



**Full Length Article**

## Correlation of Trace Mineral Profiles with Gastrointestinal Worm Burden in Rangeland Sheep of Chakwal District, Punjab, Pakistan

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### Abstract

This paper describes the relationship of trace and ultra-trace minerals with the gastrointestinal (GI) helminths burden in rangeland sheep reared in district Chakwal, Pakistan. A total of 384 sheep were randomly subjected to collect sera and fecal samples during autumn, 2014. Trace and ultra-trace minerals were determined through different spectrophotometric techniques after wet digestion of sera samples. Fecal examination revealed a variety of helminth parasites viz., *Haemonchus (H.) contortus*, *Ostertagia (O.) trifurcata*, *Trichostrongylus* spp., *Strongyloides (S.) papillosus*, *Marshallagia (M.) marshalli*, *Avitellina (A.) centripunctata*, *Fasciola (F.) gigantica* and *F. hepatica* harbored by the study population. The mean serum levels of copper (Cu), molybdenum (Mo) and selenium (Se) were within the normal ranges. However, mean zinc (Zn) level was marginally deficient while, manganese (Mn) and cobalt (Co) levels were higher than the normal ranges. The mean serum Zn, Mo and Se concentrations in sheep varied insignificantly ( $P>0.05$ ). Whereas, a significant variation ( $P<0.05$ ) in the levels of Cu, Mn and Co was observed in sheep flocks. The quantitative fecal analysis of sheep revealed lower to moderate egg per gram (EPG) range. Pearson correlation demonstrated a non-significant ( $p>0.05$ ) correlation of trace and ultra-trace minerals with EPG magnitude in naturally parasitized sheep of the study area. In conclusion, trace and ultra-trace mineral levels in naturally infected sheep flocks exhibited no significant role in reducing GI parasitic burden. © 2017 Friends Science Publishers

**Keywords:** Trace minerals; EPG; Gastrointestinal parasitism; Sheep; Chakwal

### Introduction

Sheep (*Ovis aries*) production in Pakistan has a significant economic and social impact on the rural small herd owners due to their dependence on sheep for nutrition and income. Small ruminants maintained under nomadic and transhumant systems obtain about 90% of feed from the rangelands (Khan *et al.*, 1999). Gastrointestinal helminth infections are considered a major threat to livestock all over the world (Githiori *et al.*, 2004) due to retarded growth, productivity (Perry and Randolph, 1999) and mortality (Sykes, 1994). Parasitic infections of small ruminants also decrease natural resistance to disease (Ngategize *et al.*, 1993). Sub-clinical or marginal trace mineral deficiencies are frequent and are among the major problems as compared to acute mineral deficiencies resulting into reduced growth, lower reproduction rate, poor feed utilization efficiency and depressed immune system. Different trace minerals e.g. Zn, Mn, Se and Cu are necessary for the optimum functioning of the immune system and resistance against pathogens (Galyean *et al.*, 1999). Cobalt deficiency reduced the resisting ability of animals against helminth infections

(Ferguson *et al.*, 1989). Molybdenum also plays an important role in immunity against endoparasites (McClure, 2003) and can decrease worm burden in lambs (Miller, 1984). Egg per gram of feces is an important parameter to evaluate GI helminths for livestock production and welfare. McKenna (1981) observed a logical association between worm burden and mean EPGs by describing EPG ranges of low (<500), moderate (600-2000) and high (>2000). Assessment of actual level of parasite abundance in a population needs large samples (Gasbarre *et al.*, 1996), because parasites are generally aggregated within a small fraction of host populations (Poulin, 2007). In small ruminants, GI nematode infection could be estimated directly by worm burden and indirectly by EPG (Miller *et al.*, 1998). Several studies have shown a positive and direct correlation between EPG and parasite burdens (Rieu *et al.*, 2007; Das *et al.*, 2011). The current study was planned with the objective to determine trace (Co, Cu, Mn, Zn) and ultra-trace (Mo, Se) minerals level and to establish a correlation with GI parasitic burden in sheep reared on the rangelands of district Chakwal, Punjab, Pakistan.

## Materials and Methods

### Range Site

This investigation was conducted during autumn, 2014 in district Chakwal which is situated in the south of district Rawalpindi at a distance of 97 km and lies between 32° 56' north and 72° 54' east. It comprises of four tehsils namely: Chakwal, Talagang, Kalar Kahar and Choa Saidan Shah. The total area of this district is 6524 km<sup>2</sup>. The south and south-east areas are mountainous and rocks are covered with scrub forest, interspaced with flat lying plains. The climate is sub-humid. The rainfall (350-500 mm) typically occurs during monsoon season (mid of July to the mid of September). January and June are the coldest and hottest months respectively and temperature ranges from 5.9 to 38.4°C.

### Selection of Samples

Sheep reared on the rangelands of district Chakwal were engaged for this investigation. The available farms/flocks of sheep were identified to form the sampling frame. The sample size of sheep population for screening parasitic burden and trace mineral profiles was randomly determined using standard formulae for simple random sampling (Thrusfield, 2007). Keeping in view a total of 44% prevalence reported earlier in sheep population of other districts of Punjab, a total of 384 sheep irrespective of breeds, age and sex were selected from the four tehsils of district Chakwal. A proportional allocation and map grid methods were used to take representative samples from the study sites. During the selection of population, flock owners were also interrogated about the use of any anthelmintic treatment to avoid sampling from anthelmintic-treated animals.

### Parasitological Examination

Per-rectal fecal samples from the selected sheep flocks were collected in labeled polythene bags, preserved in 10% formalin and stored in refrigerator (4°C). The samples were processed to identify GI parasites (Soulsby, 1982). Egg per gram in fecal samples was determined by modified McMaster technique (MAFF, 1986).

### Pre-treatment of Serum Samples

Blood samples (10 mL) were collected from the jugular vein of sheep into gel and clot activator vacutainer and labeled with relevant details. The collected samples were left standing (1 h), sera were separated by centrifugation (2500 rpm) and subjected to wet digestion (Richards, 1968). Briefly, one mL of serum was taken into a digestion flask and 10 mL of concentrated HNO<sub>3</sub> was added. Mixture was heated (60-70°C; 15 min.) and after

cooling, HClO<sub>4</sub> (5 mL) was added in the flask. The content of the flask was heated vigorously till the volume was reduced to 1-2 mL and finally diluted up to 25 mL by adding de-ionized water.

### Minerals Analysis

Concentrations of trace minerals (Co, Cu, Mn, Zn) from the pre-treated sera samples were analyzed by atomic absorption spectrophotometer (Hitachi Polarized Zeeman AAS, Z-8200), while ultra-trace minerals (Mo, Se) were analyzed by inductively coupled plasma-optical emission spectrophotometer (Optima 2100-DV, Perkin Elmer) according to the manufacturer's instructions. Commercially available stock solutions (Applichem®) in the form of an aqueous solution (1000 ppm) were used to prepare the calibrated standards. Highly purified de-ionized water was used to prepare calibrated standards. All glassware used throughout the analytical activities were immersed in 8N HNO<sub>3</sub> overnight and washed with several changes of de-ionized water prior to use.

### Data Analysis

The differences of elemental profile in sera were analyzed by one way analysis of variance. In case of significant ( $p < 0.05$ ) difference among treatment means, Tukey's T-test was applied. Correlation of serum trace element profiles with respective EPG values was analyzed by Pearson's correlation method (Schork and Remington, 2010).

## Results

Fecal examination of sheep (n=384) reared on the grazing sites of district Chakwal revealed eight helminths species viz., *H. contortus*, *O. trifurcata*, *Trichostrongylus* spp., *S. papillosus*, *M. marshalli*, *A. centripunctata*, *F. gigantica* and *F. hepatica*. The lowest (467.47±46.66) and the highest (660.21±48.87) EPG of mixed parasitic species were observed in sheep flocks reared on the rangelands of tehsils Chakwal and Kalar Kahar, respectively. The EPG of sheep at tehsils Choa Saidan Shah and Talagang were 506.36±44.93 and 610.16±53.02, respectively. The results (Table 1) reflected that mean serum levels (in mg/L) of Cu, Mo and Se were within the normal ranges. Serum Zn level was marginally deficient while, Mn and Co levels were higher than the normal range. Statistically, mean serum Zn, Mo and Se concentrations in sheep reared on the rangelands of different tehsils varied non-significantly ( $P > 0.05$ ). However, significant variation ( $P < 0.05$ ) was seen in case of Cu, Mn and Co serum levels in sheep. Pearson's correlation (Table 2) demonstrated no significant ( $p > 0.05$ ) correlation of EPG with trace element profiles of sheep reared on the rangelands of different tehsils.

**Table 1:** Concentration (mean±SE; mg/L) of trace elements in the sera of sheep reared on rangelands of different tehsils of district Chakwal

Tehsils	Zn	Cu	Mn	Co	Mo	Se
Chakwal	0.92±0.11	1.10±0.14 <sup>a</sup>	0.03±0.00 <sup>b</sup>	0.05±0.01 <sup>c</sup>	0.012±0.001	0.130±0.019
Talagang	0.88±0.15	0.68±0.13 <sup>b</sup>	0.59±0.14 <sup>a</sup>	0.14±0.02 <sup>a</sup>	0.011±0.001	0.105±0.014
Choa Saidan Shah	0.37±0.05	1.06±0.11 <sup>a</sup>	0.20±0.05 <sup>b</sup>	0.11±0.01 <sup>ab</sup>	0.008±0.001	0.108±0.017
Kalar Kahar	0.63±0.08	0.59±0.09 <sup>b</sup>	0.02±0.00 <sup>b</sup>	0.06±0.01 <sup>bc</sup>	0.009±0.002	0.077±0.017
Overall mean	0.78±0.06	0.81±0.15	0.21±0.04	0.09±0.01	0.010±0.001	0.105±0.009
Normal ranges	0.8-1.2	0.57-1.0	0.18-0.19	0.01-0.03	0.012	0.08-0.5

Means with similar letters in a column are non-significant (P>0.05)

**Table 2:** Correlation of EPG with trace element profile of rangeland sheep reared on rangelands of different tehsils of district Chakwal

Tehsils			Zn	Cu	Mn	Co	Mo	Se
Chakwal	EPG	Pearson Correlation	0.260	0.368	0.261	0.795	-0.281	-0.363
		Sig. (2-tailed)	0.672	0.542	0.672	0.108	0.646	0.549
Talagang	EPG	Pearson Correlation	0.244	-0.163	-0.025	0.086	-0.404	0.320
		Sig. (2-tailed)	0.239	0.436	0.905	0.682	0.500	0.600
Choa Saidan Shah	EPG	Pearson Correlation	0.176	0.207	-0.232	-0.057	0.169	-0.356
		Sig. (2-tailed)	0.401	0.320	0.265	0.787	0.786	0.557
Kalar Kahar	EPG	Pearson Correlation	-0.082	0.017	-0.263	-0.179	0.839	-0.353
		Sig. (2-tailed)	0.696	0.937	0.203	0.392	0.075	0.560

## Discussion

Blood analysis is a well established and recognized tool to estimate the trace mineral profiles of animals (Mills, 1987) and animal sera usually have less than two ppm of trace minerals (Suttle, 2010). In our study, sheep reflected normal ranges of serum Cu, Mo and Se. Nevertheless, mean Zn level was marginally deficient while, Mn and Co levels were higher than the established normal ranges. There is a vast variation in trace minerals requirements of livestock depending on age (Devi *et al.*, 2011), sex (Yatoo *et al.*, 2012), growth stage or production capabilities (NRC, 2001) and genotype and breed variation (Lukic *et al.*, 2009). Alteration in Zn level of animals can occur due to certain factors like animal age and sex, stress conditions, presence of diseases and feed availability (Kincaid and Hodgson, 1989; Ishag *et al.*, 2014). Variation in Zn level also occurs in animals during their prompt growth and existence of inhibitors in animal diet (Mills, 1981). Findings of Ortolani *et al.* (1993) indicated lower Cu level in parasite infected sheep compared to non-infected sheep. Fluctuation/deficiency in serum Cu levels were reported in different age groups and sex (Ishag *et al.*, 2014), genetic and breed varieties (Woolliams *et al.*, 1985), low concentration in feed (Suttle, 2010) and those with antagonistic elements like Mo, S and Fe in their diets (Marques *et al.*, 2003). Moreover, certain trace elements like Se have a higher capability for absorption and retention in sheep utilizing concentrate feed compared to forage (Koening *et al.*, 1997).

Egg per gram of feces is an important tool to scrutinize helminth egg level in fecal samples of flocks, assists the herd owners to decide when or whether to drench sheep. McKenna (1981) observed a rational association between worm burdens and mean EPG by describing EPG ranges of

low (<500), moderate (600-2000) and high (>2000). The EPG values of our study flocks coincide with the findings of Tramboos *et al.* (2015) and Dilgasa *et al.* (2015). However, EPG values are in partial agreement with those reported by Roy *et al.* (2013). Relatively higher EPG values in sheep infected with different GI parasites have been documented (Muhammad *et al.*, 2015). On the contrary, Martínez-Valladares *et al.* (2013) observed comparatively lower EPG values than those of our findings. This discrepancy may be due to differences in sample size (Gasbarre *et al.*, 1996), infective larvae inhibition or suppression of egg production (Bricarello *et al.*, 2004) and exposure of fecal material to the environment (Das *et al.*, 2011). Further, EPG values can be altered due to seasonal influence, being higher in summer than in winter (Ahmed, 2010). Other factors like sex (Roy *et al.*, 2013) and age (Idika *et al.*, 2012) are also responsible for variations in EPG calculations. The highest EPG in sheep has been observed at Kalar Kahar followed by Talagang, Choa Saidan Shah and Chakwal. This variation in EPG values may be attributed to variability in the acquisition of helminths by sheep from respective localities (Fakae, 1990).

The findings of the current study revealed a non-significant correlation of serum levels of trace and ultra-trace minerals with EPG magnitude in the naturally GI parasitic infected sheep. It is also noticeable that sheep of the study area have normal/higher levels of aforementioned trace elements except Zn that was just at marginally deficient level. There are evidences that trace elements revealed a non-significant correlation with the parasitic burden in small ruminants. McDonald *et al.* (1989) noted no difference in EPG magnitude in Se supplemented and un-supplemented sheep. Moreover, it was also noted that increasing Se status in deficient sheep flocks has a minor

effect against *O. circumcincta* and *T. colubriformis*. Similarly, there was a higher burden of helminths in Se treated and control groups of goat kids (Fivaz *et al.*, 1993). Our findings are also strengthened by Beriajaya *et al.* (1995) who used mineral blocks to control GI nematode infections in the naturally infected sheep. The mineral blocks only improve sodium and Zn status of treated group but Cu level in both groups remained almost similar. This trend indicates that Cu level plays no positive role in increase or decrease of parasitic burden in sheep. The findings of this study are also congruent with the results reported by Burke and Miller (2008) who observed no effect of dietary copper sulfate on the GI nematodes of goats. Likewise, Schafer *et al.* (2015) also mentioned that supplementation of Cu and Se in sheep against *H. contortus* infection did not reduce EPG and worm number. On the contrary, reduced EPG have been observed in small ruminants treated with copper oxide wire particles (COWP) (Vatta *et al.*, 2009; Soli *et al.*, 2010). Likewise, combination of Se and Cu markedly reduced worm burden in sheep (Fausto *et al.*, 2014). Vellema *et al.* (1996) documented higher EPG in lambs offered low level of Co diet compared to Co supplemented counterpart. The results reported by Camargo *et al.* (2010) and Celi *et al.* (2010) are also not in line with those observed in the present study as they have noted a significant EPG reduction in Se medicated animals. Similarly, application of Mo significantly reduced *H. contortus* burden in sheep (Suttle *et al.*, 1992). This disparity from the results of the present study could be attributed to an improved mineral profile of the experimental animals with mineral supplementation.

## Conclusion

It is concluded that analyzed trace and ultra-trace minerals have no considerable role in reducing GI parasitic burden in naturally infected sheep, despite of the fact that sheep have normal/higher levels except Zn that was just at marginally deficient level. However, the correlation of trace minerals with the quantitative helminth burden can better be studied in controlled experiments (case-control study) using standard mineral supplementation and artificial infection of model helminths on a time series scale.

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