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*Full Length Article*

# Correlation of the Gastrointestinal Parasitism with the Phytominerals in the Grazing Sheep (*Ovis aries*)

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## Abstract

Trace elements play an important role to boost the immunity and fight against parasitic infections. Concentration of trace elements like Copper (Cu), Cobalt (Co), Manganese (Mn) and Zinc (Zn) were determined in soil, forages and sera of sheep. An associative analysis was also made between the burden of Gastrointestinal (GI) parasites and concentrations of trace elements of sheep sera. For this, 384 faecal and blood samples of sheep, an appropriate number of forages and soil samples were collected. The faecal samples were subjected to determine the species and burden of GI parasites. The sera, plant and soil samples were subjected to pre-treatment (digestion) required for the determination of trace elements. The overall prevalence of GI parasites was 32.81% and the most prevalent species were *Haemonchus (H.) contortus* followed in order by *Eimeria* spp., *Strongyloides* spp., *Trichostrongylus* spp. and *Fasciola* spp. Variables like age, sex, breed and tehsils of Silakot district showed an insignificant association with GI parasitic burden. Trace elements concentration of forages showed a significant ($P < 0.05$) variation while trace elements concentration of soil showed an insignificant ($P > 0.05$) variation. In serum, Zn concentration showed significant ($P < 0.05$) results among all the tehsils of study district. Mean concentrations of Mn and Cu in serum were found inversely proportional to the mean egg count per gram of sheep faeces in tehsil Pasroor of the Sialkot district. Forages containing high concentrations of Mn and Cu can be used effectively against GI parasites. © 2021 Friends Science Publishers

**Keywords:** Sheep; Trace Elements; Forages; Soil; Sera; Gastrointestinal parasites

## Introduction

The livestock sector especially small ruminants rearing is one of the major and more secure sources of income for smallholder farmers around the world (Terefe *et al.* 2012). Small ruminants farming can comparatively require small inputs like startup investment, maintenance, feedstuffs, and expenditure as compared to the bovine population (Caroprese *et al.* 2016). The main purpose of sheep raising is to fulfill the needs of mutton and wool production (Khan *et al.* 2007). Parasitic diseases, one of the principal problems in the development of commercial livestock business, are facilitated through favorable climatic conditions and lack of awareness. About 90% of the sheep population around the world suffers from various kinds of parasitic diseases (Mohanta *et al.* 2007; Biu *et al.* 2009; Kanyari *et al.* 2009; Raza *et al.* 2014; Rizwan *et al.* 2017; Qudoos *et al.* 2017; Rizwan *et al.* 2019; Ahmad *et al.* 2020).

In the developing countries, anti-parasitic drugs are used lavishly for the control of parasitic infections especially by smallholder farmers which may lead to the development of resistance. Other factors responsible for the

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development of resistance are; poor efficacy of antiparasitic agents, low protein diet, insufficient dose level, and environmental toxicity (Smith and Sherman 2009; Khan *et al.* 2017). Development of resistance against anthelmintics and their residual effects stimulate scientists to investigate alternative sources to control parasitic infection and to improve public health (Qadir *et al.* 2010). During the past decade, uses of plants with anthelmintic properties (ethnoveterinary medicine) are under considerations around the world (Lateef *et al.* 2003; Peixoto *et al.* 2015; Tugume *et al.* 2016; Kebede *et al.* 2017; Badar *et al.* 2017).

Nutrition has the potential to affect the GI parasites because it directly affects the degree of expression of immunity and rate of acquisition which influence the survival, fecundity, and establishment of the GI parasites (Fekete and Kellems 2007). Mineral's availability in an appropriate quantity is a pre-requisite for the health and productivity of livestock, while insufficient mineral intake or unavailability decreases productivity (Khan *et al.* 2007). Under natural grazing conditions, forage plants are the major source for herbivores to obtain minerals. Apart from this source, water and soil also contribute to acquiring considerable quantities of minerals. Determination of trace elements in grazing forages and their bioavailability to the animals is important to regulate the requirement of animals (Khan *et al.* 2007; Qudoos *et al.* 2017; Rizwan *et al.* 2019; Ahmad *et al.* 2020). Grazing of animals in rangelands containing trace elements rich forages increases the resilience against parasitic infections particularly in resource-poor countries like Pakistan.

## Materials and Methods

### Study area and animals

The study was conducted in district Sialkot (32° 30'0" N/74° 31'0" E), Punjab, Pakistan which has three administrative divisions (tehsils) named; (a) Daska, (b) Pasroor and (c) Sialkot. The topography of Sialkot is plain and fertile. About 25.82% of the population lives in urban areas. The highest temperature in summer may reach 49°C and lowest -2°C during winter. The average rainfall in the study district is about 1000 mm annually. The study animals included indigenous breeds of sheep (*Ovis aries*). The total population of sheep in the Sialkot district is 87,000 (Anonymus 2016). The samples were collected from the grazing meadows of the study district by using a simple random sampling method. A total of 384 sheep of different age, sex and breeds were screened from Sialkot district. The present study was approved by Research Ethics Committee of Faculty of Veterinary Medicine, University of Agriculture, Faisalabad, Pakistan.

### Collection and coprological examination of faeces

Collection of faeces (n=384) was done using standard protocols, briefly, 10 g of faecal sample was collected directly from rectum and stored in plastic bottles containing 10% formalin. After proper labeling to, plastic bottles were transported to Molecular Parasitology Laboratory, University of Agriculture, Faisalabad for further processing. The qualitative faecal examination was done by floatation and sedimentation methods while the quantitative faecal examination was done by Modified McMaster egg counting technique briefly, 3 g of faeces was mixed in 45 mL NaCl (flotation) solution. After straining, the chamber was filled to suspension and allowed to settle for 3 to 5 min. Counting of eggs was done carefully under a compound microscope at 10x in each lane of the chamber (Soulsby 1982). Species of parasites were identified on the basis of egg size and shape. Eggs per gram faeces from 100 to 800 was considered light infection, 801 to 1200 moderate and more than 1200 high infection (Table 2).

### Collection and digestion of soil samples for elemental analysis

Representative soil samples of the selected grazing sites from each tehsil of the study district were collected. Soil samples of various depths (15, 30, 60, 90 and 120 cm) were collected with a sampling auger. Five different locations of each grazing field were selected and 200 g sample from each location was collected. All samples from each selected grazing site were mixed thoroughly to make a cumulative representative sample of 1000 mg.

Soils samples were macerated with a pestle and mortar and filtered through 0.2 mm sieve and dried at room temperature for 24 h. One g of the dried soil was taken into a 50 mL conical flask, mixed with the concentrated $HNO_3$ (10 mL) and kept for 12 h at room temperature. After that, substances in the flask were heated, $HNO_3$ (1 mL) and $HClO_4$ (4 mL) were added and heated again (200°C). After cooling, 5 mL 1:10 HCl was added followed by heating at 70°C till the volume remained 2 mL. After cooling, de-ionized water was added to make the volume of 50 mL (Amacher 1996). The mixture was filtered through Whatman filter paper No. 42 and stored until further analyses.

### Collection and digestion of forages for elemental analysis

Forages were collected from the selected grazing sites of each tehsil of the study district. Collected species of plants were packed in zip tight bags, labeled properly with all relevant information, and transported to U.A.F. Samples were identified by a professional of the Botany Department, U.A.F.

Collected forage specimens were subjected to pre-treatment for determination of mineral profile. Briefly, leaves of collected plants were washed with water and then with 1% HCl. After washing, the forage leaves were dried in the air and then in the oven at 65°C. Dried plant materials were subjected to an electric grinding machine to make powder and stored till digestion. Dried forages (1 g each) were taken in flasks mixed with 5 mL concentrated $HNO_3$ and 5 mL $HClO_4$ (5 mL) and kept overnight. The next day, five mL $HNO_3$ was added and heated until the material became clear. After cooling, 50 mL of de-ionized water was added (Miller 1998) and filtered through Whatman filter No. 42. The pre-treated plant samples were submitted to the Central Hi-Tech Laboratory, UAF for mineral profile determination using standard protocols (Haswell 1991).

### Collection and digestion of sera samples for elemental analysis

Five mL blood was collected from the selected sheep (n = 384) into commercially available gel clot activating vacutainers and labeled with the age, sex, breed and other relevant detail before transporting to the Parasitology Department, UAF. The blood was centrifuged for 15 min at 2000 x g and the serum was separated. If the serum samples were not analyzed immediately after separation; these were stored at -20°C until further processing.

Wet digestion of the collected sera samples was done as suggested by Richards (1968). Briefly, one mL serum was mixed with concentrated $HNO_3$ (10 mL) into a digestion flask and heated for 15 min at 60°C. After cooling, five mL $HClO_4$ was added and heated again till the volume remained one mL. After cooling, 25 mL de-ionized water was added and samples were subjected to determination of minerals through spectrophotometry.

The concentration of Cu, Co, Zn, and Mn from digested soil, forages and sera were analyzed with atomic absorption spectrophotometer (in triplicates) as defined by Haswell (1991).

### Statistical analyses

The relationships of independent variables like age, sex, location and breed of sheep with the prevalence of GI parasites were determined using the Chi-square test. One way analysis of variance was applied to determine the differences of trace elemental profile in soil, forages and serum samples. Each soil, plant, and serum sample was analyzed in triplicate to determine their mean values and standard error of the mean to estimate the variability between samples. Pearson's correlation method was used to correlate the trace element profile of serum with respective EPG values (Schork and Remington 2010). *P*-value of < 0.05 was considered statistically significant. All statistical analyses were performed by Minitab 17 statistical software.

## Results

### Species and burden of gastrointestinal parasites

Among 384 screened faecal samples, the overall prevalence of GI parasites was 32.81%. The species of parasites identified from the collected faecal samples were *Haemonchus (H.) contortus* (32.81%) followed in order by *Eimeria* spp. (23.70%), *Strongyloides* spp. (18.75%), *Trichostrongylus* spp. (15.36%) and *Fasciola* spp. (7.55%). The prevalence of *H. contortus, Trichostrongylus* spp. and *Fasciola* spp. were higher in adult animals while prevalence of *Eimeria* spp. and *Strongyloides* spp. were higher in young animals. The prevalence of all the identified species of parasites was significantly higher in female as compared to male. However, the prevalence of these species was insignificant in different breeds of sheep and tehsils of Sialkot district (Table 1).

The burden of gastrointestinal parasitic infection of sheep in relation to age, sex, breed and tehsils of Sialkot district, Punjab, Pakistan were insignificant (Table 2). Most of the adult and female animals showed high burden of parasitic infection followed in order by moderate and low. However, the order of the burden of parasites in young animals was high, low and moderate while in male animals was moderate, high and low. Among breeds, most of the animals of Thalli, Fat tailed and Kajli breed showed moderate infection while Salt range breed showed low infection. Among tehsils, most of the animals of all tehsils showed high burden of parasitic infection.

### Trace elements profile of forages in district and tehsils of Sialkot

Eight forage species include; *Amaranthus viridis, Cannabis sativa, Echinochloa colona, Fagonia indica, Parthenium hysterophorus, Cynodon dactylon, Brachiaria ramose* and *Cyperus rotundus* were identified from Sialkot district to be consumed by sheep. A significant variation in the concentration of selected trace elements was observed in all collected forages (Table 3). Highest concentration (39.79 ± 0.11 mg/kg) of Zn was found in *Echinochloa colona* while, minimum (19.35 ± 0.32 mg/kg) in *Amaranthus viridis*. Maximum concentration (44.83 ± 0.14 mg/kg) of Cu was found in *Amaranthus viridis* and minimum (21.53 ± 0.07 mg/kg) in *Brachiaria ramosa*. *Cannabis sativa* and *Parthenium hysterophorus* contained maximum (36.91 ± 0.17 mg/kg) and minimum (17.11 ± 0.03 mg/kg) concentrations of Mn, respectively. *Brachiaria ramose* showed maximum concentration of Co (1.48 ± 0.02 mg/kg) while *Parthenium hysterophorus* showed minimum (0.87 ± 0.05 mg/kg).

Mean concentrations of all the selected trace elements were insignificant among different tehsils of the Sialkot district. Maximum concentration (34.71 ± 6.33 mg/kg) of Zn was found in the forages of tehsil Daska while forages from tehsil Sialkot contained minimum (26.84 ± 7.51 mg/kg) concentration. Forages of tehsil Sialkot showed maximum concentration of Cu (38.35 ± 7.01 mg/kg) whereas, it was minimum (30.00 ± 7.78 mg/kg) in forages from tehsil Daska. Maximum mean concentration of Mn (33.34 ± 8.39 mg/kg) was found in forages collected from

**Table 1:** Frequency distribution of gastrointestinal parasitic species in sheep population of Sialkot district

| Variable | Level | *Haemonchus contortus* (%) | *Eimeria* spp. (%) | *Strongyloides* spp. (%) | *Trichostrongylus* spp. (%) | *Fasciola* spp. (%) |
|---|---|---|---|---|---|---|
| Age | Adult | 72 (57.14) | 34 (37.36) | 32 (44.44) | 38 (64.41) | 17 (58.62) |
| | Young | 54 (42.86) | 57 (62.64) | 40 (55.56) | 21 (35.59) | 12 (41.38) |
| Sex | Male | 41 (32.54) | 25 (27.47) | 29 (40.28) | 25 (42.37) | 11 (37.93) |
| | Female | 85 (67.46) | 66 (72.53) | 43 (59.72) | 34 (57.63) | 18 (62.07) |
| Breed | Thalli | 28 (22.22) | 18 (19.78) | 14 (19.44) | 13 (22.03) | 8 (27.59) |
| | Salt range | 34 (26.98) | 27 (29.67) | 19 (26.39) | 16 (27.12) | 5 (17.24) |
| | Fat tailed | 32 (25.40) | 21 (23.08) | 17 (23.61) | 19 (32.20) | 9 (31.03) |
| | Kajli | 32 (25.40) | 25 (27.47) | 22 (30.56) | 11 (18.64) | 7 (24.14) |
| Tehsils | Daska | 45 (35.71) | 31 (34.07) | 30 (41.67) | 24 (40.68) | 11 (37.93) |
| | Sialkot | 44 (34.92) | 35 (38.46) | 23 (31.94) | 16 (27.12) | 8 (27.59) |
| | Pasror | 37 (29.37) | 25 (27.47) | 19 (26.39) | 19 (32.20) | 10 (34.48) |

**Table 2:** Burden of gastrointestinal parasitic infection of sheep in relation to age, sex, breed and tehsils of Sialkot district, Punjab, Pakistan

| Variable | Level | Light (%) | Moderate (%) | High (%) | $\chi^2$ | *P*-value |
|---|---|---|---|---|---|---|
| Age | Adult | 14 (19.44) | 22 (30.56) | 36 (50.00) | 3.993 | 0.136 |
| | Young | 19 (35.19) | 14 (25.93) | 21 (38.89) | | |
| Sex | Male | 8 (19.51) | 18 (43.90) | 15 (36.59) | 2.053 | 0.358 |
| | Female | 24 (28.24) | 27 (31.76) | 34 (40.00) | | |
| Breed | Thalli | 8 (28.57) | 11 (39.29) | 9 (32.14) | 4.531 | 0.605 |
| | Salt range | 15 (44.12) | 7 (20.59) | 12 (35.29) | | |
| | Fat tailed | 10 (31.25) | 13 (40.63) | 9 (28.13) | | |
| | Kajli | 9 (28.13) | 12 (37.50) | 11 (34.38) | | |
| Tehsils | Daska | 14 (31.11) | 7 (15.56) | 24 (53.33) | 8.101 | 0.088 |
| | Sialkot | 7 (15.91) | 17 (38.64) | 20 (45.45) | | |
| | Pasror | 10 (27.03) | 7 (18.92) | 20 (54.05) | | |

Light = 100 - 800; Moderate = 801-1200; High = > 1200

**Table 3:** Mean concentrations of Cu, Zn, Co and Mn in forages collected from Sialkot district, Punjab, Pakistan preferred by the grazing sheep population

| Forages species | Zn (mg/kg) Mean ± SE | Cu (mg/kg) Mean ± SE | Mn (mg/kg) Mean ± SE | Co (mg/kg) Mean ± SE |
|---|---|---|---|---|
| *Amaranthus viridis* | $19.35 \pm 0.32^{d}$ | $44.83 \pm 0.14^{a}$ | $30.52 \pm 0.33^{bc}$ | $1.42 \pm 0.03^{a}$ |
| *Cannabis sativa* | $24.71 \pm 0.15^{cd}$ | $30.33 \pm 0.12^{c}$ | $36.91 \pm 0.17^{a}$ | $0.98 \pm 0.05^{a}$ |
| *Echinochloa colona* | $39.79 \pm 0.11^{a}$ | $36.28 \pm 0.12^{ab}$ | $29.29 \pm 0.06^{bc}$ | $1.32 \pm 0.01^{a}$ |
| *Fagonia indica* | $34.36 \pm 0.08^{ab}$ | $30.81 \pm 0.05^{c}$ | $25.84 \pm 0.06^{c}$ | $0.93 \pm 0.03^{a}$ |
| *Parthenium hysterophorus* | $26.81 \pm 0.08^{c}$ | $39.42 \pm 0.03^{ab}$ | $17.11 \pm 0.03^{d}$ | $0.87 \pm 0.05^{a}$ |
| *Cynodon dactylon* | $23.46 \pm 0.04^{cd}$ | $41.76 \pm 0.02^{ab}$ | $34.63 \pm 0.02^{ab}$ | $1.41 \pm 0.01^{a}$ |
| *Brachiaria ramose* | $28.51 \pm 0.08^{bc}$ | $21.53 \pm 0.07^{d}$ | $31.71 \pm 0.12^{ab}$ | $1.48 \pm 0.02^{a}$ |
| *Cyperus rotundus* | $35.82 \pm 0.07^{a}$ | $32.18 \pm 0.07^{c}$ | $34.59 \pm 0.13^{ab}$ | $0.87 \pm 0.06^{a}$ |
| Overall Mean | $30.29 \pm 3.93^{bc}$ | $35.42 \pm 4.18^{ab}$ | $29.90 \pm 3.69^{bc}$ | $1.16 \pm 0.08^{a}$ |

Mean values having same letters in a column indicate insignificant (*P* > 0.05) results

Pasroor tehsils, while minimum (24.49 ± 2.34 mg/kg) from tehsil Sialkot. The maximum concentration of Co (1.22 ± 0.26 mg/kg) was found in forages collected from Daska tehsil, while minimum (1.07 ± 0.24 mg/kg) in tehsil Sialkot (Fig. 1).

### Trace elements profile of soil

Mean concentration of Zn in grazing field soil showed an insignificant result in all tehsils of study district. Soils from grazing sites of tehsil Sialkot contained maximum (6.36 ± 1.32 mg/kg) concentration of Zn and minimum (4.84 ± 0.46 mg/kg) in the soil of tehsil Daska. The mean concentration of Cu in soils also showed an insignificant variation in different tehsils. The highest (2.16 ± 0.45 mg/kg) and lowest (1.60 ± 0.25 mg/kg) concentration of Cu was found in soil collected from tehsils Pasroor and Daska, respectively. The mean concentration of Mn of grazing sites soil varied insignificantly (Fig. 1).

### Trace element profile of sheep sera

The mean concentration of Zn showed significant results among tehsils while, Cu, Mn, and Co in serum showed insignificant (*P* > 0.05) results among all tehsils. Serum samples of sheep population belonging to Pasroor tehsils showed a maximum concentration of Zn (1.13 ± 0.90) while serum of sheep population from Sialkot tehsil showed minimum (0.59 ± 0.15) Zn concentration. Maximum (1.31 ± 0.12) mean concentration of Cu was found in serum samples collected from tehsil Pasroor while minimum (1.05 ± 0.14) from Sialkot tehsil. Serum samples of sheep collected from tehsil Daska showed maximum (0.18 ± 0.06) concentration of Mn in serum while serum collected from tehsil Pasroor showed minimum (0.15 ± 0.06) concentration. The mean concentration of Serum Co was highest (0.59 ± 0.38) in tehsil Daska and lowest (0.41 ± 0.33) in Sialkot tehsil (Fig. 1).

**Table 4:** Correlation of the selected trace elements of serum with mean parasitic eggs per gram of faeces various tehsils of district Sialkot, Punjab, Pakistan

| Tehsils | Correlations | Zn | Cu | Mn | Co |
|---|---|---|---|---|---|
| Daska | Pearson Correlation | 0.005 | -0.039 | 0.080 | 0.048 |
| | Sig. (2-tailed) | 0.949 | 0.634 | 0.336 | 0.558 |
| Sialkot | Pearson Correlation | 0.124 | -0.055 | -0.051 | 0.146 |
| | Sig. (2-tailed) | 0.211 | 0.579 | 0.607 | 0.142 |
| Pasror | Pearson Correlation | -0.002 | -0.237 | -0.200 | 0.124 |
| | Sig. (2-tailed) | 0.985 | 0.006 | 0.021 | 0.154 |

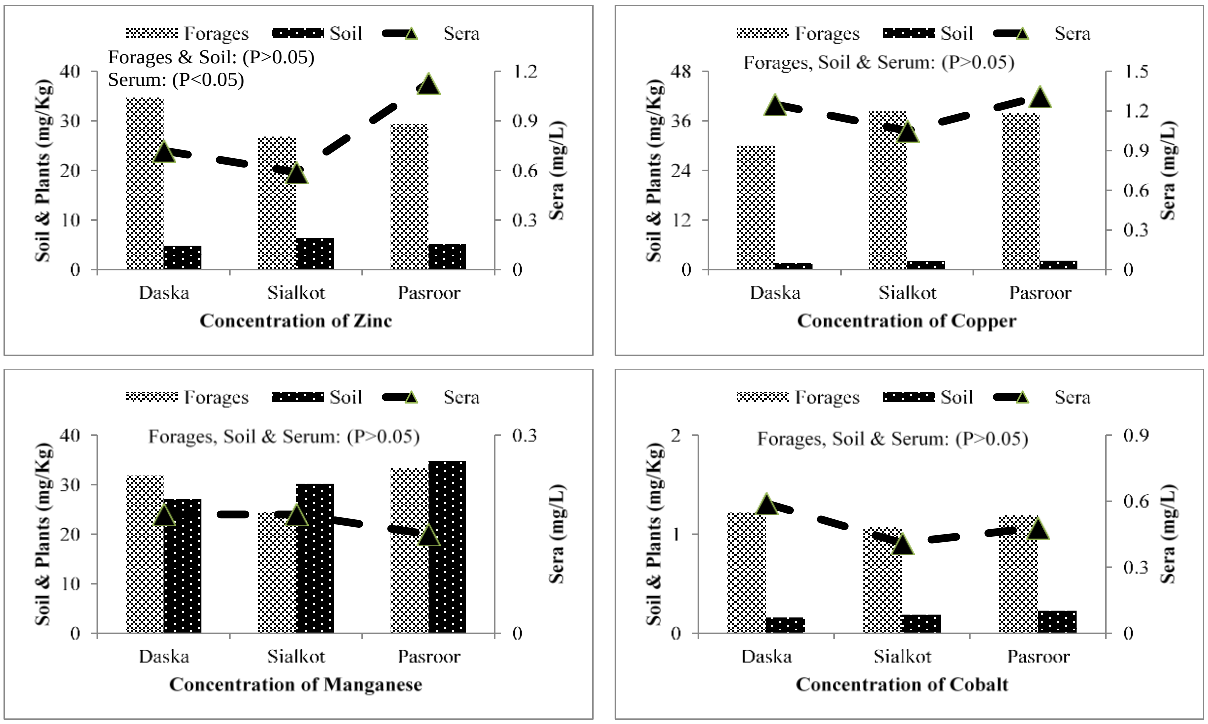


**Fig. 1:** Association of trace elements of soil-plant-serum in each tehsil of Sialkot district

**Soil-plant-serum trace element correlation analyses**

The relationship of trace elements of soil-plant-serum in each tehsil of Sialkot district is given in Fig. 1. The concentration of Zn in serum showed significant variation among all tehsils while in forages and soil showed insignificant results. However, the concentration of Cu, Mn, and Co showed insignificant results in soil-plant-animal in all tehsils. The mean concentration of Co in serum was directly related to the mean concentration of Co in forages in different tehsils of study district, while the concentration of Zn, Cu, and Mn showed variable (directly or indirectly proportional) results in all tehsils of study area.

**Correlation of serum trace elements with egg per gram of sheep faeces**

A correlation of the trace element of serum with mean EPG of each tehsil of district Sialkot is given in Table 4. Mean concentrations of serum Cu and Mn directly correlate to the mean EPG of sheep in tehsils Pasroor of Sialkot district. It showed that the high level of these trace elements decreases the burden of GI parasites, whereas, the mean concentration of Co and Zn showed insignificant results concerning the mean EPG in all tehsils of district Sialkot.

## Discussion

Parasites identified in the sheep population in the present study were also reported by various scientists from different localities of Pakistan (Ayaz *et al.* 2013; Ahmad *et al.* 2017; Qudoos *et al.* 2017; Ahmad *et al.* 2020). The same species of parasites were also reported in different parts of the world like Bangladesh (Mohanta *et al.* 2007), Kenya (Kanyari *et al.* 2009) and Ethiopia (Dagnachew *et al.* 2011). Other species of parasites have also been reported by various scientists (Khaled *et al.* 2016; Yimer and Birhan 2016; Getachew *et al.* 2017). Distribution of different parasitic species in different areas may be attributed to certain factors including age, breed, nutrition, health, availability of infective larvae, climate and management systems (Blackie 2014; Abdela and Jilo 2016). Moreover, the incidence of a particular parasitic species in a particular area is directly associated with the affected species of animal and environmental conditions.

The reason for the higher incidence of GI parasites is probably due to their grazing behaviour which enhances the acquirement of the infective parasitic stage. The higher prevalence of GI parasites may also be due to meager animal care infrastructure and the management of animals under extensive pastoralism. Relatively low prevalence of sheep GI parasites may be due to certain factors like epidemiological patterns, parasitic species, breed variation, age and sex of host, management practices and environmental conditions (Nwosu *et al.* 2007). Sheep grazed in rangelands of all tehsils of the study area exhibited almost similar prevalence of GI parasites. This similarity may be linked to the acquisition of worms by host species in these areas and may be joined with similar rainfall and humidity at these sites. The prevalence of parasites reported in sheep populations varies around the world. The prevalence of parasites can be influenced by a variety of factors such as standard of management, education level and economic capacity of the farmers, grazing habits, irrational use of anthelmintic, seasonal difference, variation in agroecology of the study area besides poorly drained land, lack of fences around the farms, combined grazing of male, female, young and adult animals, predominant agro-climatic conditions, and overstocking of animals (Lashari and Tasawar 2011; Nana 2016).

The rangelands of any country play an important role in the economy because they are used for the grazing of livestock. The topographical regions, ecological zones and climatic conditions of Pakistan are very favorable for a variety of natural vegetations suitable for the consumption by animals. Almost 60% of the livestock population of Pakistan is reared by grazing (Mirza 2007). Various forage species have been identified around the world consumed by livestock (Mashwani *et al.* 2012). The variation in plantation diversity may be linked with the topography of a particular region, structure of soil and specific conditions of climate (Nordløkken *et al.* 2015).

For determination of trace elements, analysis of forages should be a routine practice because level of trace elements of forages consumed by animals reflect the level of trace elements in grazing animals (McDowell and Arthigton 2005; Hoste *et al.* 2006). Gomide (1978) documented that composition of trace elements of forages depends on seasion, age and species of plants, soil type, and use of fertilizers in grazing areas. These factors largely cause variations in the minerals composition of forages (Ahmad *et al.* 2012). A deficiency of Zn has been reported in the livestock population subjected to graze in Zn deficient rangelands or areas having a high level of Fe, Cd, Mn, S and Mo which interact with Zn and reduce its utilization in animals (Ndebele *et al.* 2005). The low level of soil pH affects the concentration of Cu in plants because low pH increases the solubility of Fe which decreases the absorption of Cu (Beeson and Matrone 1976). The presence of Mo, Ca, and S act as an antagonist for Cu which shows that a higher level of these elements in soil decrease the level of Cu in forages (McDowell 2003). The forage Mn level depends on the level of Mn in soil, but it has also been reported that the livestock gets an adequate amount of Mn even in Mn deficient soil (Underwood 1981). The deficiency of Co in grazing animals causes severe effects (McDowell *et al.* 1984). The concentration of Co in forages depends upon the concentration of Mn, the higher the level of Mn in soil, the lower the absorption of Co in forages (McKenzie 1975).

Soil is the direct and indirect source of trace elements for livestock. The bioavailability of these trace elements to the livestock is associated with their level in the soil (Reid and Horvath 1980), lime, pH, soil quality, electrical conductivity, plant species and seasons (Khan *et al.* 2004). The presence of antagonistic trace elements can decrease or increase the uptake of other trace elements (Mitchell and Gray 2003). Reproductive problems and poor growth have been reported for animals in the areas having soil with a low level of trace elements (Tiffany *et al.* 2000). The production level of the livestock population of tropics and sub-tropical areas is severely affected by the low level of trace elements in soils (McDowell 1985). Analysis of soil to determine the level of trace elements is important to endorse the mineral supplements for grazing livestock.

For the determination of trace element level in animals, the analysis of blood is a well-established and recognized tool (Mills 1987). The overall concentration of trace elements required by animals is less than 100 mg/kg dry matter (McDowell 1992). However, the level of trace elements retained in the serum of animals is below 2 mg/L (Suttle 2010). The requirement and level of trace elements in serum vary depending upon various factors like age, sex, breed, genotype and production capabilities (NRC 2001; Marques *et al.* 2003; Lukić *et al.* 2009; Suttle 2010; Yatoo *et al.* 2012). Various factors (age, sex, disease, stress conditions and feed) have been reported to alter the level of trace elements in general and Zn specifically in animals (Devi *et al.* 2011; Ishag *et al.* 2014). Variation in the level of trace elements may also be due to prompt growth of animals and the presence of inhibitors in the food of animals (Mills 1981). A lower level of serum Zn and Cu have been reported in animals having a parasitic infection or certain other diseases as compared to healthy animals (Fouda *et al.* 2013).

In animals, trace elements act as a cofactor and are required for the proper functioning of the immune system. Adequate level of trace elements helps reduce various pathogens attack (Erdoğan *et al.* 2002; McClure 2008). A significant association of increased level of trace elements in serum like Cd (Aypak *et al.* 2016), Cu and Zn (Rizwan *et al.* 2019) and Mn (Ahmad *et al.* 2020), decreasing the level of parasitic burden is reported with GI parasitic burden.

Trace elements in serum like Mo, Zn, Se, Co, Mn, Cu (Qudoos *et al.* 2017) and P, Cu, Ca, and Zn showed an insignificant association with GI parasitic burden (Aypak *et al.* 2016). Schafer *et al*. (2015) reported a lower burden of GI parasites in animals having a higher level of Co and vice versa. Sheep population infected with *Trichostrongylus (T.) colubriformis* and *T. axei* showed a lower level of Cu in the serum (Hucker and Yong 1986). Animals with a lower level of Cu and Zn in serum showed a higher burden of *H. contortus* and *Trichostrongylus* (Silva *et al.* 1978; Abdellall 1991).

## Conclusion

Sheep population of Sialkot district is infected with a variety of parasitic infections. Most of the study animals showed a high burden of parasitic infection. The species of forages present in the study area are rich with trace elements. Trace elements like Cu and Zn were found suitable for the control of parasitic infection. However, further control studies are required to determine the role of trace elements in animals and the association of trace elements with specific GI parasites. It is also recommended to determine the presence and association of various anti-parasitic compounds of forages like tannin with trace elements and GI parasites.

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## Author Contributions

MSS, ZI and RN conceived and planned the experiments. HMR, HA, MA, AQ, AS and MS contributed to sample preparation and carried out the experiments. HMR, MSS and MS contributed to the interpretation of the results. HMR and MSS took the lead in writing the manuscript. AW identified the forages species. ZI supervised the project. MSS and GH contributed to the final version of the manuscript.

## Conflicts of Interest

The authors declare that they have no conflict of interest.

## Data Availability

We hereby declare that all data reported in this paper are available and will be produced on demand.

## Ethics Approval

Standard guidelines for the institutional animal care and use (IACU), University of Agriculture, Faisalabad, Pakistan were followed in this study.

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