The Pharma Innovation Journal 2023; SP-12(8): 971-976

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2023; SP-12(8): 971-976
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www.thepharmajournal.com
Received: 01-05-2023
Accepted: 04-06-2023

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Haemato- biochemical study of buffaloes suffering from paratuberculosis (MAP infection) in Malwa region of Madhya Pradesh

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Abstract
The present study was designed to know the haematobiochemical study of paratuberculosis in buffaloes (*Bubalus bubalis*) in Malwa region, Madhya Pradesh. In the present study, 100 (35 females and 65 males) buffaloes were slaughtered at Cantonment Board slaughter house, Mhow, which were nonproductive and the samples were examined for *Mycobacterium avium* subsp. *paratuberculosis* (MAP) infection.

In the present study the overall mean values of haematological parameters such as haemoglobin (Hb), packed cell volume (PCV), total erythrocyte count (TEC) and neutrophils showed a significant decrease, total leucocyte count (TLC) and lymphocytes were significantly high whereas overall mean values of biochemical parameters showed a significant decrease in total protein (TP) while AST and ALT were significantly high in MAP -affected buffaloes. These findings indicated the presence of anaemia and hypoproteinemia among the positive cases of MAP in buffaloes.

Keywords: Buffaloes, haematobiochemical values, MAP and paratuberculosis

Introduction
One very important disease prevalent in buffaloes is paratuberculosis (pTB). It is caused by *Mycobacterium avium* subspecies *paratuberculosis* (MAP), a gram-positive, aerobic, non-spore-forming acid-fast bacillus (AFB), that grows slowly in culture (Thorel *et al*., 1990) [14]. Paratuberculosis is a chronic contagious disease of ruminants. The primary site of affection is the lower part of the small intestine more specifically Peyer's patches at the ileum and ileocaecal junction. This disease is characterized by chronic intermittent diarrhoea, progressive emaciation, decrease in production and fertility making the animal susceptible to various other diseases (Yamasaki *et al*., 2013) [16]. The spread of the disease is critical since organisms are shed in faeces and milk even in clinically healthy animals. The organism spreads through blood via the mesenteric lymph node to the multiple internal organs (Vegad *et al*., 2008) [15].

Materials and Methods
A total of 100 buffaloes brought for slaughterhouses located in the cantonment board, Mhow were studied. Detailed ante-mortem and post-mortem examinations were performed for this study. Buffaloes were physically examined before slaughter, for their geographical location (source), breed, age, sex, the reason for slaughter (reproduction).

Ante-mortem sampling
Before slaughter, 5 ml blood was collected from each buffaloes from jugular vein in EDTA @ 2mg/ml vials for haematological and 5 ml in non EDTA vials to separate serum for biochemical estimations.

After the slaughter
Faecal sample collection
Faecal samples were collected in polythene bags, properly labelled and kept at -20 °C in a deep freezer for further examination of MAP.

Microscopic examination of faecal smears

Processing of faecal samples (for microscopic examination)

Approximately, 2 g of faecal samples were finely triturated in the sterilized pestle and mortar with the help of sterilized distilled water (10-12 ml). Triturated material was transferred into 15 ml centrifuge tubes. Tubes were centrifuged at 4000 rpm for 45 minutes at room temperature. The supernatant was discarded and the middle layer was used for the preparation of smears with the help of dropper / wooden stick on clean slides and air-dried.

Staining of faecal smear by Ziehl – Neelsen staining method

Smears were prepared by using residual faecal sediment on clean slides and stained as per the standard method of acid-fast staining. To make smears, clean, grease-free slides were drenched with carbol fuschin for 10 minutes while submerged in a steam bath to let the dye penetrate as deeply into the bacterial cell wall as possible. The slides were then carefully cleaned with tap water after being cooled to room temperature. The slides were then cleaned for 30 seconds with an uninterrupted flow of acid alcohol reagent, followed by a wash with distilled water. Before the final distilled water rinse, the slides were covered with methylene blue for a minute as a counterstain.

In the faecal smear examination, Slides displaying pink-coloured short rods (0.5–1.5 µm), indistinguishable from MAP will be considered positive for paratuberculosis / MAP infection.

Results and Discussion

In the present study, 100 (35 females and 65 males) buffaloes were slaughtered at Cantonment Board slaughter house, Mhow, all were nonproductive and the samples were examined for *Mycobacterium avium* subsp. *paratuberculosis* (MAP) infection. The age of buffaloes was between 1 to 12 years and these buffaloes were belonging to different regions of the Malwa tract, *viz*. Mhow, Dewas, Indore and Maheshwar.

The overall incidence of paratuberculosis (pTB) or MAP infection was found to be 41% (41/100), based on faecal smear examination.

The haematological changes in paratuberculosis (MAP infection)

In the present study, overall mean haematological values such as haemoglobin (Hb), packed cell volume (PCV), total erythrocyte count (TEC), total leucocyte count (TLC), differential leucocyte count (DLC) and mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) were studied in the paratuberculosis affected and unaffected buffaloes. The changes in haematological values are shown in Table (01 and 02) and Figure (01 and 02).

Table 1: Haematological changes due to paratuberculosis (MAP infection) in buffaloes

S. No.	Parameters	Positive for MAP infection	Negative for MAP infection	Normal reference range (Benjamin, 2007 and Jain, 1986)
1.	Hb (g/dl)	8.76^{a}±0.11	12.56^{b}±0.12	9-14
2.	PCV (%)	26.28^{a}±0.35	37.68^{b}±0.37	24-48
3.	TEC (million/µl)	4.38^{a}±0.05	6.28^{b}±0.06	5-10
4.	MCV (fl)	48.48^{a}±0.84	49.59^{a}±0.73	40-60
5.	MCH (pg)	13.73^{a}±0.29	14.00^{a}±0.26	11-17
6.	MCHC (g/dl)	33.80^{a}±0.34	33.83^{a}±0.34	30-36

$p \leq 0.05$ contain different superscript (a, b, c)

$p \geq 0.05$ contain same superscript

Changes in Hb, PCV, TEC, MCV, MCH and MCHC

In the present study, the mean (Mean ± S.E) value of haemoglobin concentration in buffaloes affected with pTB was 8.76±0.11 g/dl showing a significant reduction when compared with the Hb value 12.56±0.12 g/dl of non-infected buffaloes. The mean value of packed cell volume was also reduced significantly up to 26.28±0.35% when compared with the PCV value 37.68±0.37% of non-infected buffaloes. The mean value of total erythrocyte count (TEC) in buffaloes affected with paratuberculosis showed a significant reduction 4.38x106/µl as compared to the TEC value 6.28x106/µl of non-infected buffaloes.

In the present study, the mean values of MCV, MCH and MCHC were found to be (48.48±0.84 fl), (13.73±0.29 pg) and (33.80±0.34 g/dl) in buffaloes which were affected with pTB, respectively. The mean values of MCV, MCH and MCHC were found to be (49.59±0.73 fl), (14.00±0.26 pg) and (33.83±0.34 g/dl) in buffaloes which were unaffected with pTB, respectively. Non-significant changes were observed in the values of MCV, MCH and MCHC in pTB-affected buffaloes.

The overall mean value of haematological parameters in buffaloes was affected with pTB showing a reduction from the normal values.

The findings of the present investigation were almost similar to Tharwat *et al.* (2012 and 2013) [12-13], EI-Deeb *et al.*, 2014 [3], Bhardwaj *et al.*, 2011 [2] and Senturk *et al.*, 2009 [6] with a decreased level of haemoglobin concentration. Non-significant changes in the Hb values have been reported by Shakya *et al.*, 2015 [7] and Abdelaal *et al.*, 2019 [1].

Tharwat *et al.*, 2012 [12-13], Bhardwaj *et al.*, 2011 [2], Senturk *et al.*, 2009 [6], Shakya *et al.*, 2015 [7] and Abdelaal *et al.*, 2019 [1] recorded a decrease in the value of PCV and TEC. These observations have similarities with the observations of the present study.

In contrast to the present study, EI-Deeb *et al.*, 2014 [3] recorded an increase in the values of PCV.

Among RBC indices, values of MCV, MCH and MCHC, the non-significant changes were observed in infected buffaloes than non-infected buffaloes. The findings of Abdelaal *et al.* (2019) [1] also showed non-significant changes in the values of MCV, MCH and MCHC.

Table 2: Changes in total leukocyte count and differential leukocyte count due to paratuberculosis (MAP) in buffaloes

S. No.	Condition	TLC (thousand/µl)	Differential leukocyte count (%)				
			Neutrophils	Lymphocytes	Monocytes	Eosinophils	Basophils
1.	Positive for MAP infection	15.45^{b}±0.12	13.03^{a}±0.81	73.42^{b}±0.79	4.02^{a}±0.72	9.34^{a}±0.35	0.19^{a}±0.41
2.	Negative for MAP infection	10.64^{a}±0.15	27.46^{b}±0.74	61.04^{a}±0.73	3.21^{a}±0.52	8.17^{a}±0.17	0.12^{a}±0.36
3.	Normal reference range (Benjamin, 2007 and Jain, 1986)	4-12	15-45	45-75	2-7	2-20	0-2

$p \leq 0.05$ contain different superscript (a, b, c)
$p \geq 0.05$ contain same superscript

Changes in total leukocyte count

In the present study, the mean value of total leukocyte count was significantly increased up to 15.45±0.12 thousand/µl in pTB-affected buffaloes and the mean value of unaffected buffaloes was 10.64±0.15 thousand/µl.

A significant increase in TLC level in the present study was in agreement with the report of Shakya *et al.*, 2015 [7] and Siji *et al.*, 2008 [10] which showed a high value of TLC than normal and was in contrast to the study of Sharma *et al.* (2022) [9] who recorded decreased level of TLC in pTB affected buffaloes.

Changes in differential leukocyte count (DLC %)

In the present study, the mean value (Mean ± S.E.) of neutrophils in pTB-affected buffaloes was recorded as 13.03±0.81%. The neutrophil count was decreased significantly in the pTB-affected buffaloes as compared to the unaffected buffaloes (27.46±0.74%). The mean value of lymphocyte was significantly increased up to (73.42±0.79%) in pTB-affected buffaloes and the mean value of unaffected buffaloes was 61.04±0.73%.

The mean value (Mean ± S.E.) of monocyte in pTB-affected buffaloes was 4.02±0.72% where as in unaffected buffaloes which was recorded as 3.21±0.52%. The mean value (Mean ± S.E.) of eosinophils was observed as 9.34±0.35% and 8.17±0.17% in pTB-affected and unaffected buffaloes, respectively. The mean value (Mean + S.E.) of basophils in pTB-affected buffaloes was 0.19±0.41% while in unaffected buffaloes which was noted as 0.12±0.36%. No significant difference was observed in the mean values of monocytes, eosinophils and basophils.

Shakya *et al*. (2015) [7] and Sharma *et al.* (2022) [9] recorded a decrease in the value of neutrophils and these observations are in agreement with the present study. Findings of EI-Deeb *et al.* (2014) [3] were not similar with the observations of the present study and recorded a decreased level of neutrophils.

Shakya *et al.* (2015) [7] showed an increase in the value of lymphocytes which is in line with the findings of the present study. In contrast to the present study, Sharma *et al.*, (2022) [9] recorded the decreased values of lymphocytes.

Shakya *et al.* (2015) [7] showed non-significant changes in the values of monocytes, eosinophils and basophils which are matching with the findings of the present study.

The parameters taken into consideration during haematological examination includes Hb, TEC, PCV, TLC, DLC, MCH, MCV and MCHC. There was a significant decrease in the level of Hb, TEC and PCV in the affected animals, suggestive of severe anemia during the course of the disease. The anemia is mainly non – regenerative type and microcytic hypochromic in nature as paratuberculosis is predominantly chronic in nature and thus impairs bone marrow activity to a certain extent.

Although decreased MCH, MCV and MCHC values were found in the present study but statistically significant changes were not observed as both healthy and weak animals were included along with the emaciated and weak animals.

Overall increase in the TLC value was witnessed in affected animals as compared to their healthy counterparts. The leukocytosis condition can be justified due to the chronic nature of the infection associated with inflammation.

Changes in biochemical parameters due to paratuberculosis (MAP infection) in buffaloes

In the present study, 100 serum samples from buffaloes were examined for any changes in biochemical parameters such as total protein, alkaline phosphatase (ALP), aspartate aminotransferase (AST), Alanine aminotransferase (ALT) and serum creatinine. The findings of the present study are presented in Table 03 and Fig 03.

Table 3: Changes in biochemical parameters due to paratuberculosis (MAP infection) in buffaloes

S. No.	Condition	Total serum protein (g/dl)	Alkaline phosphatase (IU/L)	AST/ SGOT (IU/L)	ALT/SGPT (IU/L)	Serum creatinine (g/dl)
1.	Positive for MAP infection	4.38^{a}±0.09	104.04^{a}±2.73	153.43^{b}±1.41	52.56^{b}±0.96	1.36^{a}±0.03
2.	Negative for MAP infection	7.32^{b}±0.08	98.23^{a}±2.82	102.96^{a}±2.28	27.22^{a}±1.01	1.28^{a}±0.03
3.	Normal reference range (Benjamin, 2007 and Jain, 1986)	6.7-7.4	0-488	78-132	11-40	1-2

$p \leq 0.05$ contain different superscript (a, b, c)
$p \geq 0.05$ contain same superscript

In the present study, the results indicated that the buffaloes affected by paratuberculosis were having a significant decrease in values of total serum protein (4.38±0.09 g/dl) as compared to buffaloes, which were negative for paratuberculosis (7.32±0.08 g/dl).

The mean value (Mean ± S.E.) of alkaline phosphatase (ALP) was recorded as 104.04±2.73 IU/L and 98.23±2.83 IU/L in pTB-affected and unaffected buffaloes, respectively. No significant difference was observed in affected and unaffected buffaloes.

In the present study, the result indicated that the buffaloes were affected by paratuberculosis were having a significant increase in values of aspartate aminotransferase (AST) (153.43±1.41 IU/L) as compared to buffaloes, which were negative for paratuberculosis (102.96±2.28 IU/L). The buffaloes were affected by paratuberculosis were having a

significant increase in values of alanine aminotransferase (52.56±0.96 IU/L) as compared to buffaloes, which were negative for paratuberculosis (27.22±1.01 IU/L).

In the present study, the mean value (Mean ± S.E.) of serum creatinine was recorded as 1.37±0.03 gm/dl and 1.28±0.03 gm/dl in pTB affected and unaffected buffaloes, respectively. No significant difference was observed in affected and unaffected buffaloes.

The findings reported by Kormendy *et al.*, 1990 [4], Bhardwaj *et al.*, 2011 [2], Mohammed *et al.*, 2012 [5], Tharwat *et al.*, 2013 [13], EI-Deeb *et al.*, 2014 [3], Shakya *et al.*, 2015 [7] and Abdelaal *et al.*, 2019 [1] showed an increase in the value of total protein was in consonance with the findings of the present study.

The findings of the present study are in agreement with the findings of Kormendy *et al.*, 1990 [4] and Shakya *et al.*, 2015 [7] reported a significantly increased level of aspartate aminotransferase and alanine aminotransferase. In contrast to the findings of the present study, Abdelaal *et al.* (2019) [1] recorded non-significant changes in the values of AST and ALT.

Mohammed *et al.*, 2012 [5], Tharwat *et al.*, 2012 [12], Shakya *et al.*, 2015 [7] and Abdelaal *et al.*, 2019 [1] reported non-significant changes in the value of serum creatinine is in consonance with the findings of the present study. In contrast to the present study, Sharma *et al.* (2022) [9] recorded an increase in values of serum creatinine.

Among the various biochemical parameters taken into consideration, total protein is decreased in the affected animals. The reason for the decline is attributed to the protein losing enteropathy, that leads to reduced integrity of the digestive track mucosa or due to hyper gut motility leading to less absorption and increased protein loss. Hypoalbuminemia and hyperglobulinemia recorded could also be the consequences of the chronic nature of the disease.

Among biochemical enzymes, there was a significant increase in the levels of SGPT and SGOT which is suggestive of the impairment of hepatic function since long. However, increase in the levels of alkaline phosphatase and creatinine were statistically non-significant as both healthy and weak animals were included along with emaciated and weak animals.

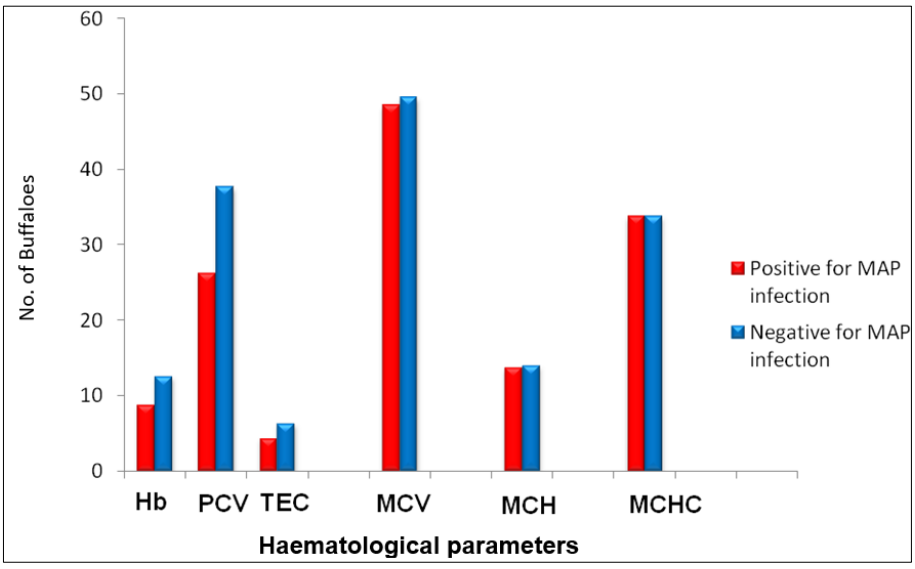

Fig 1: Haematological changes due to paratuberculosis (MAP infection) in buffaloes

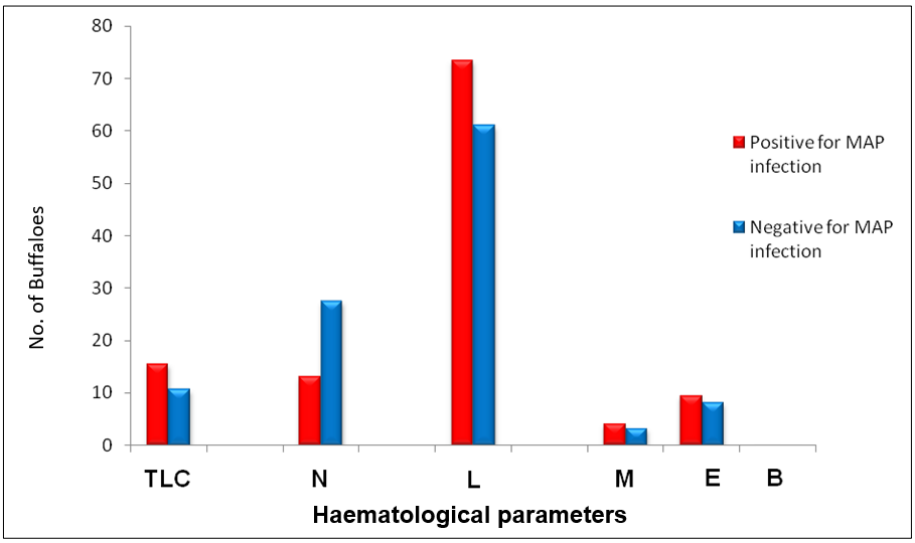

Fig 2: Changes in TLC and differential leukocyte count due to paratuberculosis (MAP) in Buffaloes

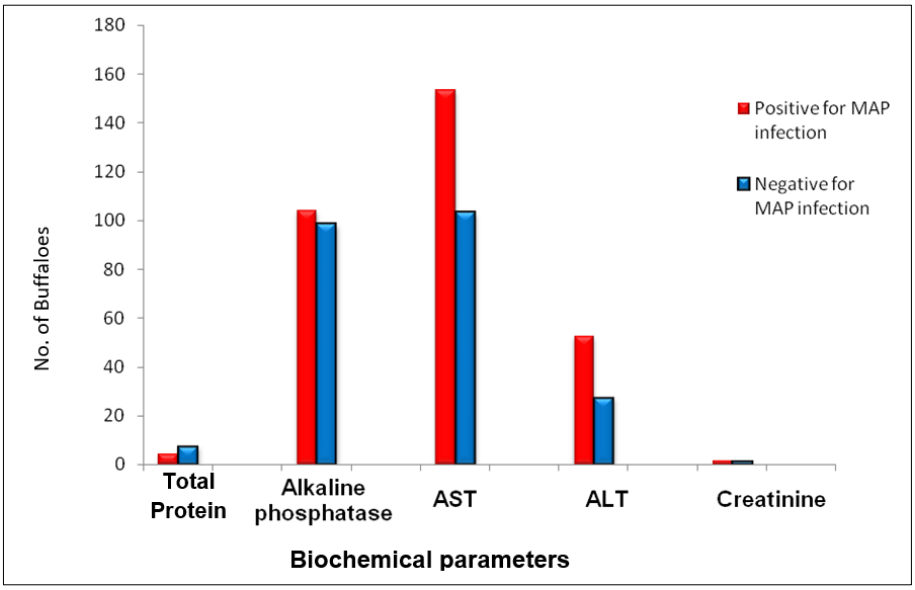

Fig 3: Changes in biochemical parameters due to paratuberculosis (MAP infection)

Conclusions

The present study results of haematobiochemical parameters indicates that the MAP infection is also affects the haematobiochemical values in buffaloes.

Acknowledgement

Authors are very thankful to the Dean of College of Veterinary Science and Animal Husbandry, Mhow for giving facility to conduct this work in the department of Veterinary Pathology.

Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

1. Abdelaal AM, Elgioushy MM, Gouda SM, El-Adl MM, Hashish EA, Elgaml SA, *et al*. Hemato-biochemical and molecular markers (IS900) of cattle infected with Johne's disease in Egypt. Slovenian Veterinary Research. 2019;56(22):421-431.
2. Bhardwaj RK, Randhawa CS, Randhawa SS, Singla VK. Haematobiochemical profiles of cows shedding acid-fast bacilli vis-à-vis normal cows. Indian Journal of Animal Sciences. 2011;81(1):19-22.
3. El-Deeb WM, Fouda TA, El-Bahr SM. Clinico-biochemical Investigation of Paratuberculosis of Dromedary Camels in Saudi Arabia: Proinflammatory Cytokines, Acute Phase Proteins and Oxidative Stress Biomarkers. Pakistan Veterinary Journal. 2014;34(4):484-488.
4. Körmendy B, Szilágyi M, Tuboly S, Nagy G. Some diagnostic features of the pathogenesis of bovine paratuberculosis (Johne's disease) and serum biochemical changes after oral reinfection. Journal of Veterinary Medicine, Series B. 1990;37(1-10):229-235.
5. Mohammed KB, Ibrahim IG, Mohamed ZA. Biochemical analysis on cattle naturally infected with *Mycobacterium avium* subspecies *paratuberculosis* Paratuberculosis, 11th Sydney. International Colloquium on Australia, 5-10, February 2012, International Association for Paratuberculosis; c2012. p. 119-121.
6. Senturk S, Metcitoglu Z, Ulgen M, Borum E, Temizel E, Kasap S. Evaluation of serum iron and iron binding capacity in cows with paratuberculosis. Tierärztliche Praxis Ausgabe G: Großtiere/Nutztiere. 2009;37(06):375-378.
7. Shakya DK, Garg UK, Shukla S, Karmore SK, Shrivastava N, Jatav GP. Studies on patho-epidemiology of Johne's disease in buffaloes of Malwa region. Indian Journal of Veterinary Pathology. 2016;40(1):8-14.
8. Shankar H, Singh SV, Singh PK, Singh AV, Sohal JS, Greenstein RJ. Presence, characterization, and genotype profiles of *Mycobacterium avium* subspecies *paratuberculosis* from unpasteurized individual and pooled milk, commercial pasteurized milk, and milk products in India by culture, PCR, and PCR-REA methods. International Journal of Infectious Diseases. 2010;14(2):121-126.
9. Sharma S, Gautam A, Singh S, Chaubey KK, Mehta R, Sharma M, Gupta S. Immunological and Hemato-biochemical alterations in diarrhoeic buffaloes screened for *Mycobacterium avium* subspecies *paratuberculosis* infection using 'indigenous ELISA kit'. Comparative Immunology, Microbiology and Infectious Diseases. 2022;87:101833.
10. Siji PC, Vijayakumar K, Tresamol PV, Saseendranath MR. Comparative clinical pathology of johnin and tuberculin reactors of bovine. Journal of Veterinary and Animal Science. 2008;39:57-59.
11. Singh SV, Sohal JS, Kumar N, Gupta S, Chaubey KK, Rawat KD, *et al*. Recent Approaches in Diagnosis and Control of Mycobacterial Infections with Special Reference to Mycobacterium Avium Subspecies. Advances in Animal and Veterinary Sciences. 2014b;2(IS):1-12.
12. Tharwat M, Al-Sobayil F, El-Magawry S. Clinicobiochemical and postmortem investigations in 60 camels (Camelus dromedarius) with Johne's disease. Journal of Camel Practice and Research. 2013;20(2):145-149.
13. Tharwat M, Al-Sobayil F, Ali A, Hashad M, Buczinski S. Clinical, ultrasonographic, and pathologic findings in 70 camels (Camelus dromedarius) with Johne's disease. The Canadian Veterinary Journal. 2012;53(5):543.

14. Thorel MF, Krichevsky M, Lévy-Frébault VV. Numerical taxonomy of mycobactin-dependent mycobacteria, emended description of Mycobacterium avium and description of Mycobacterium avium subsp. avium subsp. nov., *Mycobacterium avium* subsp. *paratuberculosis* subsp. nov. and Mycobacterium avium subsp. silvaticum subsp. nov. International Journal of Systematic and Evolutionary Microbiology. 1990;40(3):254-260.
15. Vegad JL, Katiyar AK. A Textbook of Veterinary Special Pathology. 3rd Edition International Book Distributing Co, Lucknow; c2008. p. 367-371.
16. Yamasaki EM, Brito MF, Mota RA, McIntosh D, Tokarnia CH. Paratuberculosis in ruminants in Brasil: A review. Pesquisa Veterinária Brasileira. 2013;33(2):127-140.