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RESEARCH ARTICLE

Bovine tuberculosis in buffaloes: Investigation of demographic variables and assessment of hemato-biochemical values at some selected coastal areas of Bhola district, Bangladesh

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ABSTRACT

Objectives: The objective of this study was to investigate the demographic variables and hemato-biochemical investigation of bovine tuberculosis (bTB) in buffaloes in some selected coastal areas of Bhola district, Bangladesh.

Materials and Methods: The present study was conducted in the selected coastal regions of Bhola district, Bangladesh, from July 2020 to June 2021. A total of 180 randomly selected buffaloes were investigated by a tuberculin test (CZV Bovine Tuberculin purified protein derivative). Blood and serum samples were also collected for routine blood analysis and biochemical study in the present study.

Results: The prevalence of bTB was recorded at 3.33% in buffaloes in the study area. The highest prevalence of the disease was recorded in older age (4%) and female buffaloes (4.17%). bTB was the highest in poor health buffaloes (4.21%), followed by medium (3.33%) and good health conditions (0.00%). In the present study, total erythrocyte count, hemoglobin, and packed cell volume (PCV) values were lower in infected individuals as compared with healthy buffaloes. In contrast, the PCV count was significantly lower in infected buffaloes. On the other hand, the erythrocyte sedimentation rate values were comparatively higher in disease-positive buffaloes than in healthy individuals. Total leukocyte count was relatively higher in infected buffaloes than in healthy ones. In the differential leukocyte count, neutrophil, basophil, eosinophil, and monocyte numbers were comparatively higher in infected buffaloes. Monocyte numbers were significantly higher in infected buffaloes. However, the lymphocyte numbers were more or less similar in healthy and tuberculosis-positive animals. Biochemical values of calcium, phosphorus, glucose, cholesterol, and creatinine were comparatively lower in tuberculosis-affected animals. Among the values, the glucose, phosphorus, and cholesterol levels were significantly lower in TB-affected buffaloes. The values of total protein, serum glutamic oxaloacetic transaminase, and serum glutamic pyruvic transaminase were comparatively higher in TB-affected individuals.

Conclusion: The findings of the present study might be helpful to assess the economic importance and preventive measures of bTB in the study area.

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Bovine tuberculosis; demographic variables; hemato-biochemical values; coastal areas of Bangladesh

Introduction

Livestock is one of the critical components of the complex farming system in Bangladesh. The sector serves as a source of meat protein, a significant source of farm power, and employment generation. The livestock sub-sector also provides full-time employment for 20% of the total population and part-time jobs for another 50% [1]. In a livestock production system, human life is closely associated with domestic animals [2] and faces many constraints where tuberculosis (TB) is a common disease. The disease also spotlights public health issues, especially TB originating from animals [3]. It is a highly infectious and contagious disease caused by the genus *Mycobacterium*. It is usually characterized by the progressive development of granulomatous nodules in any of the organs in most

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warm-blooded animals. In several countries, bovine tuberculosis (bTB), caused primarily by *Mycobacterium bovis*, is an endemic disease. The effect of bTB is a great threat to developing countries and remains a significant problem for the developed world's economy [4].

A high prevalence (92%) of the disease has been reported in a domesticated buffalo herd [5]. Another study showed that the prevalence of tuberculosis in buffaloes is 10.20% in Bangladesh [6]. The prevalence of bTB in cattle has also been reported as 7.78% and 5.9% in Sirajgonj and Mymensingh districts, respectively [7,8]. On the other hand, it has been reported as 27.5% in breeding bulls [9]. In addition, the prevalence of bTB was reported in Red Chittagong cattle at 30% [10]. Wild animals can transmit tuberculosis to domestic animals. It has been reported that deforestation in coastal belt areas increases the risk of exposure to wild animal diseases for human and livestock health [11]. bTB can be transmitted to bovines through the colostrums and milk of calves, ingestion of contaminated feed and water, inhalation, and direct or indirect contact with each other or other wildlife. Low lying pasture land and the hot and humid climate in the coastal region increase the risk of bTB, where feces-contaminated grass acts as a prime source of infection for other animals.

Blood and serum analysis is a component of body tissues used to investigate and assess general health, diagnose diseases, and forecast disease status [12]. However, investigation of biochemical parameters in blood serum is also beneficial to determine the animals' metabolic profile and cardinal health status. Therefore, investigating hematobiochemical values could be a good criterion for animal disease diagnosis [13]. Comparing reference values obtained from healthy animals with sick animals is useful for progressing diagnostic approaches [14]. It has been observed that variation in blood parameters might be due to changes in several physiological and environmental factors. The hematological values like, total erythrocyte count (TEC), hemoglobin (Hb), and packed cell volume (PCV) were comparatively higher in healthy animals than in the infected ones [15]. The percentage of basophils in the differential leukocyte count was significantly higher in TB patients. According to some researchers, there are no significant variations in tuberculin-infected animals' hematological and serum protein levels [16]. The literature so far available reveals that most of the studies on bTB have been performed in different districts of Bangladesh. The objective of this study was to look into demographic factors and hemato-biochemical tests for bTB in buffaloes in the coastal areas of Bhola, Bangladesh.

Materials and Methods

Ethical statement

The study was performed according to the proposed guidelines of the ethical committee of Patuakhali Science and Technology University, Dumki, Patuakhali, Bangladesh.

Study area and duration

The study area was selected based on the number and herd size of the buffalo population. The buffaloes were chosen randomly from different Bathan areas (Bhola Sadar and Borhanuddin Upazila) of the Bhola district, Bangladesh. The period of study was July 2020 to June 2021.

Sampling method

The buffaloes from the herd were selected using simple random sampling. To assess the risk factors associated with bTB, a questionnaire with demographic characteristics of buffaloes was developed. The prevalence of bTB was recorded by tuberculin tests over some time.

Animal selection

A total of 180 buffaloes (including 20 buffaloes over two years old from each Bathan) were selected for the study. The animals have no history of vaccination against tuberculosis.

Study design

The study was carried out in two selected Upazilas in Bangladesh's Bhola district (Bhola Sadar and Borhanuddin Upazila). The study areas were chosen primarily due to buffalo farming in the study regions. The cross-sectional study was performed from July 2020 to June 2021. Single intradermal tuberculin (SID) tests were done on 180 animals for the buffaloes from selected study areas. Six animals from each group were chosen from SID test positive and negative reactors for the hematological study. The whole blood sample (10 ml) was collected from the jugular vein of each affected and healthy buffalo. The tube was slightly tilted to collect serum samples to settle the blood. The serum sample was stored at −20°C until tested serologically.

Caudal fold tuberculin test (CFTT)

It is a preliminary screening test to diagnose buffaloes with bTB. A dose of bovine purified protein derivative (PPD) of 0.1 ml was injected in the caudal fold, and a reading was taken after 24, 48, and 72 h of injection. Any swelling and discoloration at the injected site were positive cases (Fig. 1).

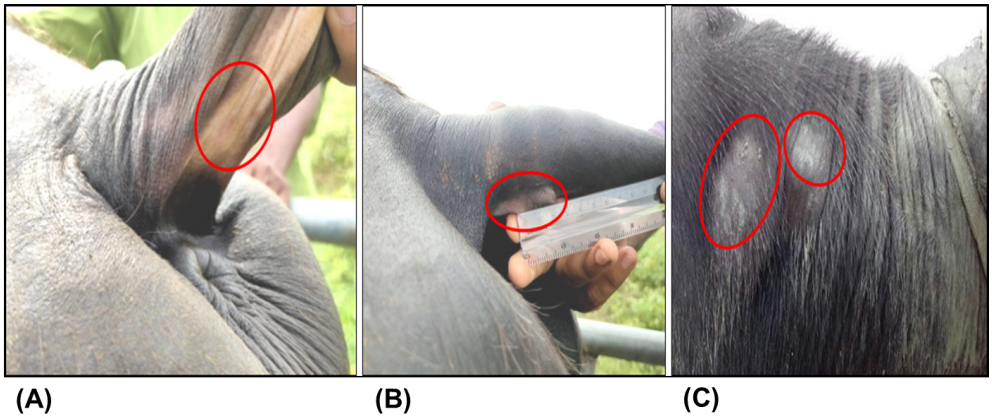

Figure 1. Tuberculin test to identify bTB. (A) No swelling at the caudal fold region, (B) Swelling at the caudal fold region, (C) Comparative tuberculin test (swelling part is TB positive-bovine TB and TB negative part indicates not swelling-avian TB).

Comparative cervical tuberculin test (CCT)

For comparative tuberculin reaction, the bovine and avian PPD were injected into the front and back clipped areas on the same side of the mid-neck of cattle, respectively (Fig. 1). The distance between the two injection sites was 12 cm [17].

Total erythrocyte count (TEC)

The method of counting total erythrocytes was described by Lamberg and Rothstein [18]. First, the well-mixed blood was drawn with a red blood cells pipette, which properly mixed the blood with diluted fluid (Hayem's solution). Then count the cells from the counting chamber. The total number of erythrocytes was expressed as millions per microliter of blood.

Total leukocyte count (TLC)

The routine hematological experiment of counting TLC was performed according to Lamberg and Rothstein [18]. The whole blood was mixed with diluents that lyse the erythrocytes and stain the nuclei of leukocytes. Then count the white blood cells from the hemocytometer chamber. The result of TLC is expressed as thousands per microliter of blood.

Packed cell volume (PCV)

The citrated blood fills the Wintrobe tube with a Pasteur pipette up to 100 marks from the bottom. Then centrifuge the tube and check for red cells at the bottom. The hematocrit or PCV was recorded by reading the graduation mark. Lamberg and Rothstein [18] came up with a way to determine how much hematocrit was in a given amount of space.

Erythrocyte sedimentation rate (ESR)

The blood without anticoagulant was taken into the Wintrobe tube, and the tube filled with blood was kept vertically on the wooden rack. Then the ESR value was recorded from the top of the column. The recorded result of the experiment was expressed in mm/1 h.

Hb concentration

Homogenized blood was taken into the Sahli pipette and immediately transferred into the tube containing hydrochloric acid. Then water was added drop by drop into the acid hematin mixture tube. The mixing solution was stirred with a glass rod until the color resembled the comparator. The resultant Hb concentration was expressed in gm/dl [18].

Total protein (gm/dl)

One milliliter of working reagent and twenty microliters of sample were collected, mixed well, and incubated at 37°C for 5 min. Read the absorbance of the samples and the standard at 540 nm against the reagent blank. An EMP Biochemical Analyzer® was used to record the reading. According to company instructions, the experimental protocol was carried out (Crescent Diagnostics®).

Calcium (mg/dl)

Working reagent, 1.0 ml, and 10 µl samples were taken. Mix and let the tubes stand for 2 min at room temperature. The reading was taken using the EMP Biochemical Analyzer®. According to manufacturer instructions, the experimental procedure was carried out (Crescent Diagnostics®).

Phosphorus (mg/dl)

At first, pre incubate the working reagent, sample, and control to reaction temperature. Then photometer was set to absorbance using distilled water. 1.0 ml of working reagent and 20 µl samples were taken into the test tube. The solution was mixed gently by inversion and incubated for 1 min. Reading of the experiment was taken using EMP Biochemical Analyzer®. The procedure of the experimental protocol was carried out according to the manufacturer instructions (Crescent Diagnostics®).

Creatine(mg/dl)

Reagents and samples were taken at room temperature, and 1 ml of working reagent and 40 µl sample were appropriately mixed. The mixture was incubated for 5 min and then proceeded for the initial absorbance reading using the EMP Biochemical Analyzer®. The experimental protocol was performed according to manufacturer instructions (Crescent Diagnostics®).

Serum glutamic oxaloacetic transaminase (SGOT) (aspartate aminotransferase) (IU/l)

Four parts of reagent I and 1 part of reagent II were taken into the test tube for stock reagent solution. Then mix the reagents thoroughly and prepare the mono-reagent. The samples were made into 10-fold dilution with 0.9% NaCl solution. 200 µl sample was properly added with 1 ml mono-reagent. The solution was mixed properly and incubated for 1 min. The analysis was performed using an EMP Biochemical Analyzer®. The experiment procedure was carried out according to company instructions (Crescent Diagnostics®).

Serum glutamic pyruvic transaminase (SGPT) (alanine aminotransferase) (IU/l)

First, a stock reagent solution was prepared using four parts reagent I and 1 part reagent II. The solution was mixed thoroughly to make a mono-reagent. The samples were made into a 10-fold dilution with 0.9% NaCl solution, and 200 µl sample was added correctly with 1 ml mono-reagent. The solution was appropriately mixed and incubated for 1 min. The biochemical analysis of the enzyme was performed using the EMP Biochemical Analyzer®. The experiment procedure was carried out according to company instructions (Crescent Diagnostics®).

Glucose (mg/dl)

The reagents and samples were taken at room temperature, and then 1 ml of working reagent and 10 µl sample were appropriately mixed. The mixture was incubated at 37°C for 10 min. The experiment was performed using an EMP Biochemical Analyzer®. The experiment procedure was carried out according to the manufacturer instructions (Crescent Diagnostics®).

Cholesterol (mg/dl)

At first, reagents and samples were brought to room temperature. Then 1 ml mono-reagent and 10 µl sample were appropriately mixed. The mixture was incubated at 37°C for 5 min. The EMP Biochemical Analyzer® correctly reads the absorbance. The study procedure was performed according to company instructions (Crescent Diagnostics®).

Statistical analysis

The laboratory and field data were imported into Microsoft Office Excel-2007. Descriptive and inferential statistics (Chi-square and *t*-test) were performed by Microsoft Office Excel-2007 software. The data was significant at a *p*-value of ≤ 0.05 and ≤ 0.01.

Results and Discussion

Prevalence of bTB in cattle according to host factor

The prevalence of bTB in buffaloes was recorded as 3.33% in the study area. Moreover, the prevalence of tuberculosis in buffaloes was 6.12% by the CFT and 4.08% by the CCT method [6], which is comparatively higher than the performed study. Another study showed that the prevalence of bTB in cattle was 5.90% in Mymensingh, Bangladesh [8], which is also higher than in the present study. The variation in the prevalence rates of bTB in buffaloes might be due to different geographical locations, management practices, and the diagnostic tests used for screening tuberculosis.

According to age-wise distribution, a comparatively higher prevalence of tuberculosis was recorded in the more than 5-year age group at 4.00% than in the less than 5-year age group at 2.50%, as shown in Table 1 and Figure 2. The buffaloes at young ages (6–30 months) were more susceptible to bTB than older ones [6], which is dissimilar to the present study. However, another study reported a higher prevalence of bTB in adults (6.41%) than in the less than 3 years of age group (2.00%), which supports the present study [8]. The highest prevalence of bTB was reported in adults (above 6 years) (10.23%), followed by > 2–6 years (7.41%) [7]. It has been found that the incubation period of the tuberculosis organism is very high. For this reason, adult cattle are highly susceptible compared to young individuals [19].

In sex-wise distribution, a higher prevalence of tuberculosis was recorded in females at 4.17% than in males at 1.67% (Table 1). As shown in Table 1 and Figure 2, a higher prevalence of tuberculosis was recorded in females (4.17%) than in male buffaloes (1.67%). It has been reported that male buffaloes (6.25%) were comparatively

Table 1. Prevalence of bTB in buffaloes according to host factor.

Factors		Tested	Positive	Prevalence (%)	*p*-value
Age group	<5-year	80	02	2.5	0.575
	Above 5 years	100	04	4	
Sex	Male	60	01	1.67	0.378
	Female	120	05	4.17	
Health condition	Good (BCS ≥3–4)	25	0	0.00	0.580
	Medium (BCS ≥2–3)	60	02	3.33	
	Poor (BCS ≥1–2)	95	04	4.21	
Total	180		06	3.33	

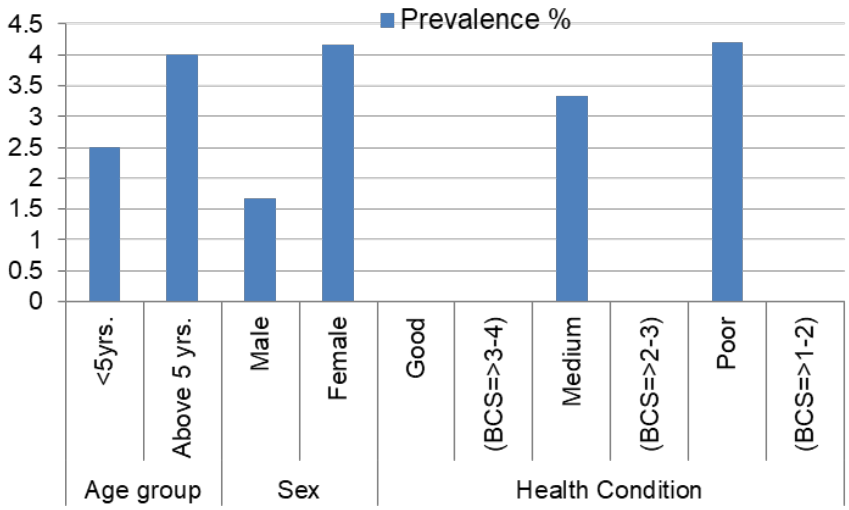

Figure 2. Prevalence of bTB in buffaloes according to demographic variables.

more susceptible than female buffaloes (3.03%) [6]. In addition, other studies also reported the prevalence of bTB in females (9.09%) and males (5.71%) [7], which is similar to the reported study.

According to health condition, a comparatively higher prevalence of tuberculosis was recorded in poor health buffaloes [Body condition score (BCS) = >1–2] at 4.21% than in medium health (BCS = >2–3) at 3.33% and in good health (BCS = >3–4) at 0.00% (Table 1 and Fig. 2). The poor health condition (20%) cattle were highly susceptible, followed by medium (4.44%) and good health (0%) cattle (8), which is in support of the present study. Individuals with good health conditions are naturally more immunocompetent than individuals with poor health conditions. For this reason, poor-health cattle might be highly susceptible to bTB.

Hematological changes in buffaloes due to bTB

The routine hematological values of TEC were comparatively lower in infected buffaloes (3.62 ± 0.11) than the healthy individual (3.76 ± 0.13). Healthy buffaloes had a relatively higher Hb concentration (8.88 ± 0.12) than infected ones (8.72 ± 0.17). The PCV values were also significantly higher in healthy buffaloes (29.52 ± 0.55) as compared with infected ones (28.0 ± 0.42). The ESR value was higher in diseased buffaloes (0.72 ± 0.11) than in healthy individuals (0.64 ± 0.08). The TLC was comparatively higher in infected buffaloes (6.67 ± 0.18) than in healthy animals (6.40 ± 0.35). In differential leukocyte count, total basophil (4.68 ± 0.36) and eosinophil (4.28 ± 0.22) numbers were comparatively higher in infected buffaloes than in healthy control animals as 4.54 ± 0.22 and 4.06 ± 0.28, respectively. The value of lymphocyte and neutrophil was also slightly higher in infected animals at 58.78 ± 1.10 and 32.42 ± 0.90, respectively than in healthy cattle at 58.76 ± 0.98 and 32.17 ± 0.94. The comparative percentage of monocytes was significantly higher in TB positive buffaloes (0.34 ± 0.07) than in healthy ones (0.18 ± 0.08) (Table 2 and Fig. 3). In the hematological study, it was found that TEC, Hb, and PCV values were higher in infected cattle

Table 2. Hematological changes in buffaloes due to bTB.

Parameters	Category	Mean ± SEM	*p*-value
TEC (10^6/µl)	Disease positive	03.62 ± 0.11	0.57
	Negative	03.76 ± 0.13	
TLC (10^3/µl)	Disease positive	06.67 ± 0.18	0.51
	Negative	06.40 ± 0.35	
PCV %	Disease positive	28.0 ± 0.42	0.02*
	Negative	29.52 ± 0.55	
ESR(mm in 1 h)	Disease positive	0.72 ± 0.11	0.43
	Negative	0.64 ± 0.08	
Hb (gm/dl)	Disease positive	08.72 ± 0.17	0.53
	Negative	08.88 ± 0.12	
Lymphocyte (%)	Disease positive	58.78 ± 1.10	0.98
	Negative	58.76 ± 0.98	
Neutrophil (%)	Disease positive	32.42 ± 0.90	0.86
	Negative	32.17 ± 0.94	
Eosinophil (%)	Disease positive	04.28 ± 0.22	0.53
	Negative	04.06 ± 0.28	
Basophil (%)	Disease positive	04.68 ± 0.36	0.61
	Negative	04.54 ± 0.22	
Monocyte (%)	Disease positive	0.34 ± 0.07	0.01*
	Negative	0.18 ± 0.08	

*Significant at 5% level.

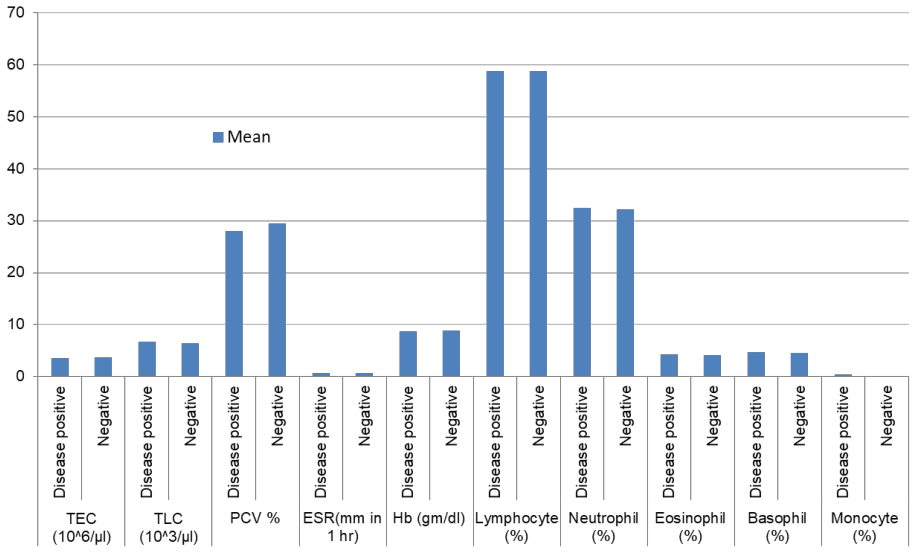

Figure 3. Changes in blood parameters due to bTB in buffaloes.

than in control counterparts [20], which was the reverse of the recorded study. The causes of the opposite result were unknown. It has been reported that TEC and PCV values were significantly lower in bTB infected cattle [16,21], which supports the present study. The TLC was slightly lower in tuberculosis-affected cattle than in healthy individuals [16], which is dissimilar to the present study. In differential leukocyte count, basophil and eosinophil values

Table 3. Changes in biochemical parameters of blood due to bTB in buffaloes.

Parameters	Category	Mean ± SEM	*p*-value
Total protein (g/dl)	Disease positive	9.11 ± 0.08	0.16
	Disease negative	8.85 ± 0.29	
Calcium (mg/dl)	Disease positive	7.66 ± 0.28	0.06
	Disease negative	8.43 ± 0.46	
Phosphorus (mg/dl)	Disease positive	6.63 ± 0.27	0.015*
	Disease negative	7.42 ± 0.18	
Creatinine (mg/dl)	Disease positive	0.84 ± 0.08	0.06
	Disease negative	0.94 ± 0.05	
SGOT (IU/l)	Disease positive	69.55 ± 2.66	0.07
	Disease negative	64.67 ± 2.60	
SGPT (IU/l)	Disease positive	33.57 ± 0.89	0.05
	Disease negative	31.20 ± 1.37	
Glucose (mg/dl)	Disease positive	53.55 ± 2.06	0.01*
	Disease negative	56 ± 1.08	
Cholesterol (mg/dl)	Disease positive	109.25 ± 1.20	0.001**
	Disease negative	121.06 ± 0.03	

* Significant at 5% level, ** Significant at 1% level.

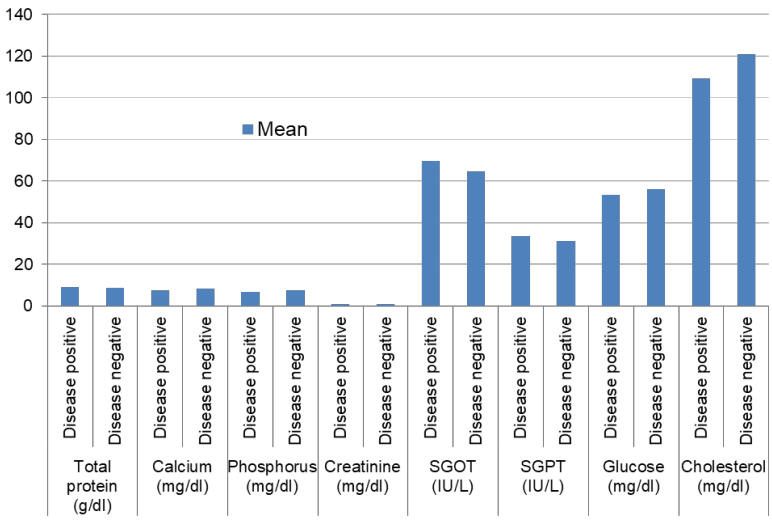

Figure 4. Changes in biochemical parameter (mean) due to bTB in buffaloes.

were significantly higher in diseased animals [20]. Allergic reactions due to tuberculosis in the body might be responsible for increased basophil and eosinophil counts.

Changes in biochemical parameters of blood due to bTB in buffaloes

Among the tested biochemical parameters, phosphorus, glucose, and cholesterol levels were significantly lower in diseased positive buffaloes at 6.63 ± 0.27, 53.55 ± 2.06, and 109.25 ± 1.20 than in healthy control animals at 7.42 ± 0.18, 56 ± 1.08 and 121.06 ± 0.03, respectively. The value of total protein (9.11 ± 0.08) was comparatively higher in diseased TB positive buffaloes than in healthy individuals (8.85 ± 0.29). Calcium (7.66 ± 0.28) and creatinine (0.84 ± 0.08) levels were also comparatively lower in diseased TB positive buffaloes than in healthy individuals

at 8.43 ± 0.46 and 0.94 ± 0.05, respectively. In the case of SGOT and SGPT analysis, the values were comparatively higher in TB positive buffaloes at 69.55 ± 2.66 and 33.57 ± 0.89 than in healthy animals at 64.67 ± 2.60 and 31.20 ± 1.37, respectively (Table 3 and Fig. 4). The total protein level was slightly higher in healthy cattle than in affected individuals [20,22], which is dissimilar to the present data. A study reported that infected cows had a significantly higher total protein level [23], consistent with the current data. On the other hand, calcium and phosphorus levels in tuberculosis-affected cattle were slightly higher than in healthy cattle [20], which is the reverse of the performed study. There was insignificant variation between tuberculin reactor and non-reactor cattle [16,24]. SGOT and SGPT values were comparatively higher in healthy cattle than in TB-positive patients [20] and inclined to the present study. In this study, glucose and cholesterol values in affected animals were alarmingly lower than in healthy cattle. It might be due to body weight loss, anorexia, and inappetence in affected animals.

Conclusions

Tuberculosis is a highly infectious and contagious disease primarily caused by *M. bovis*, which is endemic in many countries. The zoonotic significance of the disease contributes to a significant economic burden. The highest prevalence of the disease was recorded in older, female, and poor-health individuals. bTB in buffaloes also showed a significant alteration in hemato-biochemical parameters. Proper planning, research, and educational efforts are required to increase awareness of the owner, farmer, or relevant person engaged in buffalo farms or Bathan. For field screening of tuberculosis, CFTTs should be conducted regularly as a preventive approach to the disease in buffaloes. More details of molecular epidemiological investigation should be carried out to check for bTB in buffaloes in the study area.

List of Abbreviations

BCS, Body condition score; bTB, Bovine tuberculosis; CCT, Comparative cervical tuberculin test; CFTT, Caudal fold tuberculin test; ESR, Erythrocyte sedimentation rate; Hb, Hemoglobin; PCV, Packed cell volume; PPD, Purified protein derivative; TEC, Total erythrocyte count; TLC, Total leukocyte count; TB, Tuberculosis; SID, Single intradermal tuberculin; SGOT, Serum glutamic oxaloacetic transaminase; SGPT, Serum glutamic pyruvic transaminase; g/dl, gram/deciliter; mg/dl, milligram/deciliter; IU/l, International Unit/Liter.

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Conflict of interest

The authors declare that they have no conflict of interest.

Authors' contributions

Conceptualization: MSA and MR; methodology, MSA and MR; validation, MSA; formal analysis, MR; investigation, MSA; resources, MSA; data curation, MSA and MAR; writing-original drafting preparation, MSA; writing-review and editing.

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