


## Monitoring of Some Anthelmintics Against Gastrointestinal Nematodes in Sheep and Implications of Resistance in Barani Region, Pakistan

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Article ID: KVFD-2018-21009 Received: 21.09.2018 Accepted: 18.02.2019 Published Online: 21.02.2019

### How to Cite This Article

**Muhammad A, Ahmed H, Ali S, Saqlain M, Qayyum M, Simsek S:** Monitoring of some anthelmintics against gastrointestinal nematodes in sheep and implications of resistance in Barani region, Pakistan. *Kafkas Univ Vet Fak Derg*, 25 (3): 373-378, 2019. DOI: 10.9775/kvfd.2018.21009

### Abstract

The widespread prevalence of gastrointestinal nematode (GIN) infections has an important impact on livestock affecting of meat, milk, traction and manure in tropical and sub-tropical areas. The present study was aimed to monitor the efficacy of commonly available anthelmintic drugs at publicly owned sheep farms. The sheep naturally infected with nematodes were selected, aged between 10 to 18 months, for field efficacy trial. Selected sheep were divided into five groups (15 animals each group) based on their equal egg per gram (EPG) and body weight. Group-I served as control (untreated), while Group-II was treated with albendazole at the recommended rate of 8.8 mL/100 kg of body weight by oral drench, Group-III was treated with levamisole at the rate of 4.4 mL per 100 kg body weight, Group-IV was treated with ivermectin, 200 µg of ivermectin per kilogram of body weight and Group-V was treated with mixture of levamisole and ivermectin at the rate of 10 mg/kg by injecting subcutaneous, respectively. The results revealed that the highest efficacy rate (88.25%) was observed in sheep treated with mixture of levamisole and ivermectin, followed by ivermectin (86.43%), levamisole (81.32%), and albendazole (51.11%), respectively. The data has indicated that nematodes (*Haemonchus contortus* as the predominant species followed by *Trichostrongylus colubriformis*, *Trichostrongylus axei* and *Oesophagostomum columbianum*) might have developed resistance against the major anthelmintic drugs, encountered throughout this trial. Overall results showed that anthelmintic resistance resulted because non-judicial use anthelmintic drugs without considering the epidemiological knowledge related to occurrence of commonly nematode parasites.

**Keywords:** Anthelmintic efficacy, Gastrointestinal nematodes, resistance, Ivermectin, Levamisole, Albendazole

## Pakistan'ın Barani Bölgesi'nde Koyunlarda Mide-Bağırsak Nematodlarına Karşı Bazı Antelmintiklerin Etkisi ve Direncin İzlenmesi

### Öz

Gastrointestinal nematod (GIN) enfeksiyonlarının yaygın olması, tropik ve subtropikal bölgelerdeki çiftlik hayvanlarında et, süt, iş gücü ve gübre kayıplarına yol açması bakımından önemlidir. Bu çalışmada, halka elindeki koyun çiftliklerinde yaygın olarak kullanılan antelmintik ilaçların etkinliğinin izlenmesi amaçlandı. Nematodlarla doğal enfekte 10 ile 18 aylık yaştaki koyunlar, bu çalışma için seçildi. Seçilen koyunlar gram başına çıkardıkları yumurtalar (EPG) ve vücut ağırlığına göre beş gruba (her grupta 15 hayvan) ayrıldı. Grup-I kontrol olarak ayrıldı (tedavi edilmedi), Grup-II oral sonda ile önerilen dozu olan 8.8 mL/100 kg vücut ağırlığı oranında albendazol ile tedavi edilirken, Grup-III 100 kg vücut ağırlığı başına 4.4 mL oranında levamisol ile tedavi edildi, Grup-IV, kilogram başına 200 µg ivermectin ve Grup-V ise levamisol ile ivermectin karışımı ile 10 mg/kg dozda subkutan olarak uygulandı. Sonuçlar, en yüksek etkinlik oranının (%88.25), levamisol ve ivermectin karışımı ile muamele edilen koyunlarda, ardından da ivermectin (%86.43), levamisol (%81.32) ve albendazol (%51.11) uygulanan grupların takip ettiğini gösterdi. Veriler, nematodların *Haemonchus contortus*, dominant tür, *Trichostrongylus colubriformis*, *Trichostrongylus axei* ve *Oesophagostomum columbianum* ana anthelmintic ilaçlara karşı direnç geliştirebileceğini göstermiştir. Genel sonuçlar, antelmintik direncin, epidemiyolojik bilgileri dikkate almadan rastgele kullanılan antelmintik ilaçların kullanılmasından kaynaklandığını göstermiştir.

**Anahtar sözcükler:** Antelmintik etki, Mide bağırsak nematodları, Direnç, İvermectin, Levamisol, Albendazol



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## INTRODUCTION

Livestock, particularly sheep and goats help to beat economic losses in case of crop deterioration [1]. It contributes 11.8 percent to national Gross Domestic Product (GDP) and 56.3 percent to Agriculture GDP of Pakistan [2]. One of the paramount factors that influence adequate feed conversion is gastrointestinal nematode (GIN) infections. The widespread prevalence of GIN infections has infested many livestock development programs by reducing the level of output of meat, milk, traction and manure in tropical and sub-tropical areas. Same also reduces their asset value due to an increase in mortality rate, especially of young stock [3].

Although anthelmintic treatment can be used to minimize the losses by ensuring the sustainability of sheep production, other approaches, such as pasture management, could be worth mentioning. There are reports about the increasing rates of parasite resistance against chemotherapeutic agents across the globe [4-9]. When parasitic populations are not killed by the therapeutically recommended dosages of previously effective drugs, the resistance is said to have been developed [10]. Moreover, anthelmintic resistance causes great damage to both agricultural revenue and animal well-being. The loss of anthelmintic activity was established to be one of the important factors of high occurrence of GIN infections in small ruminants in Pakistan [9,11-14]. Comprehensive drugs screening studies are required to know exactly about position of currently available anthelmintic drugs. The main objectives of the present study were to find the accuracy of treatment with broad-spectrum anthelmintic using fecal egg count reduction test (FECRT) and determine the sensitivity of gastrointestinal nematodes (GINs) to benzimidazole, levamisole, ivermectin and a mixture (levamisole + ivermectin). The objective of the study was also determination of some haematological parameters in groups during post treatment period.

## MATERIAL and METHODS

**Animal's Selection:** The present study was carried out at the Small Ruminants Research Program's farm located in the National Agricultural Research Center in Islamabad which is present in Barani region, Pakistan. The sheep breed used was Bulkhi commonly known as Afghani sheep. Sheep were reared in a semi-intensive system, where they grazed on permanent pasture during the day and were housed in brick sheds with concrete-floored pens at night mixed husbandry with goats, cattle, and buffalo. The sheep were supplemented fed with pelleted concentrate (PARC Feed Technology Brand) at the rate of 0.54/kg/animal/day. While green fodder and water were provided *ad libitum* during day and night.

**Experimental Design:** A total of 75 Bulkhi sheep naturally infected with nematodes were selected, aged between

10 to 18 months, for field efficacy trial. Body weights and egg per gram (EPG) of all sheep were recorded. Animals were individually weighed before treatment on a Tru-Test scale. The accuracy of scale was checked by comparing with certified weights. Selected sheep were divided into five groups (15 animals each group) based on their equal EPG and body weight. All sheep in one group were approximately the same weight and had similar excretion rates to ensure the correct therapeutic dose. Group-I served as control (untreated), while Group-II was treated with albendazole (Albazen<sup>®</sup>) at the recommended rate of 8.8 mL/100 kg of body weight by oral drench, Group-III was treated with levamisole (Levasole<sup>®</sup>) at the rate of 4.4 mL per 100 kg body weight, Group-IV was treated with ivermectin (Ivomec<sup>®</sup>) 200 µg of ivermectin per kilogram of body weight and Group-V was treated with mixture of levamisole and ivermectin (Primisol<sup>®</sup>) at the rate of 10 mg/kg by injecting subcutaneous, respectively. The dose of each anthelmintic was calculated (dose rate per kg) as provided by the manufacturers. Fecal sampling schedule for experimental trial was 7 and 3 days pre-treatment, 0 day and 7, 14, 21, 28 and 35 days post treatment.

**Faecal Collection and Analysis:** On the above mentioned days, 5 g faecal sample of each sheep was taken directly from rectum for faecal egg count reduction test (FECRT). Faecal egg counts (FEC) were performed by using the modified McMaster method [15] with saturated sodium chloride as the flotation fluid.

**Faecal Egg Count Reduction Test:** Mean FEC, percentage reduction and 95% confidence interval (CI) was determined by using the formulae recommended by the World Association for the Advancement of Veterinary Parasitology guidelines for detecting anthelmintic resistant nematodes of sheep [16].

$$\text{Anthelmintic Efficacy} = \frac{(\text{Pre-treatment mean} - \text{Post-treatment mean})}{\text{Pre-treatment mean}} \times 100$$

An efficacy of less than 90% and 95% upper confidence levels of less than 90% was taken as indicative of the presence of anthelmintic resistant for nematodes in the sheep flock.

**Evaluation of Haematological Parameters:** Subsequently, on the above mentioned days, along with faecal samples, blood samples was also collected via jugular vein puncture into 5 mL ethylene di-amine tetra acetic acid (EDTA) coated and without (EDTA) coated vacutainer tubes for assessment of hematological parameters i.e. haemoglobin level, packed cell volume, total protein level, while total erythrocytes count, total leucocytes count and differential leucocytes count were analyzed on days 0, 7, 14, 21, 28 and 35, respectively [17].

**Coproculture Analysis:** The larvae were recovered through Baermann procedure to determine the relative composition

of specific nematode species. The identification larvae (L3) were carried out by following the keys and description given by [18].

### Statistical Analysis

Data was analyzed by statistical package POST HOC TEST (univariate analysis of variance) using SPSS version 16.0. FEC and larval culture records were transformed [ $\log_{10}(n+1)$ ] before analysis to stabilize the variance. The values of blood parameters and body weight were measured in respective units. No transformation was applied to blood parameters.

## RESULTS

The results illustrated a significant difference of FECRT on 7<sup>th</sup>, 14<sup>th</sup>, 21<sup>st</sup>, 28<sup>th</sup> and 35<sup>th</sup> days post-treatment with mixture of levamisole and ivermectin in Group V (Fig. 1) compared to control (Group I) ( $P < 0.05$ ) at 95% CI. The mean minimum FEC of mixture ( $66.72 \pm 3.3$ ) was noted at 7<sup>th</sup> day while that of ivermectin ( $81.47 \pm 4.0$ ) and levamisole ( $143.50 \pm 7.1$ ) showed the same at 14<sup>th</sup> day. No significant effects were found on the total FEC regarding albendazole

(Fig. 1). Moreover, the Group-V and Group-IV were found to be proficient enough with greatest efficacy (88.25%) and (86.43%), respectively followed by Group-III which showed moderate effectiveness with value (81.32%), while Group-II had lowest effectiveness status with low efficiency (51.11%) (Table 1).

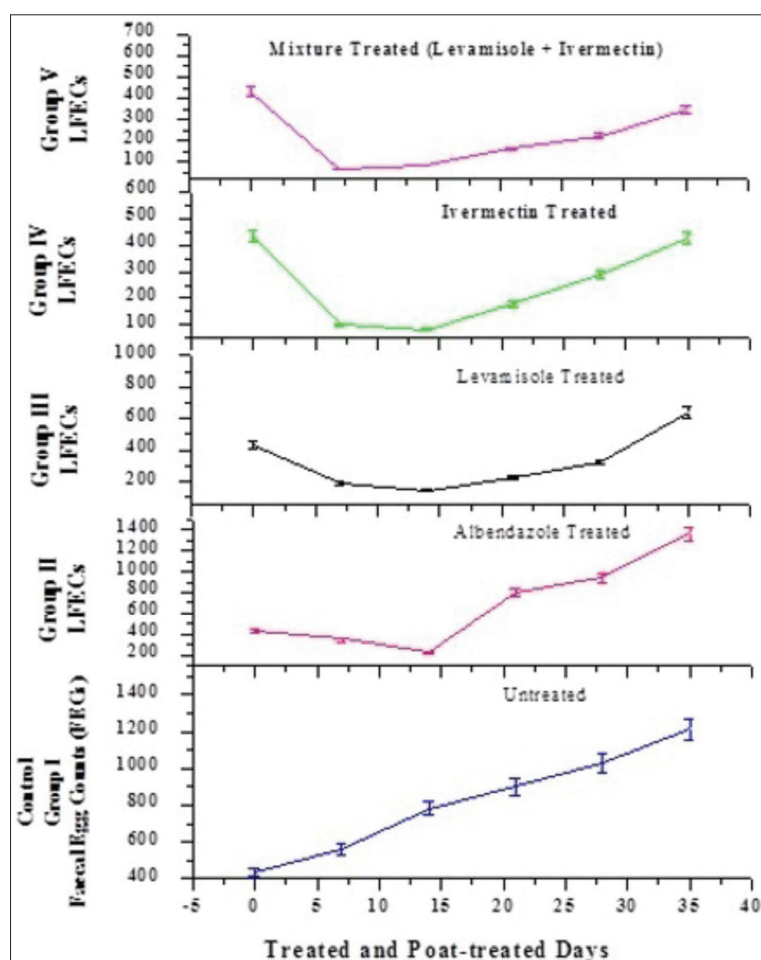
Our findings regarding the individual faecal cultures of trichostrongyles larvae (L3) pointed towards the frequencies of generic composition describing the existence of *Haemonchus contortus* as the predominant species followed by *Trichostrongylus colubriformis*, *Trichostrongylus axei* and *Oesophagostomum columbianum* throughout the study trail (Table 2).

The results of current study pointed towards an increase in live body weight in Group-V and Group-IV, while a slight body weight regain was noticed in Group III. Whereas, no significant change in body weight was observed in Group-II and Group-I (control) seemed to be losing weight continuously, being untreated (Fig. 2). A significant ( $P < 0.05$ ) increase in the haematological parameters was observed viz.; packed cell volume (PCV), haemoglobin level (Hb) and protein level (PL) was occurred in Group-IV and V as compared to the Group-I and II, in the post treatment period. Highest mean values of PCV, Hb and PL were recorded at days 7 and 14 in Group IV ( $25.70 \pm 2.1$ ;  $8.32 \pm 1.2$  and  $7.21 \pm 1.0$ ) and V ( $26.83 \pm 2.64$ ;  $8.40 \pm 1.4$  and  $7.30 \pm 1.1$ ), respectively. Furthermore, lowest mean values at day 35 in Group-I ( $19.20 \pm 1.9$ ;  $6.71 \pm 1.4$  and  $6.0 \pm 1.0$ ) and Group-II ( $22.91 \pm 2.2$ ;  $7.23 \pm 1.2$  and  $6.40 \pm 1.1$ ) were recorded (Fig. 3a, 3b, 3c).

Our data revealed a significant ( $P < 0.05$ ) increase in mean values of Total Erythrocyte Count (TEC) (Fig. 4a) and decrease in TLC within the Group IV and V after anthelmintic treatments (Fig. 4b). While in Group II, the mean values of TEC showed no significant changes followed by Group I. Reasons for the above results for all the haematological parameters were found to be the same. Furthermore, a significant decrease in differential counts ( $P < 0.05$ ) after anthelmintic treatment with mixture and ivermectin was monitored in Group IV and V but not in Group II (Table 3).

## DISCUSSION

The efficacy of various synthetic broad spectrum anthelmintic products checked by using FECRT against the GINs within the sheep flock revealed a significant difference ( $P < 0.05$ ) of FECRT on pre-treatment (0) and post-treatment (7<sup>th</sup>) days post-treatment with mixture of levamisole and ivermectin in Group V compared to control,



**Fig 1.** Group-wise anthelmintic efficacy of broad spectrum anthelmintic drugs in Bulkhi sheep

Table 1. Faecal egg count (epg) reduction (FECR) in the Bulkhi sheep before and after anthelmintic treatments					
Groups	Host (Bulkhi Sheep)				
	No of Animals Examined	Anthelmintic Drugs	Mean FEC	Mean Faecal Egg Count Reduction	
			Pre-treatments Mean±SEM	Post-treatments Mean±SEM	FECR (%)
I	15	Control (untreated)	493.2±26.3	774.2±13.5	-
II	15	Albendazole (0.75 mL/11.34 kg)	503.4±25.1	246.1±12.3	51.11
III	15	Levamisole (2 mL/45.35 kg)	796.3±39.8	148.8±7.4	81.32
IV	15	Ivermectin (1 mL/34 kg)	765.92±38.2	104.11±5.2	86.43
V	15	Levamisole + Ivermectin (1 mL/22.67 kg + 0.5 mL/17.23 kg)	859±42.9	100.9±5.0	88.25

Table 2. Post-treatment generic composition of trichostrongyles larvae (L3) recovered from faecal cultures in Bulkhi sheep

Parasite	Prevalence (%)
<i>Haemonchus contortus</i>	62
<i>Trichostrongylus colubriformis</i>	15
<i>Trichostrongylus axei</i>	12
<i>Oesophagostomum columbianum</i>	11

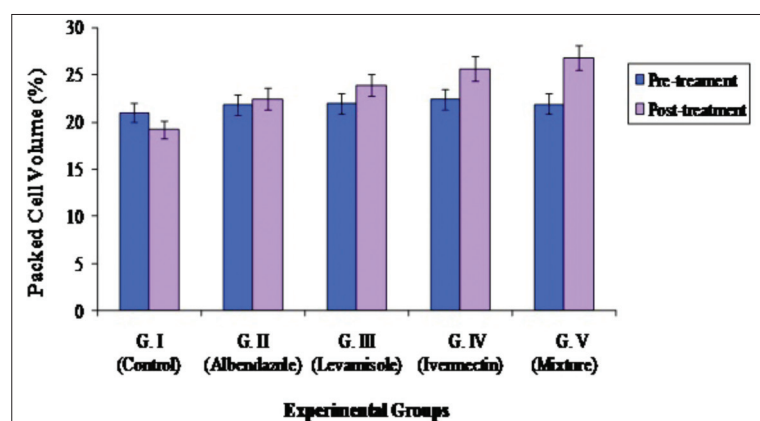


Fig 2. Effect of broad spectrum anthelmintic drugs on live body weight in Bulkhi sheep

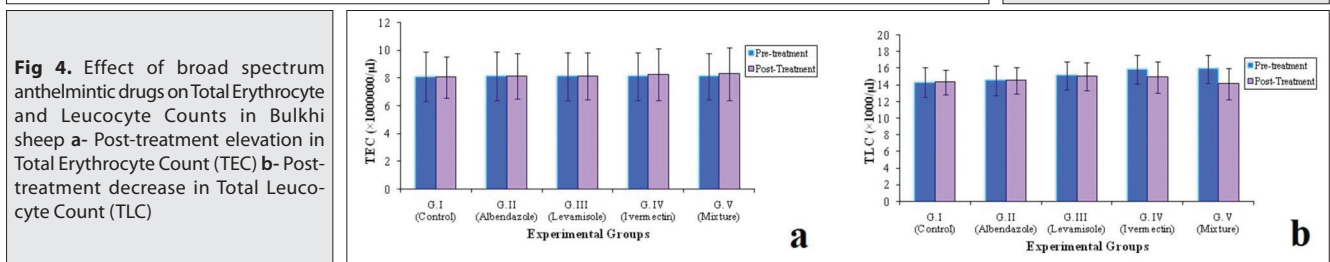
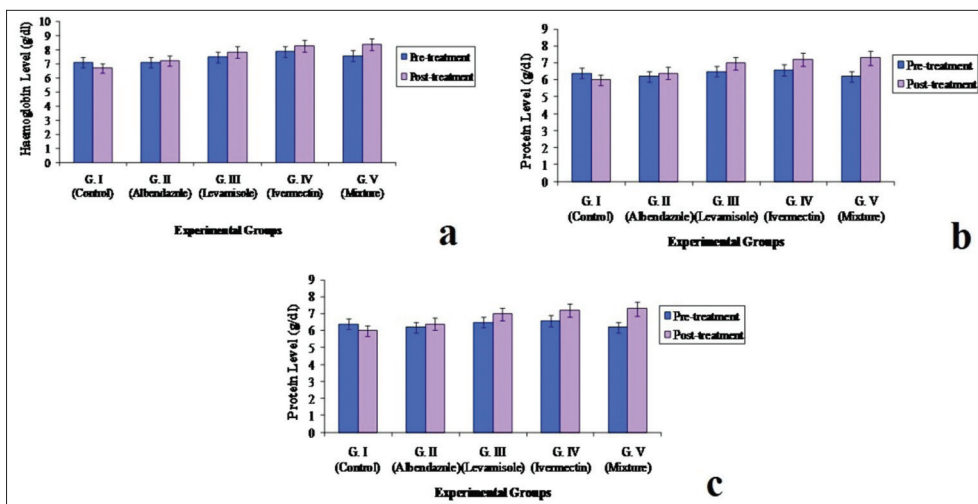
Group I. The findings in our study are in accordance with Arslan and Muhammed<sup>[19]</sup>, Islam et al.<sup>[20]</sup> and Muhammad et al.<sup>[21]</sup> as they also reported similar trend in albendazole treated sheep. The results about anthelmintic efficacy among various groups are similar to findings of Nari et al.<sup>[22]</sup> and Zajac and Gipson<sup>[23]</sup>. Uppal et al.<sup>[24]</sup> which are about 80 to 88% verified similar results in India. Many factors like genetic, biological or operational contribute in the emergence of anthelmintic resistance Raza et al.<sup>[13]</sup>. Similar observations were found in the experimental sheep flock where frequent, (6-7/annum), long term use of the broad spectrum anthelmintic drug especially the albendazole, was responsible for the development of anthelmintic resistance the continual use of the said drug might be

due to its low price, availability and easily administrable by the local farmers. Similar findings and outcome was reported by Prichard<sup>[25]</sup> and Jackson<sup>[10]</sup>.

Presently, a trend of the degree of resistance has been noted against the levamisole, ivermectin and mixture of both among the Bulkhi flock reared at NARC, Islamabad, which is alarming for veterinarians and farmers. This compels us to think about alternatives and control strategies against GINs, particularly in Barani region, Pakistan. Other approaches, such as pasture management, could be better alternative. The probable reason for the development of anthelmintic resistance might be the fact that the climate of Barani region, being humid and warm, highly supports the development and survival of free living stages of trichostrongyles and represent a reservoir of infective larvae throughout the year. The higher occurrence of GINs, might favor the development of anthelmintic resistance. Our findings of anthelmintic resistance are in accordance with Chandrawathani et al.<sup>[3]</sup> and Muhammad et al.<sup>[21]</sup>. Farmers carry on using drugs without the basic knowledge of their dosage and administration hence producing a stern anthelmintic resistance as demonstrated in our results.

The development of resistance on this study farm for instance could be facilitated by continuous grazing on permanent pasture and mixed growing with goats, cattle's and buffaloes throughout the study year by Muhammad et al.<sup>[21]</sup> According to Coles and Roush<sup>[16]</sup>, the optimal proposition is to use anthelmintic from various families one by one according to the demand of the host. However, during the past few decades there have been escalating rates of resistance of parasites to chemotherapeutic agents all over the world<sup>[6]</sup>. Correspondingly, in Pakistan, one of the important factors of high prevalence of gastrointestinal nematode infections in small ruminants





**Table 3.** Differential leucocyte count (%) in Bulkhi sheep

Groups	Differential Leucocyte Count (%)									
	Lymphocytes		Neutrophils		Eosinophils		Monocytes		Basophils	
	Day 0	Day 35	Day 0	Day 35	Day 0	Day 35	Day 0	Day35	Day 0	Day35
I	55.57	55.45	38.55	38.23	47.42	47.57	3.53	3.47	0.53	0.54
II	54.86	54.78	37.97	37.56	48.43	48.25	3.86	3.81	0.51	0.50
III	56.54	56.32	38.52	38.11	47.89	48.31	4.01	3.97	0.49	0.50
IV	55.64	53.97	38.31	35.85	48.57	47.12	3.62	3.21	0.53	0.45
V	55.12	54.23	38.43	36.67	48.26	46.87	3.59	3.12	0.54	0.43

may be failure of efficacy of anthelmintics [13]. Suggested meaningful study can provide valuable information's that may help in devising strategic guidance for the health and management in small ruminants.

The body weight regain might be a result of parasitic load removal, as parasitic free gastro-intestinal tract promotes proper digestion, absorption and metabolism of feed nutrients which make a sound base for proper weight regain. Similar findings were reported by Hussein [26] and Kenyon et al.[27]. The logical explanation for current observations might be the parasitic infection, responsible for the arrested growth.

Our results revealed a significant ( $P < 0.05$ ) increase in packed cell volume (PCV), haemoglobin level (Hb) and protein level (PL) was occurred in Group-IV and V as compared to the Group-I and II, in the post treatment period. The reason for the significant decrease in PCV, Hb and PL in Group-I and Group-II might be due to heavy

nematode burden with *Haemonchus contortus* resulting in anemia and hypoproteinemia. Whereas, the high efficacy of mixture in Group V and ivermectin in Group IV might be responsible for a significant increase in PCV, HB and PL values for their action against blood sucking parasites. Similar findings have been reported by Chaichisemsari et al.[28] and Akanda et al.[29].

A significant ( $P < 0.05$ ) increase in mean values of TEC and decrease in TLC within the Group IV and V after anthelmintic treatments was observed. The results regarding change in TEC and TLC are according to Akanda et al.[29]. Differences in leukocytes count might be a result of different levels of nematode parasites present within the host, type of sampling site, utilization of techniques used for leukocytes count and concentration of anticoagulants. In these results, again mixture and ivermectin showed the maximum ability as compared to albendazole and levamisole. Overall, results indicated that all the hematological parameters viz, PCV, Hb, PL, and TECs showed positive significant

correlation with each other in Bulkhi sheep, while inverse correlation within the host worm burden was detected, throughout study trail.

The present findings strongly suggest planning further studies on resistant nematode worms prevalent within the gastrointestinal tract among different host breeds, in different agro-ecological regions of Pakistan. Appropriate use of anthelmintic treatments concerning therapeutic dose recommended by manufactures is required, as overdose uphold homozygous and under dose promote heterozygous population of resistant worms Shalaby<sup>[30]</sup> so, it is suggested that the animals from the same sex, breed and age class must be weighed precisely for therapeutic dosage.

#### ACKNOWLEDGEMENTS

The authors are highly thankful to staff members in NARC for their technical support during this research work. We are very thankful to TUBITAK (2216-research fellowship program for international researchers) to provide an opportunity and funding. This study has been supported by a grant from Pakistan Science Foundation.

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