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MOLECULAR EPIDEMIOLOGY OF SMALL RUMINANT FASCIOLOSIS IN SELECTED REGIONS OF AZAD JAMMU AND KASHMIR

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ABSTRACT

The present study was carried out to determine prevalence of Fasciolosis in sheep and goats at three different altitudes *viz*; <3000, 3000-6000 and >6000 feet above sea level in Azad Jammu and Kashmir (AJK). Rectal faecal samples from 4662 small ruminants (n=2242 sheep; n=2420 goats) were collected during the year 2012. The samples were examined by centrifugal floatation and sedimentation techniques while egg per gram (EPG) count was performed through McMaster technique. Prevalence of Fasciolosis in sheep and goats was 26.49% and 9.91%, respectively with an overall prevalence of 17.88%. The prevalence in both the species of animals was significantly different ($P<0.05$) at altitude <3000, 3000-6000 and >6000 feet. When compared, significant difference ($P<0.05$) was observed in different season, age groups and flock sizes. The intensity of infection (EPG) at different altitudes in sheep and goats was significantly different ($P<0.05$). It was concluded that Fasciolosis was prevalent in AJK with variable prevalence and intensity at different altitudes. Further studies are required to identify the *Fasciola* species involved in the disease in different species of animals in different climates for tactical control measures.

Keywords: Fasciolosis, Small ruminants; Altitudes; Azad Jammu and Kashmir;

INTRODUCTION

The state of Azad Jammu & Kashmir (AJK) is located at the foothill of Himalayas, north east of Pakistan. It lies between 730º-750º and 330º-360º longitude and latitude (Ahmad *et al.* 2012). Azad Jammu & Kashmir has unique geographic and topographic distribution and is comprised of mountainous ranges with heights of 4734 m high in the north and with plains and valleys in the south with an average rainfall of 1500 mm (Afshan *et al.* 2011). Sheep and goats raising is a profession of tribes and nomads and many other farming communities in AJK. Sheep and goats contribute to the livelihood of the rural poor especially in high altitudes as in Himalayas. Poor management and hygiene leads to various parasitic diseases. The diverse agro climatic conditions, animal husbandry practices and pasture management practices determine the incidence and severity of various parasitic infections in grazing animals. Information on the prevalence and epidemiological pattern of the parasitic diseases in different climatic conditions provide a basis for evolving strategic and tactical control measures for such diseases (Mir *et al*. 2013). Among parasites, Fasciolosis is one of the major diseases of sheep and goats in different parts of Pakistan (Akhtar *et al.* 2012). *Fasciola* spp. are the causative agent of Fasciolosis in sheep, goats, cattle, buffalo, pig, rabbit, elephant, dog, cat and kangaroo. Human and equine are unusual hosts. Transmission depends on an intermediate host, the lymnae snail. Animal ingests metacercaria and the worm migrates to the liver where it causes extensive damage and the mature worm inhabits the bile duct (Seldemir 2000). Clinical disease is well known however, sub clinical infections are often remain unnoticed, leading to marked economic losses, reduced milk yield, weight loss, reduced fertility and immunity (Schweizer *et al*. 2005).

The disease is unique in having endemic areas that range from below sea level to altitudes of up to 13123 feet (Mas-Coma *et al.* 2008). Data are lacking with reference to its prevalence at different altitudes and geographical areas. Studies in Pakistan revealed that it is endemic in sheep, goats, cattle, buffalo and human. In the country, surveillance record showed an estimated prevalence of 14.71% in Punjab, 17.68% in Bahawalpur, 23.97% in Multan, 10.48% in Lahore and 55% in Peshawar. These reports are mostly outdated and restricted to limited study areas (Iqbal *et al*. 2002). Moreover the disease is highly susceptible to climatic factors (Fuentes *et al.* 2001) and climate change affects the transmission of the disease (Mas-Coma *et al.* 2008). This study depicts the prevalence of Fasciolosis and its association with higher altitudes in small ruminants in AJK.

MATERIALS AND METHODS

Study Areas and sample size: The study based on altitudes was carried out in three districts of AJK; Mirpur, Poonch and Neelam. Three towns from each district were randomly selected and included in the study (Fig. 1). Fecal samples from 4662 small ruminants (n=2242 sheep; n=2420 goats) were collected. Details of towns and sample size are given in Table 1. Information regarding each sheep and goat was entered in "Data Capture Form" which included altitude, species, season, gender, age, flock size and history of deworming. Altitude was categorized in to (A) below 3000, (B) 3000-6000 and (C) above 6000 feet. Two species Sheep and Goats were divided into 3 age groups (A) < 1, (B) 1-4, and (C) > 4 year. Two seasons Autumn and Spring along with flock Size ≤30 and >30 animals were analyzed for association with disease.

Collection and analysis of fecal samples: Fecal samples were collected directly from the rectum of each sheep and goat using finger covered with disposable glove. After collection, the fecal samples were transferred to separate self-sealing polythene bags, labeled and transported to the Parasitology laboratory, Faculty of Veterinary and Animal Sciences, University of the Poonch Rawalakot and Department of Zoology, Mirpur University of Science and Technology Mirpur, AJK. The fecal samples were stored at 4^{o}C till further analysis within 24 hours. The samples were analyzed using centrifugal flotation and sedimentation techniques while egg per gram (EPG) count was performed by McMaster technique (Foreyt 2001).

Statistical analysis: The GIS map of the study area was prepared using Quantum GIS 2.20 version. For test of significance Chi square analysis was used on EpiInfo^{TM} 7 (Centers for Disease Control, Atlanta, GA, USA).The strength of association of all the epidemiological factors with Fasciolosis in sheep and goats was estimated through odds ratio (OR) and the corresponding 95% confidence intervals. Significant epidemiological factors were further processed through Generalized Linear Model with binomial distribution in R program (R core team, 2014) and the coefficient of determination R^2 was calculated. The model selection was made through the evaluation of AIC value and model with lowest AIC value was selected. P-value less than 0.05 was taken as significant. Mean along with standard error was calculated in Microsoft excel (2007). Factorial Design was used in R program (R core team, 2014) to compare mean egg per gram of feces with reference to species, altitude and season.

RESULTS

Prevalence: The overall prevalence of Fasciolosis in small ruminants was 17.88%. Prevalence of Fasciolosis in sheep and goats was 26.49% and 9.90%, respectively. Statistical analysis revealed that prevalence of the disease in sheep was significantly higher (P<0.05) compared to goats. Similarly, the OR value indicated that sheep were 3.27 times more prone to Fasciolosis than goats (Table 2).The prevalence of the disease was 15.09%, 25.00% and 15.74% at altitudes <3000ft, 3000-6000ft and >6000ft, respectively. Statistical analysis showed that prevalence of Fasciolosis in sheep and goats was significantly higher (P<0.05) at an altitude of 3000-6000ft. Likewise, the OR values demonstrated that sheep and goats at altitude of 3000-6000ft have 1.84 times more chance to acquire infection than at other altitudes. The prevalence of disease in autumn (August-October) and spring (March-May) were 21.77% and 13.93%, respectively. Statistical analysis revealed that prevalence was significantly higher (P<0.05) in autumn as compared to the spring season. The prevalence in male animals was found to be 16.67% whereas, in female animals18.59%. The data showed no significant difference (P>0.05) by Chi-square analysis. The prevalence of the disease in the age group below 1 year was 4.40%, for 1-4 years was 17.73% and 4 years was 36.18%. Chi-square values showed significant difference (P<0.05) in all age groups.

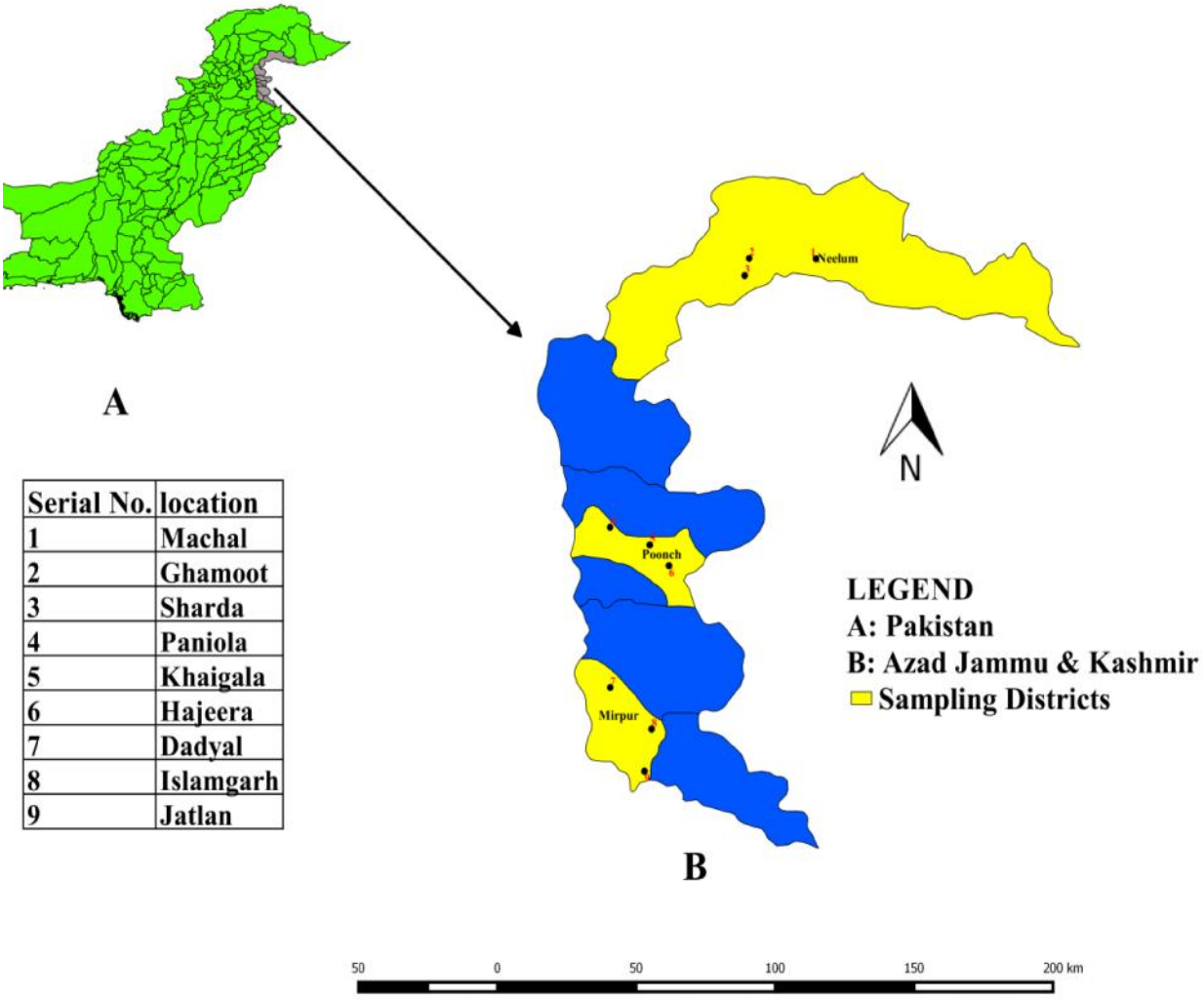
It was calculated that deworming ratio in AJK was 29.98%. 76 samples out of 1398 (5.43%) were found positive for Fasciolosis, which revealed that animals even after deworming may acquire infection. The prevalence in animals with no recent history of deworming was recorded 23.22%. The data revealed a significant difference (P<0.05) with the fact that chances of infection were more in animals that do not have an anthelmintic dose. The data showed 15.37% disease in small as compared to 18.72% in large flocks. Chi-square analysis showed a significant difference (P<0.05) in the prevalence of the disease in two flock sizes.

Generalized Linear Model: The deworming appeared to be the most significant factor in the model contributing maximum variations in disease with highest Odds followed by age groups, species, season, altitude, flock size and gender. The risk factors for Fasciolosis in sheep and goats are lack of practice of deworming, age group greater than 4 years, species sheep, autumn season, altitude between 3000- 6000 and flock size greater than 30 animals.

Intensity of Fasciolosis: Egg per Gram (EPG) of feces from all positive samples of sheep and goats (n=830) were calculated and results analyzed. The intensity of

the disease was categorized as low having EPG counts up to 200; moderate up to 300; and heavy greater than 300 eggs (Table 4). The EPG ranged 50 to 650 eggs. Goats showed higher values of heavy intensity of the disease as compared to sheep. Altitude 3000-6000 feet revealed 21.10% of infected animals having heavy infection followed by 14.46% at altitude below 3000 and 7.46% at above 6000 feet. Seasonal data revealed 26.95% heavy intensity in autumn and 13.35% in spring. The effect of season on EPG showed a higher mean values for sheep (191.49±4.92) and goats (219.72±8.13) in autumn compared to 158.04±5.28 and 180.61±8.78 in spring (Table 5). In both the seasons mean EPG for goats were found higher than sheep. The difference at three different altitudes (Table 6) was analyzed in both species. The data showed mean values for sheep 174.04±6.03, 191.87±6.07 and 168.33±7.29 at altitude below 3000, 3000-6000 and above 6000 feet respectively. The mean values for goats were 232.22±9.91, 194.95±9.32 and 170.59±12.22. The data revealed higher mean values for goats as compared to sheep at all three altitudes. The mean values of EPG for goats at an altitude below 3000 feet were found higher as compared to higher altitudes. Conversely, mean values for sheep were higher at the altitude of 3000-6000 feet.

The intensity of infection was analyzed through Factorial analysis for difference in species, altitude and season (Table 7). The difference was found significantly variable in sheep and goats. Goats revealed significantly higher ($P<0.05$) number of eggs per gram. Similarly the mean EPG at different altitudes was also found significantly different ($P<0.05$). The effect of season was found significant ($P<0.05$). The mean values of EPG in autumn season were found higher than spring in both the species of animals. The interaction between species of animal and altitude was found significant ($P<0.05$).



Serial No.	location
1	Machal
2	Ghamoot
3	Sharda
4	Paniola
5	Khaigala
6	Hajeera
7	Dadyal
8	Islamgarh
9	Jatlan

Fig. 1. Map of Azad Jammu and Kashmir showing Sampling Districts

Table 1. Sample size from different Villages/towns of each District of Azad Jammu and Kashmir.

District	Town	Altitude (feet) above sea level	Samples collected (n)
Mirpur	Jatlan	1377	613
	Islamgarh	1503	621
	Dadyal	1503	919
Poonch	Hajeera	3168	510
	Khaigala	5500	325
	Paniola	5200	397
Neelam	Sharda	6499	478
	Machal	7350	403
	Ghamoot	7800	396

Table 2. Prevalence and associated risk factors of Fasciolosis in Small Ruminants in Azad Jammu and Kashmir.

Variable	Number of Samples examined	Number of Samples Positive	Prevalence (%)	OR value	χ^2 value	P-Value
Species						
Sheep	2242	594	26.49	3.27	217.71	p<0.05
Goats	2420	240	09.90			
Sex						
Male	1721	287	16.67	0.88	2.73	P>0.05,
Female	2941	547	18.59			
Age						
<1 Year	1158	051	4.40	0.16	190	p<0.05
1-4 Years	2628	466	17.73	0.97	0.10	p>0.05,
>4 Years	876	317	36.18	3.6	245.86	p<0.05
Altitude						
<3000	2153	325	15.09	0.70	21.26	p<0.05
3000-6000	1232	308	25.00	1.84	57.64	p<0.05
>6000	1277	201	15.74	0.81	5.53	p<0.05,
Season						
Autumn	2351	512	21.77	1.72	48.83	p<0.05
Spring	2311	322	13.93			
Flock size						
≤30 animals	1158	178	15.37	0.78	6.65	p<0.05
>30 animals	3504	656	18.72			
Deworming history						
Yes	1398	76	5.43	0.19	210	p<0.05
No	3264	758	23.22			

Table 3. Generalized Linear Model with binomial distribution for risk factors assessment of Fasciolosis in sheep and goats in Azad Jammu and Kashmir

Variables	Estimate	Standard Error	Z- Value
Intercept	-3.38889	0.20814	-16.282***
Species	-1.15180	0.09235	-12.473***
Age group >4 years	1.41111	0.10522	13.411***
Age group<1 year	-1.55169	0.15814	-9.812***
Season	-1.07446	0.09229	-11.642***
Altitude <3000 ft	0.26115	0.11242	2.323*
Altitude 3000-6000 ft	0.81551	0.11559	7.055***
Deworming	2.43280	0.14384	16.913***
Flock Size	0.38318	0.11020	3.477***

Signif.codes: '***' 0.001 '**' 0.01 '*' 0.05

Table 4. Intensity of Infection with Fasciolosis in Sheep and Goats in AJK

Variables	categories	Total number of samples	Number of positive samples	Intensity		
				Low	Moderate	Heavy
Species	Sheep	2242	594	348	132	114
	Goats	2420	240	115	58	67
Altitude	<3000	2153	325	173	105	47
	3000-6000	1232	308	158	85	65
	>6000	1277	201	132	54	15
Season	Autumn	2351	512	249	125	138
	Spring	2311	322	214	65	43

Low (EPG=200), Moderate (EPG=300), Heavy (EPG>300)

Table 5. Seasonal Intensity of Infection with Fasciolosis in Sheep and Goats in AJK

Species	Season	Total Samples Examined	Total Positive Samples	Mean EPG±SE
Sheep	Autumn	1169	370	191.49±4.92
	Spring	1073	224	158.04±5.28
Goats	Autumn	1182	142	219.72±8.13
	Spring	1238	98	180.61±8.78

Table 6. Intensity of Infection with Fasciolosis in Sheep and Goats at different altitudes of Azad Jammu and Kashmir

Species	Altitude (ft)	Number of samples examined	Number of Sample positive	EPG±SE
Sheep	<3000	1034	235	174.04±6.03
	3000-6000	599	209	191.87±6.07
	>6000	609	150	168.33±7.29
Goats	<3000	1119	90	232.22±9.91
	3000-6000	633	99	194.95±9.32
	>6000	668	51	170.59±12.22

Table 7. Factorial analysis of Intensity of Infection with Fasciolosis in Sheep and Goats in Azad Jammu and Kashmir

Source of Variation	Degree of Freedom	Sumof Square	Mean Square	F-value
Species	1	105794	105794	13.30***
Altitude	2	70729	35364	4.45**
Season	1	225865	225865	28.41***
Species*altitude	2	111542	55771	7.01***
Species*season	1	387	387	0.049^{ns}
Altitude*Season	2	11347	5673	0.71^{ns}
Species*altitude*season	2	44286	22193	2.79^{ns}
Residuals	822	6534714	7950	

Signif.codes: '***' 0.001 '**' 0.01 '*' 0.05^{ns}Non Signif.

DISCUSSION

The data revealed an overall prevalence of 17.88% in both species of animals, 26.49% in sheep and 9.91% in goats. Similar results in goats were reported by Ijaz *et al.* (2008) and in sheep Njoku and Okoli, (2011). These results vary from the findings of Anjum *et al*. 2014 where 33.33% prevalence was reported in sheep and goats. These results correlate with the findings of the present study in case of sheep, however, are different to that of goats and this difference might be attributed to the different environment and management conditions. In the present study the difference in prevalence of the disease in sheep and goat was due to grazing habits of sheep. Mir *et al.* (2013) reported 5.28% disease in goats from Jammu area of Jammu and Kashmir, India. Pandit *et al*. (1989) reported 30% prevalence in sheep from the same area of Jammu and Kashmir. Their results are similar to the present investigations. Tasawar *et al*. (2007)

reported 28.75% prevalence of *Fasciola (F.) hepatica* in goats which is much higher than the present study. Similarly in a report from Kashmir, India Sharma *et al*. (1989) reported 51.30% infection rate in sheep and 14.80% in goats. The differences in prevalence rates of these findings might be due to environmental factors and the geographic locations. The environmental factors and management have strong relationship with liver fluke and its intermediate host population. Mas-Coma *et al.* (2001) has reported an important epidemiological characteristic of this disease to be the link of endemic areas to high altitudes. These researchers have established that highest prevalence of Fasciolosis occurred at altitudes 3800-4100m. Such differences of variation of the disease pattern appear to favor transmission and adaptation to high altitudes. Data are lacking regarding epidemiological reports of Fasciolosis from high altitudes of Pakistan. This is the first study of its kind from a different geographic location of the country which elaborates the extent of the disease from different climates of mountainous region. The overall prevalence rate in both species was 15.09% at altitude below 3000 feet, 25.00% at altitude 3000-6000 and 15.74% at altitude above 6000 feet. The highest prevalence was recorded at altitude between 3000-6000. The altitude between 3000-6000 showed significantly higher ($P<0.05$) prevalence of Fasciolosis in sheep and goats. These results were also in agreement with Gebreyohannes *et al.* (2013) who found 36.9% prevalence at highland and 20% at lowland as well as to those of Walker *et al*. (2008) who found that the topography of the Southern Highlands of Tanzania provided an environment where the climatic conditions favored sustenance of the intermediate host species for supporting both the *F. hepatica* and *F. gigantica*. Similar results were reported by Ahmad *et al.* (2005) from Ziarat, Baluchistan at altitude of 8346 feet. Contrarily prevalence reports are different from different climates in goats at lower altitudes. Iqbal *et al*. (1986) reported 4% prevalence from Faisalabad, Punjab however; higher rates of Fasciolosis were also reported by Durrani *et al.* (1981) and Malik *et al.* (1995). These differences in prevalence of Fasciolosis in different geographic locations were mainly because of the grazing patterns, environment of the area for flukes, their intermediate host, use of anthelmintics and management conditions.

The results of the present study revealed significantly higher prevalence of the disease in autumn (21.77%) as compared to spring (13.93%). These results are in agreement with Qureshi *et al.* (2012) and Ardo and Aliyara, (2014). Hossain *et al*. (2011) reported 26.16% prevalence in goats of Bangladesh from June-October as compared to 18.86% from March-May. Similar results were also found by Mir *et al.* (2013) in goats from Jammu and Kashmir, where prevalence was much higher in autumn than spring. Khan and Maqbool, (2012) reported 19.27% prevalence in cattle during spring and 22.20% in autumn. A huge body of data supports that Fasciolosis was more prevalent in autumn or winter. The reason for such seasonal pattern of the disease was the favorable climate of the rainy season, suitable for propagation of flukes and their intermediate host. The geographic location of the present study area indicates the favorable temperature and rainfall during summer months when animals are infected while grazing in range lands and exhibit the disease after completion of the life cycle of the parasite in September/ October. The analysis of data revealed that chances of infection with *Fasciola* species are equal in male and female animals in sheep and goats. These results suggest that no physiological or immunological phenomenon involved in the chances of getting infection in both sexes. In a study from Iran, Khanjari *et al*. (2014) and Mir *et al*. (2013), reported no sex related difference in prevalence of Fasciolosis in sheep and goats. Non-significant results were also reported by Gebreyohannes *et al.* (2013) for the sex of sheep. The data showed highest prevalence of the disease in age group of sheep and goats above 4 years and lowest in age group below 1 year. It may be concluded from these results that the age of the animal is directly correlated to the prevalence of the disease. These results are in agreement with Abdulhakim and Addis, (2012) who reported 12.7% and 7.00% prevalence in young sheep and goats as compared to 28.70% and 13.90% in adults. The reason might be the fact that young animals were kept indoors and not exposed to infection. It is probable that higher infection rate with age might be due to fall in resistance because of stress factors. Khan and Maqbool, (2012) also reported significantly higher prevalence of Fasciolosis ($P>0.05$) in adult cattle as compared to young ones. Similar results have also been reported from Ethiopia Gebreyohannes *et al.* (2013), where they found 37.6% prevalence in sheep less than 1 year and 44.4% greater than 1 year. In the present study the history of deworming in last month was taken with each sample. It was calculated that deworming ratio in Azad Jammu and Kashmir is 29.98%. The data showed a positive impact of deworming for the control of Fasciolosis under natural grazing. Even though it was not possible to collect information regarding particular group of anthelmintic drugs however, it was noted that regular deworming was necessary to control infection. Infection rates even after deworming were 5.43% which might be due to inadequate dose, improper group of anthelmintic or development of resistance against anthelmintics. Maingi *et al*. (2002) reported a marked reduction in infection with *F. hepatica* in ewes and lambs on posture after medication with dewormer in highlands of Kenya. The effect of flock size on

prevalence rate was also assessed in the present study which revealed a significantly higher infection rate 18.72% ($P<0.05$) in large herd size as compared to small 15.37%. These results were consistent with the findings of Abebaw *et al.* (2012). In a study from Bangladesh Sangma *et al*. (2012) found 7.6% prevalence of Fasciolosis in small flocks as compared to 12.7% in large and a significantly higher prevalence of helminthiasis was noted in large size flocks of sheep. This increase in rate of infection seems to be directly associated with number of animals kept under one roof as the number of animals increases, the animal have to travel long distances to explore new grazing areas or mixed with other flocks and share grazing lands and water ponds where they disseminate infection.

Intensity of Fasciolosis: The intensity of the disease was found higher in goats as compared to sheep in the study area. Factorial analyses of the data showed a significant difference ($P<0.05$) in mean EPG of the two species. These results were found in agreement with Sangma *et al.* (2012) who reported mean EPG 162.5 in sheep ranging 100-300. Chowdhury *et al*. (1994) indicated 100-400 EPG in cattle of Bangladesh. Issia *et al*. (2009) in Argentina reported mean EPG 160 in sheep and 80 in goats. The effect of season on EPG showed a higher mean values in sheep (191.49) and goats (219.72) in autumn as compared to 158.04 and 180.61 in spring. Factorial analysis showed significant difference ($p<0.05$) in intensity to be higher in autumn. The reason for higher intensity during autumn is due to the peak season of the emergence of the snail intermediate host in AJK in summer when the temperature, humidity and rain fall favors multiplication of *Fasciola* spp. New infections take place during rainy season and after completion of the life cycle new episode of egg laying starts in autumn. These findings were found similar to Qureshi *et al.* (2012) who estimated 14.2 ± 4.47 mean EPG count in spring in buffaloes from Punjab as compared to 433 ± 73.2 during autumn. Similar results were also reported by Chowdhury *et al.* (1994). The data revealed mean EPG 174.04 at altitude below 3000, 191.87 at altitude 3000-6000 and 168.33 above 6000 feet for sheep. Higher EPG values were found for goat, 232.22, 194.95 and 170.59 at three altitudes respectively. Factorial analysis showed significant difference in intensity of disease amongst altitudes. Overall intensity in both species was found higher due to heavy infection percentage 21.10% at altitude 3000-6000. These results were similar as prevalence percentage was also highest at the same altitude. It is obvious from these findings that intensity of the disease increases with rate of prevalence as new and less immune animals were exposed to infection. These results also indicate summer infection of animals when climatic factors favor the disease incidence. These results clearly indicate that one of the important risk factor for Fasciolosis was altitude 3000-6000 feet.

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