

TBD's EXPLOITING THE BLACK GOLD (BUFFALO) OF PAKISTAN

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ABSTRACT

Haemo-parasitic diseases are important diseases of livestock transmitted by ticks, which adversely affect the productive performance of animals. This study was designed with the objective to determine the frequency of haemo-parasitic diseases in central zone of Khyber Pakhtunkhwa along with associated risk factors and hematological profile. For this purpose total of 250 blood samples were collected from three districts in anti-coagulant added vacutainer. Microscopic results revealed overall occurrence of haemo-parasites as 49.6% in which anaplasmosis was recorded as 36%, whereas theileriosis was 13.6%. Haemo-parasites occurrence with respect to different predisposing factors i.e. season, location, age and gender was studied. Frequency of haemo-parasites was significantly ($P<0.05$) higher in summer season as compare to winter. Similarly frequency was higher in Peshawar (74.1%) as compare to other two districts and the difference was statistically significant ($P<0.05$). Age is also consider as an important risk factor for the occurrence of haemo-parasitic diseases and adult were found more susceptible in the current study. Frequency was higher in female as compare to female but no significant ($P>0.05$)

difference was observed. Infected animals in the study area showed significant ($P<0.05$) variation in hematological parameters and anemia was categorized as microcytic hypochromic on the basis of erythrocytic indices. Hot and humid weather favors the development and growth of ticks which may be the possible reason for the higher incidence of haemo-parasites in summer season. Hyperactive free radicals i.e. superoxide are produced in the parasitic diseases which may be the reason for anemia.

Keywords: *Bubalus bubalis*, buffalo, haemo-parasitic diseases, anaplasmosis, theileriosis, hematology, microcytic hypochromic anemia

INTRODUCTION

More than 97% of world buffalo (*Bubalus bubalis*) population are in Asia and pacific region, mainly India and Pakistan (Zia *et al.*, 2011). About 98% of buffaloes in region are raised by small farmers having less than two hectares of land and less than five buffaloes (Mudgal, 1992). Buffalo is considered as more productive, healthier and useful for the poor farmers in Asia, especially in the sub-continent (Payne and Wilson, 1999). Due to its

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important economic role, it is regarded as black gold of Asia (Memon and Khushk, 2004) and black gold of Pakistan (Bilal *et al.*, 2006). Comparative to cattle, buffalo has been much neglected in most of the research institutes.

In Pakistan, buffalo is the main dairy animal, with 36.6 million heads. Buffalo is the principal source of milk production (33,137 thous. tons) in Pakistan followed by cattle (19,412 thous. tons). More than 61% of milk in Pakistan is obtained from dairy buffaloes (Pakistan economic survey 2015 to 2016). Important breeds of buffalo in Pakistan are Nilli, Ravi, Nilli-Ravi, Aza kheli and Kundhi and are mainly used for milk production (Bilal *et al.*, 2006; Khan *et al.*, 2007). Buffalo shares about 32% in red meat supply while 2% for draught purpose (Khan *et al.*, 2007).

Despite good genetic potential, most dairy animals in Pakistan have considerably low milk production due to lack of disease control. In Pakistan, parasitic diseases including tick borne diseases are considered a major obstacle in the health and production performance of cattle and buffaloes. Ticks are important issue because they transmit diseases, induce paralysis or toxicosis and cause physical damage to livestock. Most important tick borne diseases of livestock are Theileriosis, Babesiosis and Anaplasmosis.

Theileriosis is an economically important parasitic disease, widely prevalent in Asia, north Africa, south Europe and Australia (Ali and Radwan, 2011). Two most important species which affect cattle and buffaloes are; *Theileria annulata* and *Theileria parva*. *Theileria* specie may overcome the immune system of the animal and may lead to death in severe cases. The pathology of the disease is related to intra-macrophage stage of the protozoa (schizont) (Glass, 2001). Generally, the clinical cases of theileriosis occur throughout

the year (Muhammad *et al.*, 1999). However, there is an increase in clinical cases of the disease in cattle and buffaloes during the hot and humid months of the year (Ashfaq *et al.*, 1983). *Theileria* protozoa is transmitted by the Ixodid ticks of the genus *Hyalomma* (Aktas *et al.*, 2004). The tick vectors such as *Hyalomma anatolicum*, *Hyalomma marginatum*, *Hyalomma excavatum* are well known for transmission of the *Theileria* species in tropical and sub-tropical regions (Viseras and Garcia-Fernandez, 1999).

Anaplasma is one of the most important parasite transmitted by at least 20 ticks species. There are many *Anaplasma* species but *Anaplasma marginale* and *Anaplasma centrale* are the most important (Jabbar *et al.*, 2015). *Anaplasma* parasite is responsible for severe hemolytic disease, anaplasmosis. *Anaplasma centrale* can induce a moderate degree of anemia but clinical outbreak in field may be rare (Kocan *et al.*, 2003). The disease is characterized by fever, anemia, jaundice, brownish urine, loss of appetite, dullness and depression, rapid deterioration of physical condition, muscular tremor, constipation, yellowish mucus membranes and labored breathing (Eshetu, 2015).

Keeping in mind the importance of these ticks transmitted diseases of buffalo, the present study was designed with the objective to determine the occurrence of TBD's and associated predisposing factors in central zone of Khyber Pakhtunkhwa and to correlate its hematological profile with its health status.

MATERIAL AND METHODS

Sampling area

Samples were collected from three central districts (Peshawar, Charsadda, Nowshera) of

Khyber Pakhtunkhwa, Pakistan. Climate of these districts are almost same and referred as a local steppe climate. Temperature range from 4°C in winter to over 45°C in summer.

Sample collection

Total of 250 blood samples were collected and received from different areas of district Peshawar, Charsadda and Nowshera from buffaloes in The Center of Parasitology and Poultry, Veterinary Research Institute, Peshawar. Blood samples from jugular vein were collected in anticoagulant added vacutainer and transported to lab for processing.

Parasitological examination

For detection of haemo-parasites, thin blood smears were prepared on microscopic glass slides. Blood smear was fixed in methanol for 2 minutes, stained with giemsa for 15 to 20 minutes and washed with tap water. Microscopic examination of slides was made at oil immersion lens (100 × objective) for the presence or absence of haemo-parasites (Shah *et al.*, 2017).

Hematological parameters

For estimation of hematological parameters, anticoagulant added blood was subjected to Automatic Hematological Analyser (Sysmax KX-21N, Japan) (Yasini *et al.*, 2012).

Statistical analysis

Data was compiled and analyzed through χ^2 for the prevalence of TBD's with respect to various predisposing factors. Haematological data was analyzed through student t-test and means were compared by LSD at a probability level $P < 0.05$ using statistical package SPSS 19.0 as described by Shah *et al.* (2017).

RESULTS AND DISCUSSION

Results of the study showed that overall frequency of haemo-parasites was recorded as 49.6%, in which anaplasma was observed in 36% of cases while 13.6% were positive for theileria parasites (Figure 1). Overall prevalence recorded by Buriro *et al.* (1994) was lower than that of the present study, however almost similar results about anaplasma occurrence in buffalo was also reported by Buriro *et al.* (1994). Similarly findings of Maharana *et al.* (2016) are slightly lower from the overall occurrence of haemo-parasites in the present study. Occurrence of theileria parasite in the present study is in congruent with the findings of Salem *et al.* (2014). Memon *et al.* (2016) reported higher prevalence of theileriosis i.e. 64.5% in buffalo in Hyderabad, Pakistan. This indifference might be due to geo-climatic variation and difference in animal housing.

Risk factors for haemo-parasites

Different predisposing factors (season, location, age and sex) associated with the occurrence of haemo-parasites were studied in detail in the present study (Table 1).

Occurrence of haemo-parasites was 52.4 and 23.8% in summer and winter, respectively and was found statistically significant ($P < 0.05$). These results are supported by the findings of Memon *et al.* (2016) that haemo-parasites occurrence is higher in hot and humid months of the year because it favors tick infestation which are important vectors for the transmission of disease. Similar results about season wise incidence of haemo-parasites were also reported by Maharana *et al.* (2016).

District wise incidence of haemo-parasites in the present study were 74.1%, 28.3% and 26.5% in Peshawar, Nowshera and Charsadda,

Haemo-parasites frequency

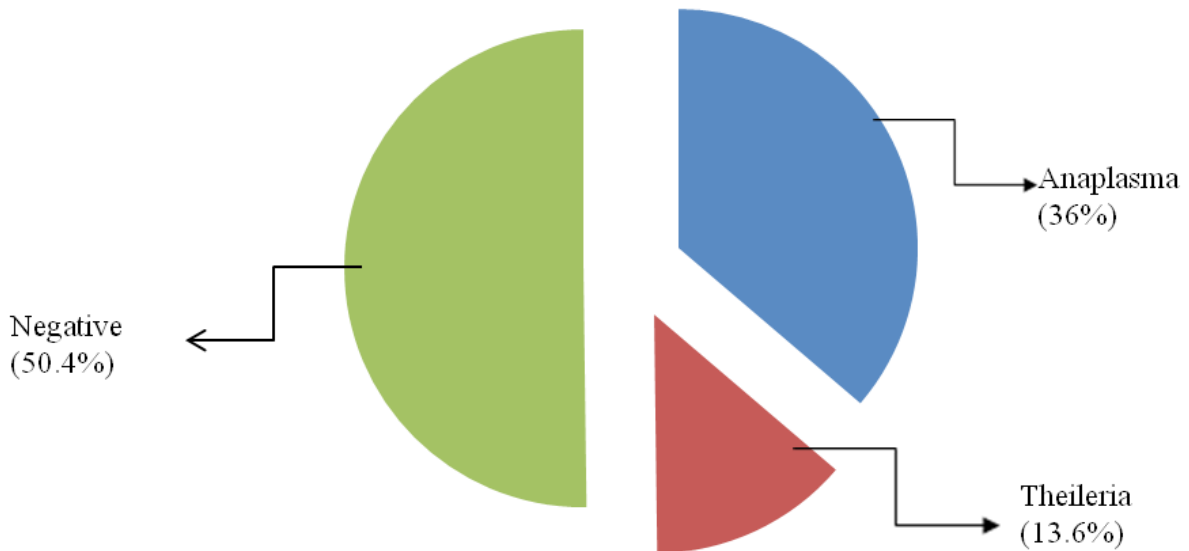


Figure 1. Overall occurrence of haemo-parasites in buffalo.

Table 1. Prevalence of anaplasmosis and theileriosis in buffalo in district Peshawar, Nowshera and Charsadda with respect to season, age and sex.

Variables	Category	N	Frequency of haemo-protozoan (%)	χ^2 -value	P-value
Season	Winter	21	23.8	6.47	0.039
	Summer	229	52.4		
Location	Peshawar	120	74.1	54.00	0.000
	Nowshera	81	28.3		
	Charsadda	49	26.5		
Age	Adult	206	55.3	13.58	0.001
	Young	44	25		
Sex	Female	217	52.1	2.87	0.238
	Male	33	36.3		

respectively. Incidence was higher in district Peshawar comparatively and this difference was found significant statistically ($P < 0.05$). In Peshawar, mostly animals are tethered and movement of animals is restricted which is the possible reason for the higher incidence of haemo-parasite infection in Peshawar as compare to Nowshera and Charsadda where animals movement is not restricted. This statement is in agreement with the findings of Iqbal *et al.* (2013); Sajid *et al.* (2014) that tethered animals are more prone to haemo-parasitic diseases.

Age wise incident was recorded as 55.3% in adult while 25% in young in the present study and this difference was statistically significant ($P < 0.05$). Higher prevalence in adult animals was also reported by Khan *et al.* (2004); Atif *et al.* (2012); Maharana *et al.* (2016). Gender wise incidence was higher in female (52.1%) as compare to male (36.35) however, no significant ($P > 0.05$) relation was found. Higher incidence of haemo-parasites in buffalo was also reported by Atif *et al.* (2012); Sajid *et al.* (2014). Possible reason for this higher incidence in females might be due to hormonal disturbances due to milk production and breeding system (Sajid *et al.*, 2009; Sajid *et al.*, 2014).

Hematological parameters

Total erythrocytic count, hemoglobin and packed cell volume of infected animals were lower from that of non-infected and were highly significant statistically ($P < 0.01$). Erythrocytic indices i.e. MCHC, MCV were also studied and were found significantly lower ($P < 0.05$). Total leucocyte count of infected animals were higher as compare to non-infected and were statistically significant ($P < 0.05$) (Table 2).

Results of hematology are in partial agreement with the findings of Durrani *et al.* (2008); Memon *et al.* (2016) who also reported significant decrease in blood parameters but classified anemia as macrocytic hypochromic whereas in the present study anemia was categorized as microcytic hypochromic. Decrease in hematological parameters was also reported by Singla *et al.*, 2013; Singh *et al.*, 2014; Maharana *et al.* (2016) and are almost in conformity with the findings of present study. Total leucocyte count was increased in the infected animals as compare to non-infected and this statement is supported by the findings of Maharana *et al.* (2016). Haemo-parasites metabolizes hemoglobin and produce free-radicals i.e. superoxide which damage erythrocytes and thus may be the possible contributing reason for

Table 2. Alteration in haematological parameters of buffalo suffering from tick-borne disease.

Parameters	Non-infected	Infected	P-value
TEC	6.84 ^a ±0.06	4.4 ^b ±0.03	0.00
Hb	11.67 ^a ±0.07	5.93 ^b ±0.05	0.00
PCV	32.43 ^a ±0.23	21.89 ^b ±0.11	0.00
MCHC	36.13 ^a ±0.26	27.1 ^b ±0.21	0.00
MCV	50.09 ^a ±0.38	47.68 ^b ±0.38	0.01
TLC	5.14 ^b ±0.04	6.34 ^a ±0.05	0.02

the anemia in haemo-parasitic diseases (Grewal *et al.*, 2005; Abd Ellah, 2013).

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