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# HEMATOLOGICAL STUDIES AMONG BOVINE TUBERCULOSIS SUSPECTED HERDS OF CATTLE IN SUBURB OF ISLAMABAD, PAKISTAN

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Various blood changes have been found associated with the onset and progression of bovine tuberculosis. In this regard study was conducted to find out changes associated with the prevalence of bovine tuberculosis (BTB) in cattle (n=200) from 10 randomly selected farms with the size ranging from 10-200 in suburb of Islamabad, Pakistan. Blood samples were collected from tuberculin positive and negative animals through jugular vein puncture. The samples were transferred to the National Veterinary Laboratories (NVL), Ministry of Food Agriculture and Livestock, located at National Agriculture Research Center (NARC), Islamabad for laboratory analysis. Of the 200 cattle tested; 3 (1.5%) cows showed positive reaction to bovine purified protein derivative (PPDB), whereas 4 (2.0%) cows showed positive reaction to avian purified protein derivative (PPDA). There was a significant ($P<0.05$) rise in white blood cells (WBC) count in mammalian PPD positive cattle ($16.0x10^3/\mu l \pm 9.85$) compared to values obtained in mammalian PPD negative ($10.75x10^3/\mu l \pm 3.81$) cattle. Mean corpuscular volume (MCV) is significantly ($p<0.05$) higher in mammalian PPD positive cattle (52.93 fL ± 1.79) compared to mammalian PPD negative cattle (49.21 fL ± 5.92). Neutrophiles percentage was significantly ($P<0.05$) lower in mammalian PPD positive cattle (28.67 %± 3.79) when compared with mammalian PPD negative cattle (31.73 % ± 9.49). Mammalian PPD positive cattle (3.0 % ± 2.0) showed significantly ($p<0.05$) lower eosinophiles percentage compared to mammalian PPD negative cattle (5.03 % ± 2.44). A significant ($p<0.05$) increase in serum total proteins (10.15 g/dl ± 3.38) and globulins (6.26 g/dl ± 3.31) in cattle, having positive reaction to mammalian PPD, as compared with total protein (8.75 g/dl ± 1.97) and globulin (4.99 g/dl ± 2.01) level in mammalian PPD negative cattle.

**Key words:** Tuberculosis, tuberculin test, mantoux test, dairy animal

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*Mycobacterium bovis* the agent that cause tuberculosis in animals, generally known as bovine tuberculosis (BTB). In addition, *M. bovis* can also cause disease in humans (Neill *et al*., 1994) and is a serious problem of public health (Morris *et al*., 1994). Its infection in cattle and other farmed animals in large areas of the world often cause significant economic loss to agricultural communities (Neill *et al*., 1994).

Bovine tuberculosis is considered as a List B disease by Office International des Epizooties (OIE), i.e. a disease which is considered to be of socio-economic or public health importance and of great significance to the international trade of animals and animal products (Cousins, 2001). The disease is considered as one of the major livestock diseases that results in high morbidity and mortality (MoA, 1984).

Bovine tuberculosis has been diagnosed by measuring the immune response of cattle to *M. bovis* infection. Among the immunodiagnostic assays developed, the intradermal tuberculin technique has been the most extensively used worldwide (Francis *et al*., 1978). The tuberculin used for the skin test is a purified protein derivative (PPD) prepared from culture

  

filtrate of a laboratory strain of *M. bovis* (Whipple *et al*., 1995). On the bases of measurement of cellular immune response, this test can be conducted by inoculation into either the caudal fold near the base of the tail or in the neck, both as a single (inoculation with only bovine tuberculin) or a comparative assay (inoculation with both bovine and avian) (Francis *et al*., 1978).

Blood is an important and reliable medium for assessing the health status of individual animal (Oduye, 1976). Haematological and biochemical values are important for assessing the health and nutrition status of the animals. Haematological tests have been widely used for the diagnosis of various animal diseases. It would help determine the nature of the disease, the response of defense mechanism of the patient and aid in diagnosing the type of possible anemia (Tibbo *et al*., 2004). Therefore, it is essential for the evaluation of clinical tests in veterinary laboratories that a base reference of normal values of clinically healthy animals is available (Sema *et al*., 2004). A relevant hematological parameter in pulmonary tuberculosis is platelet count, when high, characterizes an abnormal fibrinolytic system which leads to hypercoagulability (Bevilacqua *et al*., 1984; Kartaloglu *et al*., 2001). Little information is available on the prevalence of the disease in livestock and its zoonotic impact (Jalil *et al*., 2003; Javed *et al*., 2006). Information on the prevalence of BTB in Potohar area is non-existing. This situation calls for a comprehensive program to address this problem. Keeping in view the importance of this program, the present study was designed to compare blood indices of tuberculin positive and negative animals.

## MATERIALS AND METHODS

### *Study Area and Animal Population Studied*

Cattle (n=200) of both sexes aged 8 months with average herd size (10-200) from 10 randomly selected livestock farms in suburb of Islamabad were taken into study. The cattle were of Jersey, Sahiwal, Dhanni and cross bred breeds.

### *Blood Sampling*

Blood samples were collected from tuberculin positive and negative animals through jugular vein puncture. For hematological studies, 5 ml of blood was collected into glass tube containing EDTA, and 3 ml in anticoagulant free glass tubes for biochemical analysis of serum. The samples were transferred to the National Veterinary Laboratories (NVL), Ministry of Food Agriculture and Livestock, located at National Agriculture Research Center (NARC), Islamabad for laboratory analysis.

### *Plasma and Serum Harvesting and Storage*

For isolation of plasma, centrifugation of blood was done at 3000 rpm for 15 min and plasma was separated, labeled and stored at -20$^{o}$C until further processing. For serum harvesting 3 ml of blood was deposited in anticoagulant free glass tubes and allowed to clot at room temperature within 3 hours of collection. Centrifugation was done at 3000 rpm for 15 min and serum was separated, labeled and stored at -20$^{o}$C until further processing.

### *Haematological Studies*

Haematological studies included; total leukocyte count (TLC), total erythrocyte count (TEC), platelets count (PLC), haemoglobin (Hb), haematocrit (Hct), mean corpuscular volume (MCV = Haematocrit x 10/RBCs count), mean Corpuscular haemoglobin (MCH = Hb x 10/ RBCs count) and mean corpuscular haemoglobin concentration (MCHC = Hb x 100/ haematocrit). The values were determined on Beckman Coulter Haematological Cell Counter, USA at National Veterinary Laboratories (NVL), Islamabad.

### *Differential Leukocyte Count (DLC)*

Blood smear was made on slides by Push Wedge Slide Method, air dried, and stained with Wright Stain. After air drying, DLC was done using light microscope under oil immersion at 1000 X magnification. A total of 100 white blood cells (Monocyte, Lymphocyte, Eeosinophils, Neutrophils and Basophils) were counted and converted into percentile of the total.

### *Biochemical Studies*

Biochemical studies including determination of serum total proteins; albumin and serum globulins (by subtracting albumin from total protein values) were performed. Biochemical measurements were made by colorimetric method using AMP diagnostic

 

**Table 1:** Prevalence of Bovine Tuberculosis in Cattle and Buffalo in Islamabad area.

| Specie | N | Reaction to PPDB | | Reaction to PPDA | |
|---|---|---|---|---|---|
| | | Positive (%) | Negative (%) | Positive (%) | Negative (%) |
| Cattle | 200 | 3(1.5) | 197(98.5) | 4(2.0) | 196(98.0) |

PPDB: Bovine purified protein derivative (Mammalian tuberculin)
PPDA: Avian purified protein derivative (Avian tuberculin)

**Table 2:** Comparative haematological values in Mammalian PPD negative (n=30) and Mammalian PPD positive cattle (n=3).

| Parameters | Cattle | |
|---|---|---|
| | Mammalian PPD Negative (Mean±SD) | Mammalian PPD Positive (Mean±SD) |
| WBC ($10^3$/uL) | $10.75^b \pm 3.81$ | $16.0^a \pm 9.85$ |
| RBC ($10^6$/μL) | $8.04^a \pm 2.52$ | $7.05^b \pm 0.98$ |
| Plt ($10^3$/μL) | $266.4^b \pm 189.35$ | $369^a \pm 88.64$ |
| Hb (g/dL) | $10.17^a \pm 2.74$ | $9.8^a \pm 0.99$ |
| Hct (%) | $37.61^a \pm 11.51$ | $37.23^a \pm 4.67$ |
| MCV (fL) | $49.21^b \pm 5.92$ | $52.93^a \pm 1.79$ |
| MCH (pg) | $12.86^b \pm 1.35$ | $14.03^a \pm 1.00$ |
| MCHC (g/dL) | $26.21^a \pm 1.43$ | $26.5^a \pm 1.06$ |
| ESR mm/hr | $1.06^a \pm 0.25$ | $1.33^a \pm 0.58$ |

Means having different superscripts in each row differ significantly ($P < 0.05$).
**WBC**: White blood cell, **MCV**: Mean corpuscular volume, **RBC**: Red blood cell, **MCH**: Mean corpuscular Hemoglobin, **Plt**: Platelets, **MCHC**: Mean corpuscular hemoglobin concentration, **Hb**: Hemoglobin, **ESR**: Erythrocyte sedimentation rate, **Hct**: Haematocrit

kits (Linear Chemicals. S. L). All samples were run in Clinical Chemistry Analyzer (Metrolab 1600DR) and results were noted.

## RESULTS AND DISCUSSION

Prevalence of tuberculosis in cattle (n=200) on the basis of comparative intradermal tuberculin test was studied (Table 1). The values of prevalence of BTB in cattle in the present study were comparable to previous studies by Weinhaupl *et al*. (2000) (1.7%) and Shirima *et al*. (2003) (1.3%) in Tanzania. However results are not in line with studies of Jalil *et al*. (2003) (8.64%) in Lahore and Javed *et al*. (2006) (2.45-8.48%) in Faisalabad, Pakistan. Other studies carried out in Bangladesh (3.01%) (Samad and Rahman, 1986), India in the states of Tamil

 

**Table 3:** Variation in differential leukocyte count in mammalian PPD negative (n=30) and mammalian PPD positive cattle (n=3)

| | Cattle | |
|---|---|---|
| Parameters | Mammalian PPD Negative (Mean±SD) | Mammalian PPD Positive (Mean±SD) |
| Lymphocyte | $59.4^{a}$ ± 10.20 | $68.0^{b}$ ± 10.0 |
| Neutrophils | $31.73^{a}$ ± 9.49 | $28.67^{b}$ ± 3.79 |
| Monocytes | $3.9^{a}$ ± 1.92 | $3.67^{a}$ ± 1.58 |
| Eosinophils | $5.03^{a}$ ± 2.44 | $3.0^{b}$ ± 2.0 |

Means having different superscripts in each row differ significantly (P < 0.05)

**Table 4:** Comparative biochemical values in Mammalian PPD negative (n=30) and Mammalian PPD positive cattle (n=3)

| | Cattle | |
|---|---|---|
| Parameters | Mammalian PPD Negative (Mean±SD) | Mammalian PPD Positive (Mean±SD) |
| Total protein (g/dl) | $8.75^{b}$ ± 1.97 | $10.15^{a}$ ± 3.38 |
| Albumin (g/dl) | $3.76^{a}$ ± 0.40 | $3.89^{a}$ ± 0.09 |
| Globulin (g/dl) | $4.99^{b}$ ± 2.01 | $6.26^{a}$ ± 3.31 |
| Fibrinogen (g/dl) | $0.84^{a}$ ± 0.40 | $0.72^{a}$ ± 0.28 |
| Albumin/globulin | $2.12^{a}$ ± 1.03 | $2.59^{a}$ ± 1.50 |

Means having different superscripts in each row differ significantly (P < 0.05)

Nadu (34.58%) (Dhinakaran *et al.*, 1991) and Karnataka (30% to 35%) (Nalini *et al.*, 1998), Tanzania (13.1%) (Kazwala *et al.*, 2001), Zambia (herd prevalence of 33% and 7.4% individual animal prevalence) (Cook *et al.*, 1996), Ghana (13.8%) (Bonsu *et al.*, 2000) and Ethiopia (herd prevalence of 51% and 19% with single intra dermal tuberculin test and comparative intra dermal tuberculin test, respectively) (Laval and Ameni, 2004) showed higher prevalence compared to present study.

Various haematological values (mean ± S.D) were noted in cattle (Table 2). There was a significant (P<0.05) rise in white blood cells (WBC) count in mammalian PPD positive cattle ($16.0x10^{3}$/µl ± 9.85) compared to values obtained in mammalian PPD negative ($10.75x10^{3}$/µl ± 3.81) cattle. A significant (p<0.05) decrease in red blood cell (RBC) count in mammalian PPD positive cattle ($7.05x10^{6}$/µl ± 0.98) was observed compared to mammalian PPD negative ($8.04x10^{6}$/µl ± 2.52) cattle.

The results are in agreement with studies carried out by Samad and Rahman (1986) in cattle, and Javed *et al*. (2006) in buffaloes for leukocyte count, total erythrocyte count, erythrocyte sedimentation rate and hemoglobin. An appreciable drop in RBC count has been reported (Amin *et al*., 1990; Rao *et al*., 1992; Kumar *et al*., 1994). No reference was found on effect of tuberculosis on platelets, MCV, MCH and MCHC. It was reported that erythrocyte sedimentation rate serves as a valuable diagnostic test for inflammation in some species but is not demonstrable in cattle (Olsen, 1966; Rankin, 1955).

**Differential count:**

Results of differential leukocyte counts in cattle were analyzed (Table 3). Lymphocyte percentage was significantly (P<0.05) higher in mammalian PPD positive cattle (68.0 % ± 10.0) as compared to mammalian PPD negative cattle (59.4 % ± 10.20). Whereas, neutrophiles percentage was also significantly (P<0.05) lower in mammalian PPD positive cattle (28.67 %± 3.79) when compared with mammalian PPD negative cattle (31.73 % ± 9.49).

**Biological values:**

 

Mean ± SD values of serum proteins in cattle were determined (Table 4). Results revealed a significant ($p<0.05$) increase in serum total proteins (10.15 g/dl ± 3.38) and globulins (6.26 g/dl ± 3.31) in cattle, having positive reaction to mammalian PPD, as compared with total protein (8.75 g/dl ± 1.97) and globulin (4.99 g/dl ± 2.01) level in mammalian PPD negative cattle. Albumin/globulin (0.72 ± 0.28) was significantly ($P<0.05$) lower in mammalian PPD positive cattle when compared with value of mammalian PPD negative cattle (0.84 ± 0.40). Kichelberger and McCluskey (1927) also reported that during inflammatory diseases like tuberculosis serum proteins increases and ratio of albumin/globulin decreases. Information is not available so far for the association of protein values with tuberculosis in cattle.

**REFERENCES**


1. Amin, S., M. A. Khan, H. A. Hashmi, J. Rashid and M. S. Khan. (1990) Effect of tuberculosis on certain blood indices in buffaloes. Pakistan Vet. J 10, 92-93.
2. Bevilacqua, M. P., J. S. Pober, G. R. Majeau, R. S. Cotran and J. R. Gimbrone. (1984) Interleukine 1 (IL-1) induces biosynthesis and cell surface expression of procoagulant activity in human vascular endothelial cells. J. Exp. Med. 160, 618-23.
3. Bonsu, O. A., E. Laing and B. D. Akanmori. (2000) Prevalence of tuberculosis in cattle in the Dangme-West district of Ghana, public health implications. Acta. Tropica 76, 9–14.
4. Cook, A. J., L. M. Tuchili, A. Buve, S. D. Foster, P. Godfrey-Fausett, G. S. Pandey and K. P. McAdam. (1996) Human and bovine tuberculosis in the Monze District of Zambia-a cross-sectional study. Br. Vet. J 152(1), 37-46.
5. Cousins, D. V. (2001) Mycobacterium bovis infection and control in domestic livestock. Rev. Sci. Tech. 20, 71-85.
6. Dhinakaran, M., V. N. Appaji Rao and S. Nedunchellian. (1991) Immunological response in tuberculin reactor cattle. Compendium of Indian Society for Veterinary Medicine Decennial Convention and National Symposium on Recent advances in control of diseases of crossbred and companion animals, 19-21 December, Konkan Krishi Vidyapeeth. Bombay Veterinary College, Department of Medicine, New Campus, Goregaon, Mumbai. 4. Monaghan M.L., Doherty M.L.
7. Francis, J., R. J. Seiler, I. W. Wilkie, D. O'Boyle, M. J. Lumsden amd A. J. Frost. (1978) The sensitivity and specificity of various tuberculin tests using bovine PPD and other tuberculins. Vet. Rec. 103, 420-435.
8. Jalil, H., P. Das and A. Suleman. (2003) Bovine tuberculosis in dairy animals at Lahore, Threat to the Public Health. Metropolitan Corporation Lahore, Pakistan.
9. Javed, M. T., M. Usman, M. Irfan and M. Cagiola. (2006) A study on tuberculosis in buffaloes: some epidemiological aspects, along with haematological and serum protein changes. Veterinarski Arhiv. 76 (3), 193-206.
10. Kartaloglu, Z., K. Cerrahoglu, O. Okutan, A. Ozturk and R. Aydilek. (2001) Parameters of blood coagulation in patients with pulmonary tuberculosis. J. Intern. Med. 2, 2.
11. Kazwala, R. R., D. M. Kambarage, C. J., Daborn, J. Nyange, S. F. H. Jiwa and J. M. Sharp. (2001) Risk factors associated with the occurrence of bovine tuberculosis in cattle in the Southern Highlands of Tanzania, Vet. Res. Commun., **25,** 609–614.
12. Kichelberger, L. and K. L. McCluskey. (1927) Arch. Int. Med. 40, 831.
13. Kumar, G. S., P. K. R. Lyer, M. C. Prasad and A. K. Sharma. (1994) Tuberculosis in cattle: haematobiochemical studies. Ind. J. Vet. Pathol., 38, 20-42.


 

14. Laval, G. and G. Ameni. (2004) Prevalence of bovine tuberculosis in zebu cattle under traditional animal husbandry in Boji District of western Ethiopia. Rev. Med. Vet. 155, 494-499.
15. MoA. (1984). Livestock Sector Review. Annex, Vol. 1. Ministry of Agriculture, Addis Ababa, Ethiopia.
16. Morris, R, S., D. U. Pfeiffer and R. Jackson. (1994) The epidemiology of Mycobacterium bovis infections, Vet. Microbiol., 40, 153-177.
17. Nalini, T. S., R. N. Sreenivas Gowda, S. K. Vijayasen and S. Rao. (1998) Autopsy incidence of TB in bovines. In: Scientific Proceedings Vol. II. Second Pan Common wealth Veterinary Conference on animal health and production in rural areas – the essential role of women at all levels, 22-27 February, Bangalore. Pp. 112-124.
18. Neill, S. D., J. M. Pollock, D. B. Bryson and J. Hanna. (1994) Pathogenesis of Mycobacterium bovis infection in cattle. Vet. Microbiol., 40, 41–52.
19. Oduye, O. O. (1976) Haematological values of Nigerian goats and sheep. Trop. Anim. Health Prod., 8(3), 131-6.
20. Olsen, R. E. (1966) Determining the erythrocyte sedimentation rate of cattle. J. Am. vet. med. Ass. 148, 801-803.
21. Rankin, J. D. (1955) The erythrocyte sedimentation rate in normal cattle and in cattle injected with Mycobacterium johnei. Br. vet. J. 111, 480-483.
22. Rao, V. N. A., P. Ramadas and M. Dhinakran. (1992) A study on the effect of tuberculosis on body weight and haemogram values in cattle. Cheiron. 21, 19-22.
23. Samad, M. A. and M. S. Rahman. (1986) Incidence of bovine tuberculosis and its effect on certain blood indices in dairy cattle of Bangladesh. Ind. J. Dairy Sci. India. 39(3), 231-234.
24. Sema, Y., T, Pahun., N. Pindak., U. U. Fahri and Y. Reklu. (2004) Investigation of Some Hematologic and Biochemical Parameters in the Serum of Gazelles (Gazella subgutturosa) in Ceylanpinar, Şanliurfa, Turkey. Turk. J. Vet. Anim Sci. 28, 369-372.
25. Shirima, G. M., R. R. Kazwala and D. M. Kambarage. 2003. Prevalence of bovine tuberculosis in cattle in different farming systems in the eastern zone of Tanzania, Prev. Vet. Med., **57:** 167–172.
26. Tibbo, M., Y. Jibril., M. Woldemeskel., F. Dawo., K. Aragaw and J. E. O. Rege. 2004. Factors affecting haematological profiles in three Ethiopian Indigenous goat breeds. Intern. J. Appl Res. Vet Med, 2 (4): 297-309.
27. Weinhaupl, I., K. C. Schopf, D. Khaschabi, A. M. Kapaga and H. M. Msami. 2000. Investigations on the prevalence of bovine tuberculosis and brucellosis in dairy cattle in Dar es Salaam region and in zebu cattle in Lugoba area, Tanzania, Trop Anim. Health Prod.**, 32:** 147-154.
28. Whipple, D.L., A. J. Davis, J. L. Jarnagin, D. C. Johnson, R. S. Nabors, J. B. Payeur, D. A. Saari, A. J. Wilson and M. M. Wolf. 1995. Comparison of the sensivity of the caudal fold skin test and a commercial g-interferon assay for diagnosis of bovine tuberculosis. Am. J. Vet. Res., 56: 415-419.

 