeds	Angora	10.7±0.5[a]	8.9±0.6[a]	18.2±3.4[a]	10.8±0.6[a]	21.2±1.5[a]	60.2±5.7[a]	18.4±6[a]
	Beetal	10.2±0.6[a]	7.2±0.5[a]	15.6±2.5[a]	12.2±4.1[a]	21.1±0.7[a]	52.6±6.2[a]	16±3.5[a]
	Kaghani	9.5±0.5[a]	8.5±0.6[a]	14.9±1.4[a]	10.8±0.7[a]	21.6±1.2[a]	68.2±3.5[a]	17.5±6[a]
P-value		0.02	0.03	0.03	0.03	0.04	0.04	0.04
Age	Young (<1 year)	9.3±0.7[a]	7.8±0.5[b]	13.1±1.8[a]	10.6±0.7[a]	20.1±1[a]	52.2±5[a]	16.4±4[a]
	Mid age (1-3 years)	9.4±0.4[a]	8.04±0.9[a]	15.02±3[a]	11.3±2[b]	21.4±1[b]	61.3±6[a]	17.3±3[a]
	Adult (>3 years)	11.6±1[a]	11.4±1[a]	16.11±4[a]	12.4±4.1[a]	21.3±1[b]	69.7±7[a]	18.4±6[a]
P. value		0.02	0.03	0.04	0.03	0.06	0.09	0.7

Table 2: *Effect of Gender on the hematological parameters in goats.*

Factors	Categories	RBC (10^{12}/L)	Hb (g/dl)	HCT (%)	MCH (pg)	MCV (fL)	MCHC (g/dl)	TLC (10^9/L)
Sex	Female	9.2±0.8[a]	8.1±0.7[a]	14.5±2.3[a]	11.2±0.4[a]	21.2±0.8[a]	62.5±6.2[a]	15.4±4.2[a]
	Male	10.5±0.5[a]	10±0.9[a]	16.2±3.4[a]	12.1±1.2[a]	21.4±0.5[a]	59.2±8.4[a]	17.6±5.1[a]
P. value		0.2	0.03	0.2	0.3	0.2	0.4	0.3

Table 3: *Effect of breed and age on the hematological parameters in sheep.*

Factors	Categories	RBC (10^{12}/L)	Hb (g/dl)	Hct (%)	MCH (pg)	MCV (fL)	MCHC (g/dl)	TLC (10^9/L)
Breeds	Kaghani	13.6±1.6[a]	9.1±1.7[a]	17.3±3.1[a]	11.2±1.7[a]	42.4±5[a]	51.2±7[a]	14.2±1[a]
	Ramboullet	11.4±2.4[a]	8.3±1.2[a]	14.5±2.4[a]	11.8±2.4[a]	41.2±5[a]	58.4±6[a]	13±1.5[a]
	Ramghani	10.5±1.8[a]	9.2±1.3[a]	16.2±2.3[a]	10.8±1.5[a]	45.6±7[a]	48.5±5[a]	12.5±2[a]
P-value		0.02	0.03	0.03	0.02	0.04	0.04	0.03
Age	Young (<1 year)	8.3±0.5[a]	8.1±0.9[b]	16.4±3[a]	11.2±1.7[a]	40.1±2[a]	42.5±7[a]	12.1±3[a]
	Mid age (1-3 years)	8.9±0.7[a]	9.07±0.6[a]	14.2±2.5[a]	12.4±2[b]	45.4±4[b]	61.4±3[a]	14.3±2[a]
	Adult(>3 years)	10.2±2[a]	10.2±0.9[a]	15.2±3[a]	11.6±1[a]	43.3±3[b]	66.1±5[a]	15.8±4[a]
P. value		0.02	0.03	0.04	0.03	0.04	0.04	0.4

Table 4: *Effect of Gender on the hematological parameters in sheep.*

Factors	Categories	RBC (10^{12}/L)	Hb (g/dl)	Hct (%)	MCH (pg)	MCV (fL)	MCHC (g/dl)	TLC (10^9/L)
Sex	Female	9.8±0.6[a]	9.2±0.8[a]	15.1±3[a]	10.3±0.5[a]	44.5±8[a]	52.4±7[a]	13.6±2[a]
	Male	10.4±0.8[a]	11±0.4[a]	16.8±4[a]	11.8±1[a]	49.2±5[a]	64.5±8[a]	16.9±6[a]
P. value		0.2	0.02	0.3	0.3	0.4	0.4	0.2

Gender wise effect of the *Ostertagia* spp. on hematological profile shows that there was a significant difference in both male and female sheep in all the parameters of the hematological profile of the sheep of both genders (Table 4).

Discussion

Sheep and goat are prone to GIT helminth parasites in developing countries, resulting in lowered feed intake and absorption which ultimately impaired health and affected production traits and reproduction adversely (Ayaz *et al.*, 2013; Kanyari *et al.*, 2009). Heavy endoparasitic load leads to nutrients deficiency resulting in stunted growth and reduced production (Terefe *et al.*, 2012) and cause anemia and ultimately death plague (Hassan *et al.*, 2011). Due to this pressure, a parasitic plague decreases the animal's resistance and renders it subject to other parasitic infections; at last this might affect in serious financial losses (Garedaghi *et al.*, 2011). Endoparasites of small ruminants are widely distributed in Asian content (Sharma *et al.*, 2020). The losses in ruminants in Pakistan run into billions of rupees in the form of retarded growth, milk, wool and hair production, death especially in young stock and parasite control measures (Tehmina *et al.*,

2014). For control of parasites in small ruminants especially kept by small farmers, it is better to identify the burden and types of helminths along with specific risk factors associated with Helminthosis of the specific area (Ayana and Ifa, 2015).

In current study overall prevalence of *Ostertagia* spp. was 58.32%, 34.41% (sheep) and 23.91% (goats) respectfully. Our results are supported by (Malathi *et al*., 2021) that goats are resistant to parasitic infections as compare to sheep. Gender wise prevalence of *ostertagia* parasite is higher in female goat than male goats in current study which is not agree with study of (Ayaz *et al*., 2013).

Gender wise prevalence of *Ostertagia* spp. in sheep in current is higher in female sheep than in male sheep which is not agree with study of (Craig *et al*., 2006) that the prevalence of ostertagia parasite are higher male sheep than female sheep.

Age wise prevalence of *ostertagia* spp. in current is higher 3 to 4 year of goats. Which is agreed with the results of (Tariq *et al*., 2010; Zeryehun, 2012) that the adult goats were parasite infested, and in sheep higher prevalence in sheep above 4 years (Hafeez, 1996; Sajid *et al*., 1999).

In the current study, breed wise prevalence of *Ostertagia* spp. in sheep is higher in Ramboullet which is agreed with the study of (Soulsby, 2005). That breed of host seems to have influence on the prevalence of *Ostertagia* spp., infection. But their result show that Among these 3 breeds maximum infection (87.09%) was present in Lohi followed by Awassi and Hisardale which was (76.9%) and (74.2%), respectively. But their results showed Lohi breed had higher prevalence than Awassi and Hisardale breed Natural resistance based on genetic background and genetic variations might be responsible for the differential prevalence of *Ostertagia* spp., among different breeds of sheep.

In the current study, breed wise prevalence of *ostertagia* spp. in goat which is 75.09% (n=35) samples were negative while remaining samples 23.91% (n=11) exhibited infestations in different breeds of goats. Among the positive cases, eggs/oocysts of *Ostertagia* spp. were found in Kaghani (18.18%), Angora (9.09%) and Beetal (72.73%). Results revealed that the prevalence of *Ostertagia* spp. was found highest in Beetal breed followed by Kaghani and Angora. No such result found in previous.

Age wise data showed that erythrocytes count, Hb, hematocrit and MCH concentrations were significantly ($P<0.05$) differed with age of goats infected with *Ostertagia* spp while MCV, MCHC and TLC concentration of young kids was non-significantly ($P>0.05$) lowered as compared to mid age and adult goats which is not correlated with the study of Esmaeilneiad *et al*. (2012). Hematological findings showed significant decrease in value of TEC, Hb, PCV, MCH and MCV while in MCHC and TLC no significant changes were observed in sheep. Hematological parameters were almost similar in both sheep and goats except MCHC which was found statistically significant decrease in goat

In the current study, the effect of *Ostertagia* spp. on the hematological parameters of sheep was studied. For this purpose, certain factors like breeds and age of the sheep was also being considered for probability. Breed wise results revealed significant ($P<0.05$) effect on the hematological parameters of sheep between the three selected breeds. Age wise data showed that there was a significant difference found in all the parameters of the hematology of three selected breeds of sheep along with their age is not agree with the study of Khan *et al*. (2015), Ahmadi-Hamadan *et al*. (2012). On the basis of erythrocytic indices anemia can be classified as macrocytic normochromic in sheep with increase in MCV and MCHC in normal range. In goats significant increase was observed in MCV while statistical significant decrease was noticed in MCHC values and anemia can be classified as macrocytic hypochromic.

Conclusions

In current study it was concluded that various species of gastro-intestinal parasites were variably prevalent in sheep and goats reared at alpine pastures of Livestock Experiment Station Jaba Mansehra. It is concluded that the *Ostertagia* spp., were more prevalent in female as compared to male. The parasitic burden increased as the gender and age of sheep and goats increased and it also affected the hematological parameters of the animals.

Acknowledgments

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Veterinary research and disease investigation center Abbottabad and Livestock Experiment Station Jabba Mansehra in all aspects of study.

Novelty Statement

Although several studies have evaluated growth and hematological parameters in small ruminants, limited data are available on their relationship with parasitic load under local field conditions. This study is novel as it simultaneously correlates EPG with hematological parameters across different breeds and age groups. The findings provide new evidence for using hematological indices as indicators of parasitic burden.

Author's Contribution

MM, MS and SK conceived the idea of study. SK collected samples and analysed. MS supervised the sample analysis in laboratory. AA helped in sample collection. MS, MA, and NS did the write-up and data analysis.

Generative AI and AI-assisted technology statement

No AI tool was used for data analysis, result generation, or decision-making. All interpretations and conclusions are the sole responsibility of the authors.

Conflict of interest

The authors have declared no conflict of interest.

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