Research Article

Epidemiological and Hematological Investigations of Tick-Borne Diseases in Small Ruminants in Peshawar and Khyber Agency, Pakistan

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Abstract | Parasitism is considered as an important risk factor for the productive capacity of small ruminants and it causes huge economic losses in most parts of the world, including Pakistan. This study was designed with the objective to determine the hematological profile and prevalence of haemo-parasitic diseases in small ruminants and to correlate it with its health status. Total of 300 blood samples (Sheep, n=249, Goat, n=52) were collected from different areas of Peshawar and Khyber agency. Samples were processed for the presence of haemo-parasites and different hematological parameters were estimated. Prevalence of haemo-parasitic diseases was recorded as 40, 7 and 6% for anaplasmosis, babesiosis and theileriosis, respectively. Different risk factors were studied but no statistical significant changes ($p>0.05$) were observed in prevalence of haemo-parasitic diseases with respect to season, location, specie, age and sex. Hematological profile of diseased animals showed significant decrease ($p<0.05$) in TEC, Hb, PCV, MCH and MCV while no significant changes ($p>0.05$) were observed in MCHC and TLC. On basis of erythrocytic indices in sheep anemia can be classified as macrocytic normochromic while in goats anemia was recorded as macrocytic hypochromic. It can be concluded from the results that anaplasmosis was the most prevalent haemo-parasitic disease followed by Babesiosis and Theileriosis in the stdudy area and along with general health status of the animal it also affect the hematological profile of animals.

Keywords | Haemo-parasitic diseases, Prevalence, Hematology, Risk factors, Anemia

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INTRODUCTION

Pakistan is an agricultural country having a huge population of livestock which are well adapted to the environmental condition of the country. Our livestock mainly consist of buffalo, cattle, goats and sheep with the population of 33.7 million, 38.3 million, 64.9 million and 28.8 million, respectively. Sheep plays a major role and occupies a premier place in the livestock industry, contributing significantly to the economy of the Pakistan (PES, 2013-14).

Parasitism is considered as important risk factor for the production of small ruminants and it causes huge economic losses in most of the developing and underdeveloped parts of the world (Skyes, 1994). Small ruminants are highly exposed to variety of parasitic infections including intestinal parasitism and haemo-parasitic infections (Anaplasmosis, Theileriosis and Babesiosis) (Maske et al., 1990). Onset of parasitic diseases is mostly high in tropical and subtropical regions of the world (Muraleedharan, 2005).

Anaplasmosis in small ruminants is caused by Anaplasma marginale and Anaplasma phagoctophilum. Most common clinical signs of anaplasmosis include fever, anemia, jaundice, anorexia, respiratory signs, drop in milk production and reproductive disorders in small ruminants (Stuen et al., 2002, 2003; Garcia-Perez et al., 2003; Razmi et al., 2006). There are huge economic losses due to this disease

because of reduced weight gain and productivity losses (Matsumoto et al., 2006).

Theileriosis is a tick-borne disease of domestic animals present in tropical and subtropical regions of the world, transmitted mostly by Hyalomma. Clinical signs of theileriosis include lacrimation, anorexia, swelling of superficial lymph nodes, enlargemen of spleen, liver and icterus (Naz et al., 2012).

Babesiosis caused by Babesia ovis, is a tick transmitted intra-erythrocytic protozoan parasite. Clinical signs of babesiosis include febrile condition, anemia and haemoglobinuria. Babeia ovis is transmitted by Rhiphicephalus species of ticks in small ruminants.

Pakistan being located in the subtropical zone offer favorable environment for the proliferation of ticks population which are important indicator of haemo-parasitic diseases. Different ticks species like Hyalomma, Rhiphicephalus, Ixodidae and Boophilus play an important role in transmission of haemo-parasitic diseases (Eshetu, 2015), while Hyalomma species being the most abundant in Pakistan (Atif et al., 2012).

Most of the studies in the past have been carried out on the prevalence of haemo-parasitic diseases in cattle in Pakistan but there is limited data available on the epidemiology of tick borne diseases in small ruminants. Hematological and serum biochemistry profile can be used as an important indicator for prognosis of Tick borne diseseas. Due to scarcity of data about haemo-parasitic infection in small ruminants in Pakistan, the present study was designed with the objective to determine the epidemiological and hematological investigations of tick-borne diseases in small ruminants.

MATERIAL AND METHODS

Study Area

This study was conducted in Peshawar and Khyber Agency. Peshawar district lies at 34.04° N Latitude and 71.5° E Longitude with an altitude of 359m and has area of 1257 km^2. Khyber agency lies at 34.02° N latitude and 71.28° E longitude with an altitude of 1070m and has area of 2,567 km^2. Average temperature range from 25°C (77 °F) to over 40°C (104 °F) in summer and 4°C (39 °F) to 18.35°C (65.03 °F) in winter.

Sample Collection

During the year 2015 total of 300 blood samples (Sheep=247, Goat=53, Adult=267, young=33, Male=129, Female=171) were collected from different areas of Peshawar and Khyber agency. Blood samples collected were screened for haemo-parasites (Anaplasma, Theileria and Babesia) and for estimation of hematological profile hematology was performed.
Prevalence of Haemo-parasites were estimated using formula.

$$P = \frac{d}{n} \times 100$$

Where:
P: Prevalence; d: No. of animals found positive; n: Total no. of animals sampled (Thrusfield, 1995).

Microscopic Examination

Thin blood smears were prepared and fixed in methanol for 5 min and stained for 30 min in Giemsa stain as described by Jalali et al. (2013).Slides were then examined for haemo-protozoan like Anaplasma, Theileria and Babesia spp. at 100× objective magnification.

Hematology

For finding of hematological profile, 3ml of blood was collected in sterile vacutainer tubes and processed for hematological studies including, Total Erythrocyte Count (TEC), Total Leukocyte Counts (TLC), Hemoglobin levels (Hb) and Packed Cell Volume (PCV) by using Hematology Analyzer (Sysmax KX-21N, Japan). Erythrocytic Indices were also estimated including Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH) and Mean Corpuscular Hemoglobin Concentration (MCHC) (Yasini et al., 2012).

Statistical Analysis

Data collected was arranged in Ms Office Excel and statistically analyzed by Chi square (χ^2) for prevalence while hematological data was analyzed through t-test: paired two sample means and means were compared by Duncan's multiple range tests at a probability level ≤ 0.05 using Statistical Package for Social Services (SPSS) version 16.0.

RESULTS AND DISCUSSION

Prevalence Of Haemo-Parasites In Small Ruminants

Out of total 300 blood samples 159 (53%) samples were positive for Haemo-protozoan's in which Anaplasma, Babesia and Theileria was recorded as 40, 7 and 6%, respectively (Table 1).

Table 3: Hematological profile of sheep and goats suffering from tick borne diseases in district Peshawar and Khyber Agency (Mean ± standard error)

Parameter	Sheep			Goats		
	Non-infected	Infected	P-value	Non-infected	infected	P-value
TEC	11.29[a]±0.081	5.57[b]±0.08	0.00	11.53[a]±0.16	5.35[b]±0.16	0.00
Hb	10.66[a]±0.14	6.73[b]±0.08	0.00	11.23[a]±0.15	5.74[b]±0.18	0.00
PCV	34.21[a]±0.31	20.69[b]±0.35	0.00	35.09[a]±0.54	20.52[b]±0.67	0.00
MCH	9.61[b]±0.13	12.46[a]±0.15	0.00	9.79[b]±0.28	10.9[a]±0.34	0.01
MCV	30.53[b]±0.5	38.26[a]±0.58	0.00	31.23[b]±0.9	39.62[a]±1.12	0.00
MCHC	32.286[a]±0.39	32.66[a]±0.45	0.53	31.66[a]±0.86	28.38[b]±1.08	0.02
TLC	9.14[a]±0.08	9.16[a]±0.1	0.89	9.04[a]±0.18	9.36[a]±0.23	0.29

[a,b]: means with different superscript with in row are significant at P-value 0.05; **TEC:** Total erythrocytes count; **Hb:** Hemoglobin; **PCV:** Packed cell volume; **MCV:** Mean corpuscular volume; **MCH:** Mean corpuscular hemoglobin; **MCHC:** Mean corpuscular hemoglobin concentration; **TLC:** Total leukocytic count

Table 1: Overall prevalence of Haemo-parasites in different small ruminants in District Peshawar and Khyber Agency

Parasitism	Parasites	N	Prevalence (%)
Haemo-parasites	Anaplasma	300	40
	Theileria	300	6.0
	Babesia	300	7.0

Table 2: Prevalence of Haemo-protozoan in small ruminants in District Peshawar and Khyber Agency with respect to season, location, specie, age and sex

Variables	Category	N	Prevalence of Haemo-protozoan (%)	χ^2 value	P value
Season	Winter	16	31.25	8.16	0.47
	Spring	28	53.5		
	Summer	225	53.3		
	Autumn	31	61.2		
Location	Peshawar	244	55.7	5.37	0.14
	Khyber agency	56	41		
Specie	Sheep	247	55	7.62	0.05
	Goat	53	43.3		
Age	Adult	267	52.8	3.17	0.36
	Young	33	54.5		
Sex	Female	171	46.7	6.43	0.09
	Male	129	61.2		

Risk Factors For Haemo-Parasitic Diseases

Season wise prevalence of haemo-parasites was 31.2, 53.5, 53.3 and 61.2% in winter, spring, summer and autumn, respectively. Prevalence was recorded as 55.7% in Peshawar while 41% in Khyber agency. Regarding species, prevalence was 55% in sheep and 43.3% in goats whereas, 53.8 and 54.1% prevalence was recorded in adult and young animals, respectively. Sex wise prevalence was 46.7% for female and 61.2% for male animals. It is evident from Table 2 that despite of slight differences in variables no significant differences was recorded with respect to season, location, specie, age and sex.

Hematological Findings

Hematological profile (Table 3) of infected and non-infected animals shows significant decrease in TEC, Hb, PCV, MCH and MCV while no significant changes were observed in MCHC and TLC. On basis of erythrocytic indices in sheep anemia can be classified as macrocytic normochromic while in goats anemia is macrocytic hypochromic.

Pakistan being an agricultural country has huge population of livestock, in which goats and sheep population are 64.9 million and 28.8 million, respectively (PES, 2013-2014). Despite of this huge population productive capacity of these animals are not according to the need. Haemo-parasitic diseases (Anaplasmosis, Theileriosis and Babesiosis) are important parasitic diseases of small ruminants throughout the world but results are more dreadful in tropical and sub-tropical regions of the world, including Pakistan (Sathaporn et al., 2004; Silke, 2009).

Among the haemo-parasitic diseases anaplasmosis was the most prevalent diseases of small ruminant in the present study and these findings are in agreement with the results of Takeet et al. (2009), Puvarajan et al. (2014) that anaplasmosis is the most prevalent haemoprotozoan in small ruminants. In the present study prevalence of Anaplasmosis, Babesiosis and Theileriosis was 40, 7 and 6%, respectively. Anaplasmosis was recorded higher *i.e.* 40% which is not in accordance with the findings of Khan et al. (2015) where prevalence of Anaplasma was 28%. Possible reason for this indifference might be due to the fact that Khan et al. (2015) carried out their study only in sheep and study

area was district Peshawar while in the current study Khyber agency was also included in the sampling area. Other possible reasons for this disagreement might be difference in sampling size and inclusion of goats in the present study. Prevalence of Theileriosis was almost in congruent with the findings of Naz et al. (2012). Babesia prevalence recorded in the present study was lower from the study conducted by Esmaeilnejad et al. (2012). This might be due to the geographical differences and also PCR was used as a diagnostic tool by Esmaeilnejad et al. (2012) which is more sensitive and specific diagnostic test as compare to microscopy which is used in the current study.

Prevalence of parasitic diseases was found to be non-significant with respect to season, location, specie, age and sex. These findings were partially in congruent with the study carried out by Naz et al. (2012) that age, sex and season had no effect on prevalence of parasitic diseases in goats while in sheep prevalence was affected by age and sex. In current study prevalence in both sheep and goats was not significant with respect to season, location, age and sex. Haemo-parasitism though non-significant, was recorded higher in autumn, summer and spring as compare to winter. This statement is supported by the findings of Velusamy (2015) that prevalence was recorded higher in hot months of the year. Higher prevalence of haemoprotozoan in hot and humid months of the year can be due to the availability of favorable environment for the infestation of arthropod vectors in these months which play pivot role in the transmission of these diseases (Sajid, 2007; Durrani, 2008; Atif et al., 2012).

Hematological findings showed significant decrease in value of TEC, Hb, PCV, MCH and MCV while in MCHC and TLC no significant changes were observed in sheep. Hematological parameters were almost similar in both sheep and goats except MCHC which was found statistically significant decrease in goats. Study carried out by Esmaeilnejad et al. (2012) endorsed the statement that MCHC was statistically significant in goats affected with haemo-parasites. Results of hematology were almost similar to the findings of Khan et al. (2015), Ahmadi hamedani et al. (2012), Younis et al. (2010). On the basis of erythrocytic indices anemia can be classified as macrocytic normochromic in sheep with increase in MCV and MCHC in normal range. In goats significant increase was observed in MCV while statistical significant decrease was noticed in MCHC values and anemia can be classified as macrocytic hypochromic.

CONCLUSION

Based on these findings, it can be concluded that anaplasmosis is the most prevalent haemo-parasitic disease in small ruminants, followed by Babesiosis and Theileriosis in the studied area. Along with general health statuses, hematological profile of diseased animals can also be affected and thus should be considered as markers of parasitism.

CONFLICT OF INTEREST

There is no conflict of interest.

AUTHORS CONTRIBUTION

SSAS and MIK conceived idea of the manuscript. SSAS and HUR collected and processed blood samples. SSAS analyzed the data and draft the manuscript. All authors reviewed the final version of the manuscript.

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