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Haematological and Serum Protein Values in Tuberculin Reactor and Non-Reactor Water Buffaloes, Cattle, Sheep and Goats

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ABSTRACT

The diagnostic accuracy of tuberculosis can be enhanced by new tests like gamma interferon used in conjunction with tuberculin test but these tests are quite expensive to be conducted on all animals in resource poor countries. The present study was conducted to clarify the usefulness of haematological parameters and changes in serum proteins along with tuberculin test in different animal species. In buffaloes, significantly lower RBC count, WBC count, Hb concentration, neutrophils and eosinophils while significantly higher monocytes percentage along with serum total proteins, albumin and globulins were observed in tuberculin positive reactors. In cattle, significantly lower RBC count, PCV, neutrophils and serum albumin, while lymphocyte percentage was significantly higher in positive reactor animals. In sheep, PCV was lower in positive reactors. In goats, basophils percentage was significantly higher (P = 0.002) in positive reactors. A significant positive correlation of Hb with RBC and PCV in positive reactor buffaloes, goats and sheep was observed. The correlation statistics for differential leukocyte percentage did not differ between positive and negative reactor animals of these species. In cattle and goats, albumin showed significant positive correlation with globulins in negatively reacting animals, while this correlation was non-significant in positive reactor animals. In buffaloes, albumin showed a significant positive correlation with globulins and this correlation was non-significant in negative reactor buffaloes. It was concluded that tuberculosis caused decrease in erythrocyte parameters including RBC count, PCV and Hb concentration, while it caused an increase in monocytes, eosinophils and lymphocytes.

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INTRODUCTION

Bovine tuberculosis is one of the most important zoonotic diseases of cattle and other farm animals. It is an infectious disease caused by *Mycobacterium bovis* that has a broad host range (Wedlock *et al*., 2002). This disease has socio-economic or public health importance and is of great significance to international trade of animals and animal products (OIE, 2004). Not only, disease poses a greater threat to the economies of developing countries but also remains a problem in developed world (Khan *et al.*, 2008). It is a contagious chronic debilitating disease of animals associated with progressive emaciation and tubercle (granuloma) formation that are mostly confined to respiratory system, primarily in the lungs, lymph nodes and occasionally in other organs (Menzies and Neill, 2000). The causative organism not only produces tubercles but also affects hemato-biochemical parameters as various diseases have an adverse effect on the haematological parameters (Sattar and Mirza, 2009). According to Samad and Rahman (1986), hemoglobin concentration, total erythrocytes and neutrophils significantly decrease whereas values of erythrocytes sedimentation rate, total leukocyte counts and lymphocytes significantly increase in positive reactors than healthy cattle. Moreover, depending on the stage and progress of the disease, leukocytosis, leukopenia, lymphocytosis, lymphopenia, neutrophilia and monocytosis can occur (Kumar *et al*., 1994).

The diagnosis of this disease in routine is made by intradermal tuberculin test, while the gold standard is histopathological examination of morbid tissues and

culture isolation of organism. Recently, new tests have been developed like gamma interferon ELISA but these tests are quite expensive to be conducted routinely in resource poor countries. These tests are reported to be used in conjunction with tuberculin test for more accurate diagnosis. In the absence of these tests for poor countries, the use of haemato-biochemical parameters could be of value in enhancing the diagnostic accuracy. To have the idea of its usefulness in different animal species, the present work was conducted involving a larger sample size in buffaloes, cattle, sheep and goats along with comparative tuberculin test.

MATERIALS AND METHODS

A total of 2057 buffaloes, 2273 cattle, 4983 sheep and 1987 goats were tested using the single comparative intradermal tuberculin test at the neck region, using bovine (50,000 IU/ml) and avian (25,000 IU/ml) PPDs produced at the authorized laboratory at the Istituto Zooprofilattico Umbria e Marche, Italy. These animals were from Government Livestock Experiment Stations of Punjab and villages of two districts of Punjab. Buffalo and cattle studied in two cities were from the same locality as for sheep and goats, while a total of 15 Government livestock farms were included in the study. The negative animals included were from small holdings at villages and small number was taken from a farm where animals were newly purchased and were tested negative. The protocols for the production of PPDs, the execution of the test and its interpretation were carried out according to the criteria described in the OIE Manual of Standards for Diagnostic Tests and Vaccines (OIE, 2004).

Blood was collected from all the positive reactors including, 137 buffaloes, 134 cattle, 36 sheep and 33 goats. Almost same numbers of negative animals of about same status (age, parity etc.) were also included and blood samples were collected from these animals (140 buffaloes, 130 cattle, 36 sheep and 33 goats). About 15 ml of whole blood was collected from jugular vein of each animal. Out of this, 5 ml was separated in a clean glass tube having sufficient quantity of EDTA and 10 ml of blood was separated in another glass tube without EDTA to collect serum. The haematological studies were carried out as described by Benjamin (1978). Serum total proteins were determined by the Biuret method, as described by Oser (1976). The serum albumin was determined following the method of Varley *et al*. (1980). The globulin was estimated by subtracting serum albumin from serum total proteins.

The results obtained were subjected to statistical analysis by using General Linear Model procedure and means were compared by Dunett's test on personal computer using SAS statistical software (SAS, 2004). Correlation statistics was also computed.

RESULTS

In buffaloes, the results on haematological parameters showed significantly lower red blood cell count (P=0.0026), white blood cell count (P<0.0001), haemoglobin (P<0.0001) concentration, neutrophils and eosinophils (P=0.01), while monocyte percentage was higher in positive reactor than negative reactor animals (Table 1). Serum total proteins, albumin and globulins were significantly (P<0.0001) higher in positive reactor animals (Table 1).

In cattle, haematological parameters revealed significantly lower values of red blood cell count (P=0.005), packed cell volume (P<0.0001) and neutrophil percentage (P=0.0004), while lymphocyte percentage (P=0.0003) was significantly higher in positive reactor cattle (Table 2). Among serum proteins, only serum albumin was significantly lower (P=0.02) in positive reactor cattle (Table 2).

In sheep, only packed cell volume (PCV) differed significantly between positive and negative reactor animals, while all other parameters of haematology and serum proteins did not differ statistically (Table 3). PCV percentage was lower (P=0.02) in positive reactor sheep (Table 3). Similarly, haematological parameters and serum proteins in goats did not differ statistically between positive and negative reactor animals, except basophil percentage which was higher (P=0.002) in positive reactor animals (Table 4).

Table 1: Comparison of mean haematological parameters and serum proteins in tuberculin positive and negative reactor buffaloes

Parameters studied	Negative animals (n = 140)	Positive reactors (n = 137)	P value
RBC (10^6/µl)	5.77 ± 1.34	5.09 ± 1.81	0.002
WBC (10^3/µl)	5.88 ± 2.89	2.59 ± 3.44	<0.0001
Hb (g/dl)	13.86 ± 6.34	11.05 ± 2.07	<0.0001
PCV (%)	31.76 ± 4.59	32.91 ± 7.14	
ESR (mm/hr)	79.54 ± 43.52	77.24 ± 44.17	
Differential leukocyte count			
Neutrophils (%)	40.82 ± 7.30	38.05 ± 8.62	0.01
Lymphocytes (%)	48.80 ± 7.30	50.62 ± 9.26	
Monocytes (%)	3.92 ± 2.69	5.99 ± 3.35	<0.0001
Eosinophils (%)	6.40 ± 2.61	5.26 ± 3.99	0.01
Basophils (%)	0.27 ± 0.58	0.41 ± 1.11	
Serum proteins			
Serum total proteins (g/dl)	7.55 ± 1.61	10.18 ± 3.22	<0.0001
Serum albumin (g/dl)	3.77 ± 0.82	4.73 ± 1.60	<0.0001
Serum globulins (g/dl)	3.55 ± 0.88	5.07 ± 2.61	<0.0001

Table 2: Comparison of mean haematological parameters and serum proteins in tuberculin positive and negative reactor cattle

Parameters studied	Negative animals (n = 130)	Positive reactors (n = 134)	P value
RBC (10^6/µl)	6.04 ± 1.54	5.32 ± 1.51	0.005
WBC (10^3/µl)	6.26 ± 1.03	6.06 ± 1.71	
Hb (g/dl)	9.71 ± 2.04	9.78 ± 1.71	
PCV (%)	33.47 ± 2.91	30.56 ± 4.05	<.0001
ESR (mm/hr)	2.74 ± 8.30	2.80 ± 1.77	
Differential leukocyte count			
Neutrophils (%)	33.64 ± 7.97	27.57 ± 10.84	0.0004
Lymphocytes (%)	58.27 ± 6.97	64.00 ± 10.19	0.0003
Monocytes (%)	2.88 ± 1.19	2.89 ± 0.95	
Eosinophils (%)	5.33 ± 1.70	5.22 ± 1.81	
Basophils (%)	0.16 ± 0.42	0.30 ± 0.48	
Serum proteins			
Serum total proteins (g/dl)	11.93 ± 3.43	11.23 ± 2.94	
Serum albumin (g/dl)	5.76 ± 1.13	5.17 ± 1.32	0.02
Serum globulins (g/dl)	5.97 ± 2.93	6.18 ± 2.58	

Table 3: Comparison of mean haematological parameters and serum proteins in tuberculin positive and negative reactor sheep

Parameters studied	Negative animals (n = 36)	Positive reactors (n = 36)	P value
RBC (10^6/µl)	8.17 ± 1.28	7.83 ± 1.59	
WBC (10^3/µl)	4.59 ± 2.11	8.47 ± 2.59	
Hb (g/dl)	7.44 ± 3.25	8.05 ± 1.71	
PCV (%)	26.80 ± 3.68	24.91 ± 2.95	0.02
ESR (mm/hr)	4.58 ± 8.34	2.30 ± 3.07	
Differential leukocyte count			
Neutrophils (%)	38.72 ± 7.23	37.94 ± 9.63	
Lymphocytes (%)	38.32 ± 7.37	40.48 ± 8.68	
Monocytes (%)	3.00 ± 1.97	3.57 ± 2.47	
Eosinophils (%)	16.60 ± 11.07	15.57 ± 10.24	
Basophils (%)	1.16 ± 1.72	1.45 ± 1.96	
Serum proteins			
Serum total proteins (g/dl)	10.31 ± 2.45	11.16 ± 3.51	
Serum albumin (g/dl)	6.28 ± 2.36	6.23 ± 2.41	
Serum globulins (g/dl)	3.99 ± 1.68	4.97 ± 2.17	

Table 4: Comparison of mean haematological parameters and serum proteins in tuberculin positive and negative reactor goats

Parameters studied	Negative animals (n = 33)	Positive reactors (n = 33)	P value
RBC (10^6/µl)	8.52 ± 1.41	7.91 ± 1.34	
WBC (10^3/µl)	6.47 ± 2.48	6.52 ± 2.39	
Hb (g/dl)	7.83 ± 1.42	8.53 ± 1.32	
PCV (%)	24.56 ± 3.74	23.96 ± 2.88	
ESR (mm/hr)	1.40 ± 0.71	1.53 ± 0.96	
Differential leukocyte count			
Neutrophils (%)	38.78 ± 7.15	37.91 ± 7.61	
Lymphocytes (%)	45.86 ± 10.17	43.89 ± 11.03	
Monocytes (%)	3.17 ± 1.52	3.07 ± 2.30	
Eosinophils (%)	12.04 ± 11.01	13.96 ± 10.50	
Basophils (%)	0.41 ± 0.71	1.76 ± 1.56	0.002
Serum proteins			
Serum total proteins (g/dl)	11.28 ± 6.65	9.68 ± 3.27	
Serum albumin (g/dl)	6.19 ± 3.87	4.82 ± 1.94	
Serum globulins (g/dl)	5.08 ± 3.24	4.86 ± 2.22	

Correlation

The results of correlation statistics were different in positive and negative reacting animals of each species. A significant positive correlation of haemoglobin with red blood cell count and PCV in positive reacting buffaloes (r=0.348, P<0.0001; r=0.438, P<0.0001), goats (r=0.805, P<0.001; r=0.424, P =0.02) and sheep (r=0.731, P<0.0001; r=0.353, P =0.03) was observed, while this correlation was non-significant in negative animals of these species. The correlation statistics for differential leukocyte percentages did not differ between positive and negative reactor animals of these species.

The correlation statistics of serum proteins revealed similar findings in cattle and goats with albumin showing significant positive correlation with globulins in negatively reacting cattle (r=0.413, p=0.01) and goats (r=0.745, p<0.0001), while this correlation was non-significant in positive reactor animals of these species. However, in buffaloes, albumin showed a significant positive correlation with globulins (r=0.255, p=0.03), while this correlation was non-significant in negative reactor buffaloes.

DISCUSSION

Haematological and serum protein variations in 18 tuberculin positive buffaloes have previously been reported (Javed *et al*., 2006), while the data about epidemiological factors in sheep and goats have been presented in another paper (Javed *et al*., 2010). This study reports data of 137, 134, 36 and 33, positive reacting buffaloes, cattle, sheep and goats, respectively to avian and bovine PPD. Therefore, this data gives a better picture about haematological and serum proteins variations among tuberculin positive and negative reacting animals under tropical conditions.

The results of haematological and serum proteins parameters in buffaloes and cattle show species differences. However, some of the results were similar in both species like the decrease in red blood cell count and neutrophil percentage. Positive reactor buffaloes showed significant decrease in RBCs, WBCs, Hb concentration and eosinophils, while monocytes percentage along with serum total proteins, albumin and globulins showed significant increase. However, in cattle, lymphocyte percentage was higher, while PCV and serum albumin were lower in positive animals. This suggests a species variation. However, the results indicate that RBC count and neutrophil percentage can be taken as subjective indicator along with results of PPD testing for confirmation of tuberculosis in these species. The monocyte percentage in buffaloes and lymphocyte percentage in cattle can be considered supportive indicators of disease in these species. There was however, a clear variation in serum proteins pattern in both the species with increase in buffalo and decrease in cattle. The results on studied parameters can however, vary with the stage of the disease in both the animal species (Lepper *et al*., 1977). We were unable to find any clinical sign indicative of tuberculosis in these animals nor we had any such history, therefore, experimental studies are needed to better correlate these results in both buffaloes and cattle with severity, acuteness or chronicity of the disease. Previously, a drop in red blood cell count and haemoglobin concentration (Samad and Rahman, 1986; Amin, 1989; Rao *et al*., 1992; Kumar *et al*., 1994), PCV (Kumar *et al*., 1994) and neutrophils (Samad and Rahman, 1986) has been reported in positively reacting cattle. The increase in monocytes (in buffaloes) and lymphocytes (in cattle) correlated to some extent with earlier finding in bison, where these were slightly higher than control animals (Miller *et al*., 1989). A significant decrease in leukocytes in buffaloes, while non-significant decrease in cattle, was another supportive finding to tuberculin results that has been previously reported and related with peracute to acute form of the disease (Lepper *et al*., 1977). Similarly, increase in lymphocyte percentage and also total leukocyte count (contrary to our results) has been reported (Samad and Rahman, 1986). Kumar *et al*. (1994) also reported that depending on the stage and progress of the disease, leukocytosis, leucopoenia, lymphocytosis, lymphopenia, neutrophilia and monocytosis can be observed. Our results also confirm the earlier findings (Kumar *et al*., 1994) about no utility of ESR in animals in the diagnosis of tuberculosis or supportive diagnosis of tuberculin testing and thus our results disagree with the increase in ESR in positive animals reported earlier (Samad and Rahman, 1986; Amin, 1989).

The results on these parameters in both sheep and goats were almost similar with non-significant difference between positive and negative reactor animals, with exception of PCV which was significantly lower in sheep, while non-significantly in goats. This suggests that these species behave differently than large ruminants. However, a significantly lower PCV in sheep was also observed in positive cattle, thus supporting the results for this parameter to be of some utility in sheep and cattle in conjunction with results of tuberculin test.

The correlation statistics appears to have some more meaningful results in multi animal species. A significant positive correlation existed of Hb with RBC and PCV in three species of animals (buffaloes, goats and sheep), while a significant negative correlation of albumin with globulins was observed in two species (cattle and goats). The latter correlation in buffaloes was also significant but positive. This suggests usefulness of the correlation statistics along with haematology and serum proteins in strengthening the results of PPD testing in these species. This kind of data would be very helpful in the diagnosis of tuberculosis along with tuberculin testing in resource poor countries where advanced diagnostic facilities are costly and mostly unavailable and animal test and slaughter policy along with strong abattoir vigilance is missing to confirm the tuberculin results. Further, the results also suggest that buffaloes behave differently than cattle and these parameters (haematology and serum proteins) are probably of less significance in small ruminants.

Conclusions

It can be concluded from the present study that tuberculosis causes decrease in erythrocyte parameters including RBC count, PCV and Hb concentration, while it causes an increase in monocytes, eosinophils and lymphocytes. Further, the results of haemato- logy need to

be interpreted carefully in different animal species as species variation does exist.

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