International Journal of Veterinary Sciences and Animal Husbandry

ISSN: 2456-2912
VET 2024; 9(1): 262-264
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www.veterinarypaper.com
Received: 01-11-2023
Accepted: 05-12-2023

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Sub clinical status of a murrah buffalo in an organized livestock farm for Paratuberculosis and its hemato-biochemical values

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Abstract
A clinical study on the prevalence of *Mycobacterium avium* subspecies *paratuberculosis* (MAP) was carried out in an organized livestock farm in buffalo population, Jamdoli, Rajasthan state using acid fast method, SID, ELISA followed by hemato-biochemical parameter studies. Out of 12 buffaloes one adult buffaloe showing weight loss was found positive with the above diagnostic tests. Detailed studies of this sub clinically infected buffaloe was carried out for hemato-biochemical variations for better management of the rest of the population of the livestock farm. The hematological and biochemical studies revealed significant decreases in RBCs, Hb concentration, PCV, and MCHC level and in the total protein and albumin contents respectively.

Keywords: Murrah buffalo, livestock farm, *Mycobacterium avium* subspecies *paratuberculosis*

Introduction
Johne's disease (J.D), known as Para tuberculosis is a severe production – limiting disease with significant economic implications for the global cattle industry caused by *Mycobacterium avium* subspecies *paratuberculosis* (MAP). JD manifests as chronic enteritis in infected cattle. The lack of effective treatment options, such as vaccine, has hampered JD control resulting in its increasing global prevalence. The disease was first reported in 1895, and the recent research facilitated enhanced understanding of the pathogen factors influencing disease pathogenesis. (Sanjay Mallikarjunappa *et al* 2021) [6]. The severity of disease and the rate of progression depend on several variables, among the most important of which are the doses of MAP and the age of the animal at exposure. Large doses might be necessary to infect older animals. After uptake by the M cells, MAP bacilli are transferred to underlying lymphoid tissue. Dissemination via the bloodstream can then occur, with subsequent localization to secondary sites – the liver, spleen, and peripheral lymph nodes (Sweeney, 1996) [9]. The spectrum of disease in domesticated animals is explained by many authors. JD is characterized by vague and often variable clinical signs; Whitelock and Buergelt (1996) [11] have described the progression of disease in cattle as stage 1 as silent infection, stage 2 as subclinical disease, stage 3 as clinical disease and stage 4 as advanced clinical stage. The animals in subclinical disease are adult animals that are carriers of MAP. The animals do not exhibit clinical signs typical of JD, but they sometimes have detectable antibodies or exhibit altered cellular immune responses. In a small percentage, disease can be detected by fecal culture, by altered cellular immune response, by serum antibodies, or by histopathology. Ahmed *et al* (2019) reported hemato-biochemical changes in Johne's disease infected cattle. Nuzhat Hassan *et al* (2022) [4] reported lower level of total protein and albumin in clinical JD animals.
Regular screening program on the prevalence of JD infection in this farm was carried out using Acid fast staining method using rectal pinch, Johnin by SID as per standard protocol. All the 12 buffaloes tested with Acid fast and Johnin methods were found negative. This animal suffered for its clinical signs in sub clinical status i.e. loss of weight and anorexia for more than 2 months. Then the serum sample was subjected to ELISA test which revealed the JD infection status. Based on this back round the buffaloes' serum sample was subjected to hemato-biochemical analysis and the details are discussed here.

Results and Discussions

Table 1: Hematology

Sl.no	Parameter	Finding	unit	Normal value	Diagnostic interpretation
1	Haemoglobin	5.40	g/dl	8.0-15.0	Anemia
2.	Total leucocyte count	5.60	thou/cu.mm	4.0-12.0	
3	Differential leucocyte count				
	Neutrophils	35	%	15.0-33.0	High
	Lymphocytes	60.0	%	45.0-75.0	
	Eosinophils	4.00	%	0.0-20.0	
	Monocytes	1.00	%	0.0-8.0	
	Basophils	0.00	%	0.0-3.5	
4	RBC parameters				
	RBC	3.70	106/cu.mm	5.0-10.0	Anemia
	PCV	19.30	%	24.0-46.0	Anemia
	MCV	52.20	fl	40.0-60.0	
	MCH	14.60	pg	11.0-17.00	
	MCHC	28.00	g/dl	30.0-36.0	Hypochromic anemia
	Platelet count	292.00	103/cu.mm	100-800	

Table 2: Bio-chemical studies

Sl.no	Parameter	Finding	unit	Normal value	Diagnostic interpretation
1	ALT (IU/L)	31.30	U/L		
2	AST(IU/L)	88.00	U/L	45.3-110.2	
3	ALP (IU/L)	42.80	U/L	17.5-152.7	
4	CGT (IU/L)				
5	TB(mg/ml)	0.10	mg/dl	0-0.8	
6	TP (g/dl)	5.39	g/dl	6.2-8.2	Low
7	Albumin (g/dl)	1.32	g/dl	2.8-3.9	Low
8	Globulin (g/dl)	4.07	g/dl	2.9-4.9	
9	A/G Ratio	0.32			
10	BUN (mg/dl)	19.86	mg/dl	10-25	
11.	Blood urea	42.50	mg/dl	18.8-55.4	
12	Creatinine(mg/dl)	1.31	mg/dl	0.6-1.8	
13	Calcium	8.30	mg/dl	8.0-11.4	
14.	Phosphorus	4.35	Mg/dl	4.3-7.8	
15	Sodium	140.9-	mEq/l	132-152	
16	Potassium	4.50	mEq/l	3.9-5.8	
17.	Chloride	105.20	mEq/l	97-111	
18	Glucose random	62.90	mg/dl	40-100	

Haematology

In this study the Hb level, RBC count, PCV, MCHC level were decreased. Ahmed *et al* (2019) [1] studied the haematological changes in cattle infected with JD bacilli and reported significant decreases in RBCs, PCV, MCHC level. Decreased Hb concentration revealed a non-significant change which reflects the microcytic hypochromic anaemia. Muhammad Anwarullah *et al* (2021) [5] reported lower levels of RBCs, Hb, and PCV and non-significant level of MCHC in their studies. SumanVinod kumar jain *et al* (2016) [8] reported significant anaemia and lower levels of Hb, probably as a result of in-appetence or anorexia leading to poor body condition. Higher DLC showed neutrophilia along with lymphopenia due to normal defence mechanism against infection. This observation coincides with the present studies, when lymphopenia is not observed in this study. Similarly Arafa *et al* (2008) [2] reported significant decrease in total RBCs counts, Hb values, MCH and MCHC due to coexistence of clinical marginal anaemia with deficiency of minerals. Shalini Sharma *et al* (2022) [7] reported significant differences in creatinine, globulin, total leucocytic, and lymphocyte numbers in JD infected buffaloes which are not observed in this infected Murrah buffalo.

Biochemical reactions

In this study the liver function tests revealed decreased total protein and albumin levels and the other parameters did not altered. The low level of protein is attributed to protein-losing enteropathy which might be due to the impaired integrity of the mucosa of the gastro-intestinal tract as reported by Sweeny *et al* (2012) [10]. Brady *et al* (2008) [3] reported low values of albumin with normal globulin level which are in agreement with this study. The low level of albumin could be due to decreased liver function. The kidney function tests revealed no changes in all concerned parameters. Ahmed *et al* (2019) [1] studied the biochemical changes in cattle infected with JD bacilli and reported that clinically affected cows showed non-significant changes in the levels of albumin, ALT, AST & ALP as enzymes markers of the liver function, & the urea and creatinine as the markers of renal damage. Nuzhat Hassan *et al* (2022) [4] reported low levels of total protein andalbumin in buffaloes infected with JD bacilli. These observations confirm the findings of these studies in buffaloes with JD bacilli.

Conclusion

Studies on the confirmations of J.D infection in a murrah buffalo was carried out by its clinical signs & ELISA, followed by hemato-biochemical parameter studies. The study observed haematological changes in Hb, RBC, neutrophil count, PCV and MCHC values. The biochemical studies revealed changes in the total protein and albumin levels. The hemato-biochemical changes observed in this study may be taken as additional parameters for confirmation of sub clinical status of Paratuberculosis infection for better management of J.D infected buffalo population.

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