Hematological and Serum Biochemical Alteration in Cattle and Buffaloes Suffering from Natural Infection of Black Quarter

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Abstract

Hematological and serum biochemical changes in cattle suffering from natural outbreak of Black quarter (BQ) in different areas of Punjab, Pakistan were studied. Blood samples from infected cattle were subjected to TLC, TEC, DLC, hemoglobin and PCV while serum samples for estimation of Cpk, ALT and AST (n=50). It was found that mean erythrocyte count decreased significantly ($P < 0.05$) while mean leukocyte count increased significantly ($P < 0.05$) in diseased animals. On the other hand mean Hb, platelets count and PCV in diseased animal did not differ significantly ($P > 0.05$) as compared to healthy animals.Average DLC values were found varying to great extent. It was found that mean neutrophils and lymphocytes (

Index terms— black quarter, cattle, buffalo, serum chemistry, hematology.

1 Introduction

lack quarter is important disease of cattle and buffaloes causing significant mortality in Pakistan. Cattle and buffaloes are domesticated ruminants facing lot of challenges in Pakistan like production, management, nutrition and health care problems. Clostridia are commonly found in environment as well as in the intestinal tract of humans and of many animals. Several Clostridium species are pathogenic to humans, domestic animals and/or wildlife and are responsible for well knownclostridial diseases such as gas gangrene, botulism, pseudo-membranous colitis and food borne illness (Hatheway, 1990).

Clostridiumchauvoei is a gram-positive, sporeforming anaerobe that has strong hemolytic activity. Within the space of few days disease occurs and within a herd it is more likely to be affected a number of animals. The disease is enzootic in particular areas, especially when they are subject to flooding; such an area may vary in size from a group of farms to an individual field. The case fatality rate in blackleg approaches 100% **??**Radostitset al., 2006). It causes serious toxemia and high mortality in cattle, sheep and many other ruminants associated with spore contaminated soil. It is considered the most important Clostridium producing economic losses in livestock (Smith and Williams, 1984).

Knowledge and understanding of the epidemiological profile of contagious diseases is quite necessary in order to devise strategies to eradicate diseases of sporadic nature. Seasonal prevalence of Black Quarter in different areas of Punjab can be proved as a useful tool to understand the pattern and mode of transmission of disease. The parameters like geographical and seasonal distribution of contagious diseases were recorded and analyzed during scanning surveillance (Khan, 2010).

This disease caused huge economical losses in the form of mortality of cattle and buffaloes. Lack of research on this pathogen in Pakistan created many difficulties in the control, prevention and management. Hence the present study was planned to find suitable solution for the problem. The objective of this study was to study hematology and serum biochemistry of infected animals in different districts of the Punjab, Pakistan.

2 II.

3 Materials and Methods

A survey of prevalence of black quarter was conducted in different districts of the Punjab province and samples were collected from suspected and infected animals to explore the hematology and serum chemistry.

Approximately 2 ml blood sample was collected from each of the suspected animals with the help of sterile needle and poured in ethylenediaminetetra-acetic acid (EDTA) mixed vaccutainers. haematological parameters studied were total erythrocyte count (TEC), haemoglobin (Hb), haematocrit, thrombocyte count, total leukocyte count (TLC) and differential leukocyte count (DLC). All these parameters were simultaneously performed in an automated hematology analyzer (Beckhim and Coleman, USA). The results were obtained in printable form with the help of printer attached with the instrument.

Animal were divided in four experimental groups and three groups were infected artificially with Clostridium cha obtained from field. Serum samples were collected from animals in all groups at scheduled (0, 10, 20, 30) hours post infection for three days consecutively. Blood glucose was measured by taking a drop of blood from each animal on code free strip and reading was noted by Glucometer (code free, China). a) Creatinine Phospho-Kinase (CpK) Creatinine kinase was assayed from serum samples using Fortress Diagnostics kit BXC0452 -CK-MB. The readings of each sample were recorded using spectrophotometer.

4 III.

5 Results

Hematological data revealed a significant decrease ($P < 0.05$) in leukocyte count on second and third day of sampling and is non-significant ($P > 0.05$) at 1stday of sampling (Fig. **??**). Mean values of total erythrocyte count were significantly decreased ($P < 0.05$) while there was no significant difference ($P > 0.05$) observed on 1stof sampling (Fig. **??**). There was significant decrease ($P < 0.05$) of hemoglobin level in all treatment groups as compared to healthy animals (Fig. **??**). Mean platelets count was significantly increased ($P < 0.05$) on 3rd day of sampling but it was nonsignificant ($P > 0.05$) on 1st and 2nd day of sampling (Table 1). Data were analyzed through ANOVA and DMRT was used for the comparison of means of different treatment groups using SAS (SAS Int. Cary, North Carolina, V 9.1). 2 indicate there was a significant increase in neutrophils count ($P > 0.05$) noted as compared to healthy buffaloes at each sampling day but they were not found significantly different ($P<0.05$) among each other. Similarly Lymphocyte count was significantly decreased as compared to normal cattle while not significant ($P<0.05$) with other treatment groups (Fig. **??**). Differential leukocyte count for buffaloes is presented in Table 3 andthe results show that there was significant increase in neutrophils count in all treatment groups ($P < 0.05$) as compared to normal while there opposite trend i.e. decrease in lymphocyte count. Monocytes count was also showing increase as compared to normal groups (Fig. 4).

6 Serum Biochemical Tests

Results of biochemical test are given in Table 4. Means with same superscripts in a column are not significantly different ($P <0.05$) as shown in Table 4. It can be inferred from the results that the mean values of Blood glucose were increased significantly ($P <0.05$) on 1st day of sampling and then decreased gradually and were non-significant among each other during 2nd and 3rd day of sampling (Fig. **??**). Mean values of CPK also increased much significantly ($P >0.05$) in infected cattle as compared to normal during 1st day of sampling and then showed a gradual decrease in the subsequent sampling days having non-significant difference among each other (Fig. **??**).Mean ALT conc. Was also found significantly higher ($P >0.05$) in all treatment groups as compared to healthy animals (Fig. **??**), while interestingly AST levels were significantly higher at 1st sampling day ($P >0.05$), insignificant at 2nd sampling ($P <0.05$) and significantly lower than normal at 3rd Sampling day (Fig. **??**). Data regarding serum enzyme level estimation for healthy and infected buffalo is shown in Table 5. A significantly higher level of CPK and ALT ($P < 0.05$) was observed in 1st sampling group subsequently decreasing in next treatment groups while the values of AST also increased in 1st sampling group significantly while it continued to decrease in 2nd and in 3rd group it was significantly decreased than normal (Fig. **??**, 8, 9).

V.

7 Discussion

Although previously reported literature emphasized on the fact that hematological values are not much significant to tell about the course of infection and disease pathogenesis. Yet some parameters have some clinical importance. In the present study, significant leukopenia was noted, however, decreasing tendency was observed with the passage of time. **??**ingh et al. (1991) reported somewhat similar pattern of leukopenia in experimentally infected hill bulls. The results of present studies are also in agreement with the findings of Usehet al. **??**2008). The studies conducted in our experiment reveal that during day 1 and 3 mean WBC's count was significantly decreased while during day 2 it was found non-significant with the healthy animals. The possible reason behind leucopenia may be attributed to the production of neuraminidase by Clostridiumchauvoei. The outcome of this neuraminidase is to deacylate leukocytes, leading to their decrease concentration in peripheral blood (Esievo and Saror, 1983).

Observations regarding Erythrocyte count revealed a significant decreased tendency during 1st and 3rd day of sampling while it was not significantly different from normal during 2nd day of sampling. These results are in contrary with the findings of Singh et al. (1993), who reported an increase in TEC in the all the infected groups of cattle. The reason for this variability may be the course and nature of disease occurring naturally versus the experimental inoculation of the infectious agent. The decrease in erythrocyte count may also be the consequence of hemorrhages and hemolysis due to the effect of toxins produced by the bacteria (El-sawiet al., 1989).

Mean Hb Concentration and PCV values were found significantly increased as compared to normal healthy cattle attributing to the effect of loss of Plasma volume as a result of dehydration (El-Sawiet al., 1989). These results were corroborated by the studies reported by Usehet al. **??**2008). Another possible reason for increase in PCV may be due to the activity of neuraminidase consequential to enhance vascular permeability leading to edema, hypovolemia, hemoconcentration and ultimately leading to decreased PCV in infected animal **??**Useh, 2002; **??**sehet al., 2006). The significance of present findings were strengthened by the results of Singh et al. (1993) which also concluded his studies reporting a significant increase in PCV and Hb. Concentration in the experimentally infected bulls.

Platelets count in all the sampling days was found to be increased ($P < 0.05$) as compared to normal group. The results of this finding about this particular parameter are in contrast to the results of studies of some other researchers **??**El-sawiet al., 1989;Singh et al., 1993) have shown thrombocytopenia in their respective studies. One of the hypotheses behind this discrepancy may be the nature of infection and healing tendency of edema found more pronounced in the present studies relating to an increased production of platelets.

Differential leukocyte count was observed with variable trend in all infected groups when compared to normal healthy group. Neutrophils count was significantly much higher ($P> 0.05$) in all sampling days while the values of lymphocytes and monocytes were observed gradually increasing throughout from day 1 to day 3 of infection. On the other hand values of eosinophil count were significantly higher on day 1 decreasing gradually subsequently. The results of present study were in somewhat agreement with the findings of (Singh et al., 1993; **??**sehet al., 2008). The conclusion of their findings was based on the fact that there was lymphopenia and neutropenia (El-sawiet al., 1989), lymhopenia (Singh et al., 1993),Eosinopeniaand monocytopenia (Usehet al., 2008). These findings are as a possible result of migration of leukocytes towards the site of infection resulting in their decrease in peripheral circulation **??**Rodostitiset al., 2006). [1]

Figure 1:

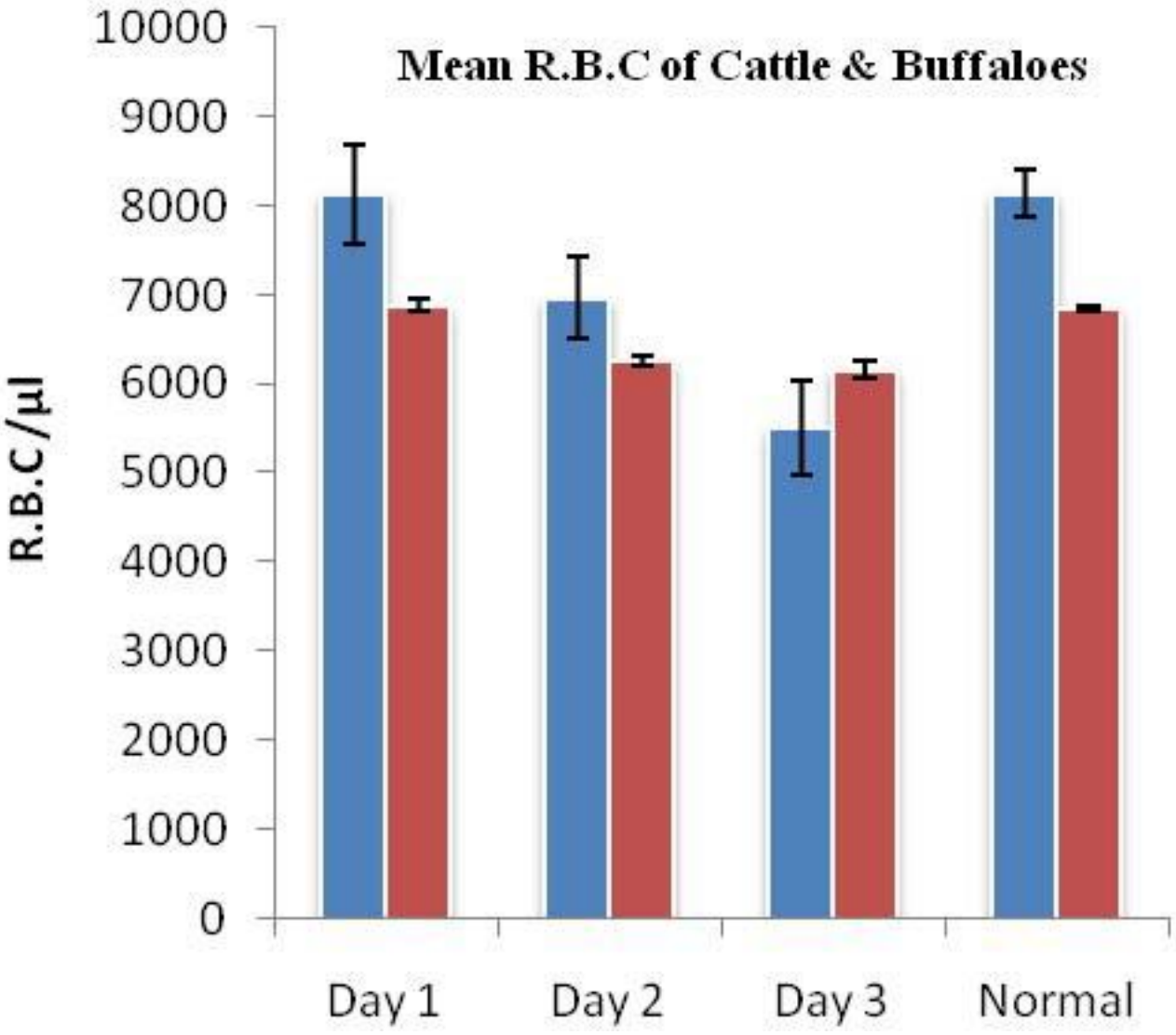

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Figure 2: Figure 1 :Figure 2 :Figure 3 :

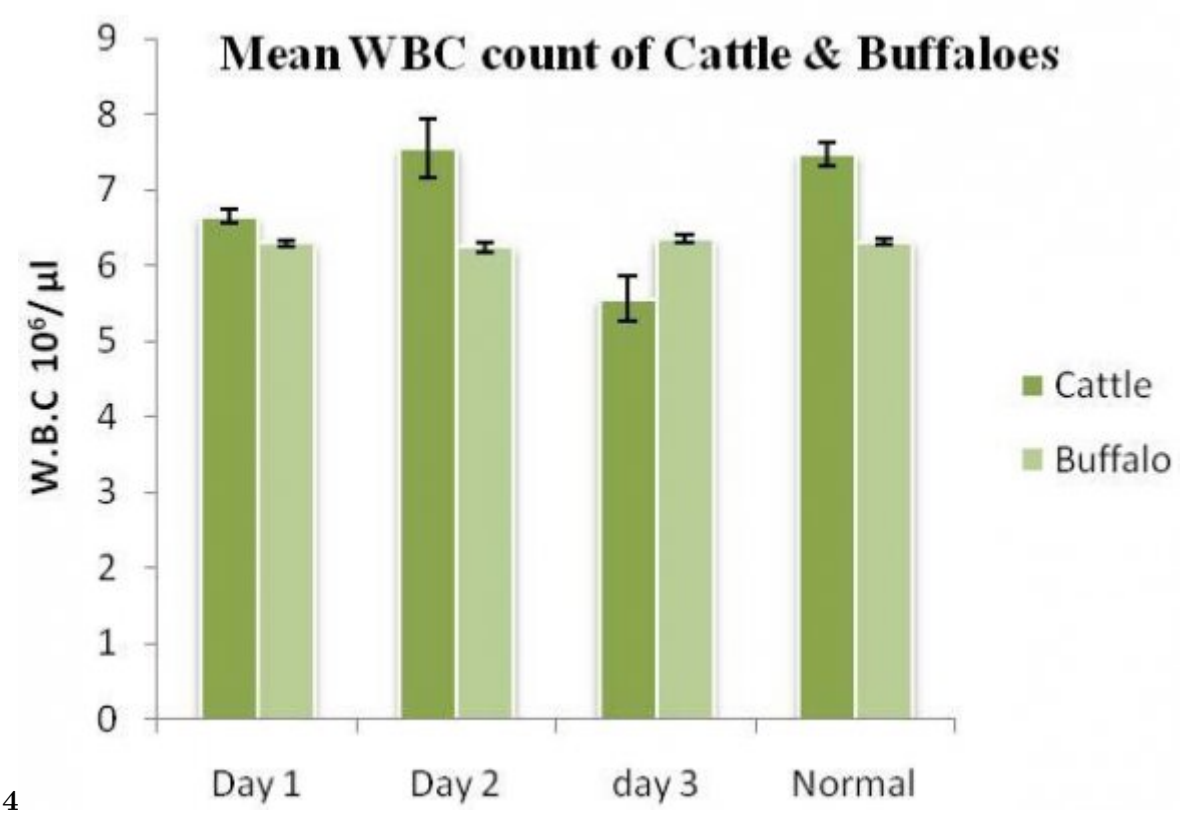

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Figure 3: Figure 4 :

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Figure 4: Figure 5 :

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Figure 5: *Figure 6 :Figure 7 :Figure 8 :Figure 9 :

Figure 6:

1

Days of Infection	Blood White Cells / µl	Red Cells 10 6 / µl	Blood g/dl	Hemoglobin	Packed Cell Volume %	Platelets 10 3 / µl

Figure 7: Table 1 :

2

Days of Infection	Neutrophils %	Lymphocytes %	Eosinophils %	Monocytes %
1 st Sampling	64.55 ± 2.28 a	21.30± 1.46 b	9.15± 0.90 b	5.35± 0.6 a
2 nd Sampling	66.85± 1.42 a	22.1± 1.45 b	7.65± 0.34 c	3.40± 0.30 a
3 rd Sampling	68.1± 1.13 a	20.90± 1.21 b	7.75± 0.35 c	3.25± 0.29 a
Normal	32.80 ± 4.76 b	55 ± 8.1 a	3.70± 1.30 a	8.50 ± 2.49 b

*Means with different superscripts in columns are significantly different at ($P < 0.05$)

Figure 8: Table 2 :

3

Days of Infection	Neutrophils %	Lymphocytes %	Eosinophils %	Monocytes %
1 st Sampling	58.15 ± 1.77 a	24.90± 1.04 b	7.45± 0.61 a	9.65± 0.56 a
2 nd Sampling	61.3± 1.56 a	22.15± 1.24 b	8.0± 0.53 a	8.55± 0.45 a
3 rd Sampling	62.05± 1.45 a	19.88± 1.21 b	7.88± 0.49 a	8.41 ± 0.49 a
Normal	34.0 ± 1.17 b	54.45 ± 0.81 a	6.80± 0.61 a	4.75 ± 0.41

[Note: b Means with different superscripts in columns are significantly different at ((P < 0.05)]

Figure 9: Table 3 :

4

Days of Infection	Blood Glucose m.mol/ l	CPk IU/ l	ALT IU/l	AST IU/l
1 st Sampling	8.85 ± 0.56 a	702.19±116.77 a	43.85 ± 0.48 a	55.0 ± 9.52 a
2 nd Sampling	7.80 ± 0.42 ab	555.75 ± 87.79 ab	42.87 ± 0.48 a	33.26 ± 5.28 b
3 rd Sampling	7.07 ± 0.21 bc	325.17 ±55.68 bc	43.10 ± 0.67 a	20.00 ± 1.99 bc
Normal	6.19 ± 0.82 c	114.2 ±16.25 c	18.85 ± 2.53 b	38.60 ± 3.86 c

*[Note: *Means with different superscripts in columns are significantly different at (P < 0.05)]*

Figure 10: Table 4 :

5

Days of Infection	Blood Glucose m.mol/ l	CPk IU/ l	ALT IU/l	AST IU/l
1 st Sampling	3.87 ± 0.19 a	149.80 ± 6.0 a	39.93 ± 2.37 a	139.60 ± 7.34 a
2 nd Sampling	4.03 ± 0.22 ab	111.08 ± 6.73 ab	38.35 ± 2.37 a	105.34 ± 4.87 b
3 rd Sampling	3.58 ± 0.22 ab	134.63 ± 9.88 ab	36.61 ± 1.97 a	

Figure 11: Table 5 :

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