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Mixed Infection of Tick-borne Haemo-parasites in Water Buffalo and Associated Pathological Responses and Treatment

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ABSTRACT

Background: Water buffaloes are important milch species of tropical and subtropical countries. In general, they are considered to more resistant to diseases compared to crossbred cattle. However, a recent problem of tick-borne diseases in the water buffaloes by the field veterinarians and farmers causing production losses was observed. The study was conducted to explore the spectrum of tick-borne diseases (TBDs) infections in buffaloes and analyze the associated risk factors.

Methods: Acute-phase response, cytokine and oxidative stress in infected buffaloes with TBDs were evaluated and compared with the negative buffaloes (control) to elucidate their role in pathogenesis and outcome of infection. The study was undertaken in 107 tick-infested water buffaloes. The conventional Giemsa stained blood smear (GSBS) based confirmation and classification of infection of haemo-parasites were made. The statistical model was used to understand their relevance with TBDs.

Conclusion: Tick-borne disease in water buffaloes must be looked upon seriously to maintain good productivity. The buffaloes are often accompanied by low and un-yielding clinical symptoms due to associated co-infections of haemo-parasites. Our study showed that the buffaloes had a high prevalence (47.66%) of haemo-parasites transmitted by the ticks associated with co-infections and a low level of parasitemia. Most buffaloes responded symptomatically with the different modalities administered. Production parameter was not restored post 15 days of treatment. Oxidative stress is one of the important mechanisms of production losses in infected buffaloes.

Key words: Acute phase response, Buffalo, Cytokine, Giemsa staining, Mixed infection, Tick-borne.

INTRODUCTION

Tick-borne diseases (TBDs) of bovine are of immense concern to livestock farmers. Among different TBDs, buffaloes in the tropical region can suffer from anaplasmosis, theileriosis and babesiosis depending on the association of the type of vector ticks. In general, the distribution of different types of ticks in the region affecting bovine includes *Hyalomma* spp., *Boophilus* spp., *Rhipicephalus* spp., *Haemophysalis* spp., *etc.* (Ghosh *et al.* 2006). These ticks play a central role in transmitting many TBDs of bovine, causing substantial economic losses. TBDs often remain subclinical and are associated with multiple pathogens. It renders diagnosis difficult and unyielding based on the clinical symptoms which is the essential tool for farmers to present the animal for veterinary examination. Economic losses from TBDs are due to high mortality rates, reduced milk production and body condition loss due to anemia or stress. An estimated loss from TBDs in cattle is around 498.7 million US$ per annum (Minjauw and Mc Leod 2003). Buffaloes are considered comparatively resistant to TBDs than exotic and crossbred cattle due to many intrinsic and extrinsic factors (Ponnudurai *et al.* 2017). Indian farmers have indicated a positive trend towards buffalo rearing in most parts of the country. There has been a 31.23% increase in the buffalo population mostly in rural areas from 2003 to 2012. The 2019 livestock census showed a 1.06% increase in the buffalo population compared to 2012 livestock census data, DAHD, Government of India (Basic Animal Husbandry Statistics, 2019). In India, the prevalence of theileriosis and babesiosis in bovine has been documented in most parts of the country, while anaplasmosis is constrained to only some parts of the country (Ghosh *et al.* 2006). In the middle Indo-Gangetic plains of Bihar, established reports of co-infection of *Theileria* and *Anaplasma* in crossbred cattle is available

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(Prabhakaran *et al.* 2021). Contemplating these, we intended to explore the prevalence of TBDs in buffaloes based on conventional methods and analyze the associated risk factors. Acute phase and oxidant-antioxidant response in TBD infected buffaloes were evaluated and compared with the negative buffaloes (control) to elucidate their role in pathogenesis and outcome of infection.

MATERIALS AND METHODS

Ethics

The study involved drawing of 6 ml blood from jugular vein aseptically from buffaloes with the consent of the owners. These buffaloes were either brought for treatment to veterinary hospitals or as a call for treatment at the farmer's door by the local veterinarian. There are no specific ethical guidelines for a blood sample collection from clinical cases, and hence no prior approval was mandatory. Treatment was provided to all buffaloes under the study.

Study area and sampling

The study has been conducted in peri-urban blocks of the middle Indo-Gangetic plains from Jan 2018 to Jan 2019. The study area's selection has been made considering the inputs of the veterinarians working in the state government hospitals. These areas have a higher demand for buffalo milk due to a higher fat percentage and proximity with the state capital market. The blood samples of selected buffaloes collected from the farmer's door, veterinary hospitals or directly sent to the laboratory for investigation. The buffaloes selected were either suffering from persistent fever and/or not responding to conventional antibiotics. Besides, buffaloes with tick infestation and/or recently purchased from the common market place and/or have any overt signs like fever, inappetence, lacrimation, enlarged peripheral lymph node, red urine, respiratory distress, corneal opacity and history of decreased milk production were also selected. Blood samples in both K2EDTA vacutainer (J K Diagnostics®) and BD Vacutainer® serum collection tubes were aseptically collected from 107 adult female lactating buffaloes suspected to suffer from TBDs. Ticks were also collected from infested animals and stored in a glass vial with muslin cloth covering for identification based on morphology (Kaiser and Hoogstraal, 1964; USDA, 1976). A pre-tested questionnaire was used to collect the data from the farmers on management practices, frequency of acaricidal used, grazing pattern, disease history, *etc*.

Sample processing

Serum was harvested from the blood and stored at -20°C for biochemical estimations and enzyme-linked immunosorbent assay (ELISA). The haematological and blood smear examination was completed on the same day, whereas the biochemical parameters were done within two days of sampling. Before the analysis, frozen serum samples were thawed at room temperature for 30 min and gently homogenized.

Diagnosis of TBDs

Thin blood and lymph node smears were prepared and fixed in methanol for 5 min and stained by 10% Giemsa. Two blood smears were made from each sample and a minimum of 100 fields of each stained blood smear was examined under oil immersion to determine parasitemia. The level of parasitemia was rated on the percentage of RBCs parasitized and calculated based on the method described by Haron *et al.* (2014). These were categorized as low (less than 1% parasitized RBCs), mild (1-3% parasitized RBCs) and high (>3% parasitized RBCs). The samples which were negative for haemo-parasite in duplicate slides were considered negative.

Biochemical and haematological estimation

The total protein concentration (g/dL) in serum samples was measured spectrophotometrically (550 nm wavelength) by the Biuret method using a commercial kit from the Coral clinical system, Goa, India. Serum aspartate aminotransferase (AST/GOT) and alanine aminotransferase (ALP/GPT) enzymes were estimated using methods described in the commercial kits procured from Span diagnostics Ltd. Surat, India and expressed in U/L.

The haemoglobin (Hb) concentration (g/dL) in the blood was measured using HemoCue® cuvettes and its analyzer.

Acute-phase response protein, cytokine and cortisol

Serum amyloid A (SAA) was estimated to understand the acute phase response to inflammation and tissue injury. The estimation was carried out using a commercial diagnostic bovine ELISA kit (G-Biosciences, USA) with a detection range of 3.125-200 mg/mL. Similarly, bovine tumor necrosis factor-alpha (TNF-α) is a potent multifunctional cytokine estimated in serum using a commercial ELISA kit (G-Biosciences, USA). Serum cortisol level as an indicator of stress, infection and adrenal function was estimated in serum using a commercial diagnostic bovine ELISA kit (G-Biosciences, USA).

All samples were assayed in duplicate and the optical density (OD) was recorded at 450 nm using a microplate reader (Electronic Corporation of India, Hyderabad).

Oxidant and antioxidant

Lipid peroxidation (LPO) as malondialdehyde (MDA) were assessed using commercial bovine ELISA kits (G-Biosciences, USA) and antioxidants glutathione (GSH) was estimated using a commercial ELISA kit (Wuhan Fine Biotech Co Ltd. China). The plasma catalase (CAT) activity (kU/L) were measured using the method described by Goth (1991). Briefly, based on the ability to decompose hydrogen peroxide, OD was measured at 405 nm wavelength against a blank containing all the components except the enzyme on a spectrophotometer (Schimadzu UV1800 UV-VIS, Japan).

The ELISA based parameters were assessed in equal numbers (n=14) of random serum samples from three groups categorized based on Giemsa stained blood smear (GSBS) observations of hemo-parasite infection. Group 1 buffaloes were infected with *Theileria* spp. Group 2 buffaloes had co-infections of hemo-parasites or mixed infection. Group 3 buffaloes as control were negative for haemo-parasites.

Therapeutic management

Initial therapeutic management was based on administering oxytetracycline long-acting (200 mg/mL) at the rate of 1 mL/ 10 kg bodyweight along with antipyretic by the field veterinarian or animal health worker when the buffaloes were brought or contacted for veterinary consultation. However, therapeutic management was provided to all buffaloes from the second day onwards with different modules or their combinations, based on GSBS diagnosis, clinical symptoms, and general body condition of the buffaloes (Table 1). Administration of antipyretic drugs, anti-allergic drug, loop diuretics and fluid-electrolyte infusion to buffaloes was also recommended based on the requirement and response. The response was recorded concerning improvement in body temperature, appetite, respiration rate, milk yield, tick control and other associated symptoms. After treatment, there was no follow up for pathogen presence.

Statistical analysis

Oxidative stress activities between groups were compared using single-step multiple comparisons using Turkey HSD by ANOVA post hoc test. Data on observed symptoms recorded and other parameters measured were statistically tested using a regression model. Significance was observed at $^*P<0.05$ and $^{**}P<0.01$. STATA version 11.0 statistical software was used for analysis.

RESULTS AND DISCUSSION

Disease history and symptoms

The clinical signs and disease history were indicative of possible tick-born infection, however, these observed signs were mild, varied and overlapping with TBDs. It is attributed to the low severity of parasitemia and milder pathogenicity (Zwart, 1985). The major clinical signs or management practices associated with different pathogens of TBDs are enlisted in Table 2. The case fatality rate was highest (20.0%) in buffaloes with infection of only *Theileria* spp. compared to co-infected (7.69%) buffaloes. No deaths were recorded in buffaloes infected with *Anaplasma marginale*, *Babesia* spp. and negative buffaloes. Co-infection of pathogens resulted in a higher proportion of animals showing key symptoms of TBDs recorded under the study. Provision for wallowing had lesser haemo-parasitic infected buffaloes but no proportional effect on the type of pathogens in TBDs infected buffaloes. Red urine due to hemoglobinuria was only observed in buffaloes found infected with *Babesia* spp. Redwater (hemoglobinuria) and associated symptoms of high fever, anorexia, anemia, decreased milk production and physiological state were highly suggestive of babesiosis which is in concurrence with published reports (Singla *et al.* 2002; Rani *et al.* 2010). However, without hemoglobinuria babesiosis is difficult to differentiate from other TBDs infection based on clinical symptoms and epidemiology. Peripheral lymph node enlargement was associated with *Theileria* infection (40.00%) or co-infected (7.69%). Corneal opacity and conjunctivitis were–also observed in few buffaloes found infected with *Theileria* spp., or co-infected with *A. marginale*. Anaplasmosis and theileriosis are also difficult to differentiate clinically in adult buffaloes, though superficial lymph node is suggestive of the early stage of

Table 1: Treatment module in water buffaloes for hemo-parasites and tick control.

Hemo-parasites	Modules	Drugs and Dosing
T	I	Buparvaquinone by deep intramuscular route @ 2.5 mg/kg body weight along with module IV
A	II	Imidocarb dipropionate @ 3.0 mg/kg body weight by a subcutaneous route along with module IV
B	III	Diminazene accurate @ 5 mg/kg body weight by the intramuscular route along with module IV
T + A	I + II + IV	
A + B	II + IV	
B + T	I + III + IV	
Negative	IV	Antioxidants containing vitamin E and selenium and extract of Ocimum sanctum orally @ 20 ml per day and oral hematinics containing ferrous fumarate 1500 mg, vitamin B12 75 mcg and folic acid 7500 mcg per dose for 7 days.
Ticks	V	All buffaloes and cattle: Topical spraying amitraz (12.5% EC Solution) and the surrounding environment by applying deltamethrin (1.25% solution)

Where T= *Theileria* spp.; A= *A. marginale*; B= *Babesia* spp.

Theileria spp. infection. TBDs are economically important diseases of buffaloes, though the occurrence reported is comparatively lesser than crossbred cattle. Ticks collected from buffaloes were grossly identified based on key features belonged to different species of the *Ixodidae* family namely *Hyalomma* spp., responsible for the transmission of *Theileria* spp. and *Rhipicephalus* spp. responsible for the transmission of *Babesia* spp., *Theileria* spp. and *A marginale* in the region (Jithendran, 1997). Disease history and clinical findings and association of tick's vectors in the surrounding as well as suspected buffaloes were indicative of tick-borne diseases but are often overlapping and diverse (Ghosh and Nagar, 2014). The use of acaricidal by farmers also did not have a proportional effect on TBDs positive or negative animals. Acaricidal use by farmers is sometimes rendered ineffective due to the availability of infected vectors in the surroundings and premises shared with other animals. Response to empirical treatment with oxytetracycline had a beneficial symptomatic effect in both *Anaplasma* and *Theileria* infections and their co-infection. The oxytetracycline LA is reported useful against *Anaplasma* and *Theileria* infection (Bansal and Sharma, 1986; Cranwell, 1990; Yousef *et al.* 2020). Significant (P<0.05) positive coefficients was observed for increased body temperature and lacrimation in TBDs buffaloes using probit regression (Table 3). These symptoms may help in the screening and selection of animals for such conditions.

Prevalence and spectrum of TBDs by Giemsa stained microscopy

The buffaloes had a high prevalence (47.66%) of parasites causing TBDs including *Theileria* spp., *A. marginale* and *Babesia* spp. (Fig 1). The high prevalence of TBDs positive buffaloes in the region is attributed to the selection of suspected buffaloes and the availability of vector in the environment. However, the majority of these infected buffaloes (86.28%) had low severity of infection. Amongst the buffaloes found positive for infection of hemo-parasites, most of them (56.52%) had co-infection of parasites or mixed infection (Table 4). It may be associated with multiple genus of tick infestation associated with one or more different parasite. Earlier reports of mixed infection in buffaloes of Egypt associated with *Anaplasma*, *Babesia* and *Theileria* infection supports our findings (Nagwa *et al.* 2016; Amira *et al.* 2020). The most prevalent hemo-parasite infection in buffaloes was *A. marginale* followed by *Theileria* spp. and *Babesia* spp. (Table 4). The present findings of the prevalence of only *Theileria* spp., infection (4.67%) is less, while associated with other TBDs (26.17%) is high than the earlier reports of the prevalence of theileriosis 9.33% from suspected buffaloes of Patna (Kala and Deo, 2018). This difference may be due to the geographical restriction of sampling and considering only one pathogen of TBDs. In mixed infection of buffaloes, the most prevalent concurrent pathogens identified were *A. marginale* and *Theileria* spp. (43.14%) infection. We did not observe any buffalo infected

Table 2: Clinical signs, history and management practices (%) in buffaloes associated with tick-borne infections.

Observations	T (n=5)	A (n=18)	B (n=2)	Co-infected (n=26)	Negative (56)
Increase body temperature	80.00	50.00	100.00	69.23	19.64
Anemia	100.00	77.78	100.00	96.15	35.08
Inappetence	100.00	94.44	100.00	96.15	60.71
Decrease milk yield	100.00	72.22	100.00	92.31	57.14
Lacrimation	80.00	11.11	0.00	61.54	3.57
Peripheral lymph node swelling	40.00	0.00	0.00	7.69	0.00
Red urine	0.00	0.00	50.00	0.00	1.79
Animal sharing	60.00	55.56	100.00	80.77	73.21
Wallowing option	0.00	22.22	0.00	19.23	67.86
Use of acaricides	80.00	66.67	0.00	53.85	82.41
Response to Oxy-tetracycline LA	80.00	94.44	0.00	84.62	3.57

Table 3: Probit regression for the relationship between variables as signs and practices and tick-borne infections in buffaloes.

Variables	Coefficient	Standard error	P-value
Increase body temperature	0.8017557	0.4075537	0.049
Anemia	-0.4629887	0.6148052	0.451
Inappetence	0.7758669	0.5475456	0.156
Decrease milk yield	-0.3723748	0.4754377	0.433
Lacrimation	1.388923	0.4885448	0.004
Animal sharing	0.0712827	0.3731284	0.848
Wallowing	-0.2882004	0.3838722	0.453
Use of acaricides	-0.3299074	0.3836209	0.390

Log likelihood = -39.71443; LR chi2(9)= 66.79; Prob > chi2 = 0.0000; Pseudo R2 = 0.4568; Number of obs = 107.

with all three pathogens. The parasitic load was low in most (96.15%) of the buffaloes diagnosed co-infected with hemo-parasites. The low parasitic load and milder symptoms in the buffaloes diagnosed with mixed infection of TBDs indicate that buffaloes may have innate immunity to substantially reduce parasites from circulation (Tuli *et al.* 2015; Benitez *et al.* 2018) and endemic stability (Mckeever, 2009) in the study area.

Oxidative stress and biochemical response

The comparative increase oxidant and decrease anti-oxidants concentration in the TBDs infected buffaloes is indicative of oxidative stress. The significantly increased level of LPO measured as MDA in group I buffaloes infected with *Theileria* alone or group II associated with mixed infection of haemo-parasites versus group III negative buffaloes give insight to oxidative stress as an important aspect of disease pathogenesis. This is supported by a significant reduction in the level of the antioxidants GSH and CAT in buffaloes of group I and II compared to haemo-parasite negative buffaloes of group III (Table 5). Oxidative stress in cattle infected with tick-borne pathogens has been extensively

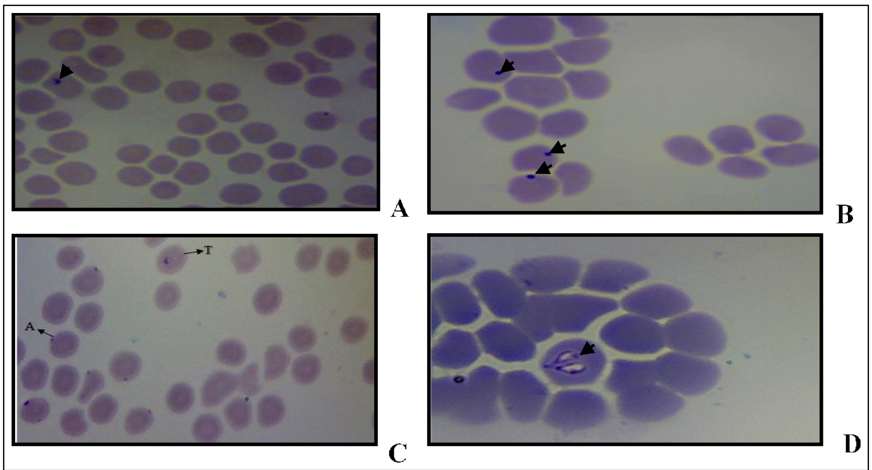

Fig 1: GSBS based diagnosis of tick-borne infection in buffaloes. A. Giemsa stained blood smear showing piroplasm of *Theileria* spp. in RBC. B. GSBS showing *A. marginale* in RBCs of buffalo. C. GSBS showing mixed infection (Arrow T: *Theileria* spp., piroplasm; A: *A. marginale*) in RBCs. D.: Giemsa stained blood smear showing *Babesia* spp. (paired) in RBC. Representative photographs were taken under oil immersion microscope 1000x magnification.

Table 4: Prevalence (%) of tick-borne infection in suspected buffaloes (n=107) based on microscopic examination of blood.

Pathogens observed	Frequency	Frequency of severity categorized			Prevalence in suspected population	Prevalence in infected buffaloes
		Low	Mild	High		
TBDs positive	51.0	44	6	1	47.66	
TBDs negative	56.0	-	-	-	52.34	
Theileria spp. (T)	5.0	3	2	-	4.67	9.80
A. marginale (A)	18.0	15	2	1	16.82	35.29
Babesia spp. (B)	2.0	1	1	-	1.87	4.35
Mixed infection	26.0	25	1	-	24.30	56.52
Pattern of mixed infection						
T + A	22.0	21	1	-	20.56	43.14
T + B	1.0	1	-	-	0.94	1.96
B + A	3.0	3	-	-	2.80	5.88

Table 5: Blood biochemical parameters estimated in buffaloes (mean±S.E).

Variable	Group 1 (n=14)	Group 2 (n=14)	Group 3 (n=14)
MDA (ng/ml)	1252.13^{b}±86.85	1192.43^{b}±77.29	363.41^{a}±37.42
GSH microgram/ml	60.46^{b}±3.92	82.84^{b}±8.94	119.63^{a}±10.24
CAT (U/L)	0.99^{b}±0.06	1.28^{b}±0.14	2.51^{a}±0.19
ALT (Units/L)	43.25^{b}±3.58	37.53^{ab}±3.87	26.26^{a}±3.98
AST (Units/L)	113.85^{b}±9.50	108.85^{b}±8.72	62.25^{a}±5.91
Cortisol (ng/ml)	7.03^{b}±0.61	24.85^{a}±3.28	27.22^{a}±3.45
Total protein (gm/dl)	5.55^{b}±0.24	5.64^{b}±0.31	6.76^{a}±0.13
Hb (gm%) in blood	6.53^{b}±0.59	7.78^{ab}±0.50	9.02^{a}±0.51

Values with different superscript varies significantly (P<0.01) between groups.

reported worldwide which is in concurrence with our observations (AL-Hosary, *et al.* 2015; Esmaeilnejad *et al.* 2020). Similarly, in buffaloes, oxidative stress and parasitic burden-dependent oxidative damages to erythrocytes by *T. annulata* infection in buffaloes have been reported (Molayi-Jabdaragi *et al.* 2020). Logistic regression between oxidant MDA and antioxidant CAT in TBDs infected buffaloes showed significant positive (0.004±0.002) and negative (-2.449± 1.150) coefficients, respectively (Table 6). This indicates that an increase in MDA and a decrease in CAT level can be a useful indicator for screening TBDs infected buffaloes.

Table 6: Logistic regression between TBDs infection in buffaloes and variables estimated.

Variables	Coefficient	Standard error	P-value
SAA	-0.003	0.032	0.930
TNF alpha	-0.032	1.161	0.978
MDA	0.004*	0.001	0.012
GSH	0.022	0.019	0.235
CAT	-2.449*	1.150	0.033
ALT	-0.008	0.034	0.822
AST	0.027	0.016	0.091
Cortisol	0.148*	0.062	0.016
Protein	-1.368*	0.660	0.038
Hb	0.247	0.282	0.381

Log likelihood= -22.39; LR chi2(10)=47.50; Prob > chi2=0.00; Pseudo R2=0.52.

The biochemical response during the infection was measured in terms of ALT, AST, total protein and cortisol concentration in buffaloes (Table 5). The concentration of ALT increased significantly in group I buffaloes and only non-significantly higher in group II buffaloes compared to group III buffaloes. The concentration of AST was also significantly higher in group I and II compared to group III buffaloes. The total serum protein was significantly reduced in both group I and II buffaloes compared to group III buffaloes. The biochemical response in TBDs infected buffaloes was indicative of damage to the tissues by the parasitic invasion particularly of liver, muscle, RBCs. This damage brought hypoxic changes in organs. It was reflected by an increase concentration of liver and tissue enzymes (ALT, AST) and a decrease in total serum protein (Singh *et al.* 2001; Hasanpour *et al.* 2008). A similar observation in cattle infected with *A marginale* (Ganguly *et al.* 2018), with *T. annulata* (Omer *et al.* 2003) and with *B. bigemina* (Ganguly *et al.* 2017) has been reported. The level of Hb was lower in all the groups of buffaloes. However, in group I buffaloes, the concentration of Hb were significantly lower compared to other groups. Anemia due to low haemoglobin level in most of the buffaloes sampled might be due to endemic infections of TBDs and sample selection criteria. The cortisol level was significantly lower in group I buffaloes compared to both group II and III buffaloes which may be indicative of hormonal dysfunction of adrenal gland favouring the dissemination of parasite (Forsyth *et al.* 1999). This finding

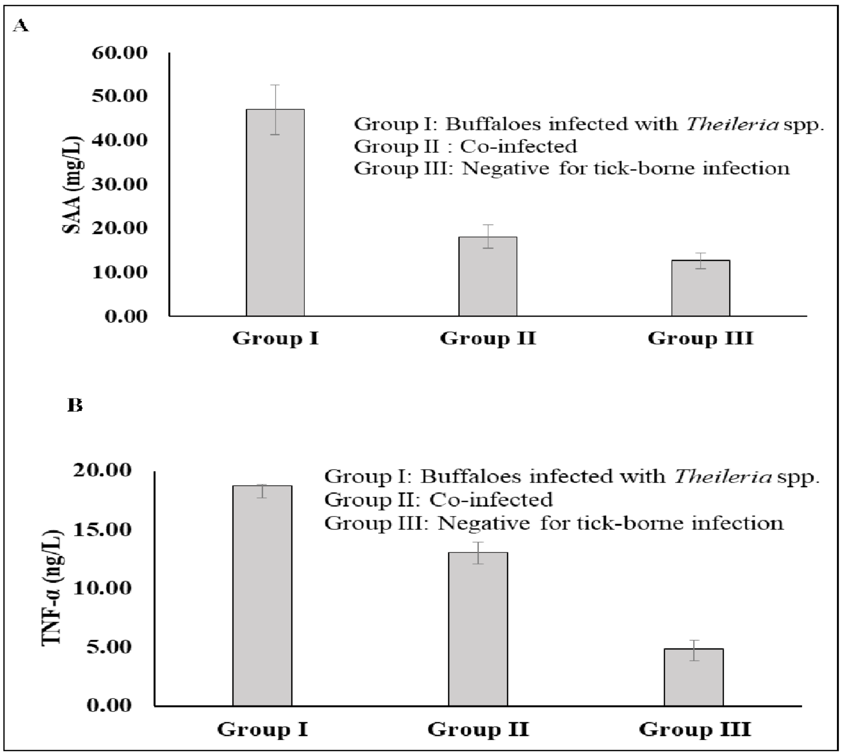

Fig 2: Graphs of acute phase response and cytokine in water buffaloes. A. Acute-phase response measured as SAA (Serum amyloid) (A) In hemo-parasite negative and positive buffaloes; (B) Cytokine response measured as TNF alpha in hemo-parasite negative and positive buffaloes.

corroborates with previous reports of decreased cortisol level in *T. annulata* infected cattle (Sangwan *et al.* 2002). A significant logistic regression coefficient was observed only for cortisol (0.148±0.062) and total serum protein (-1.368 ±0.066) and TBDs infected buffaloes and may be useful as prognostic and diagnostic markers (Table 6).

Acute-phase response and cytokines

Acute-phase response is a complex systemic early defence system activated by infection and inflammation mediated by pro-inflammatory cytokines with the release of acute-phase protein (Glass *et al.* 2003). These proteins are involved in controlling inflammation against a wide range of pathogens and often the severity of infection is quantified based on their level. Acute-phase response was measured in terms of the serum SAA in haemo-parasite negative and positive buffaloes and especially in *Theileria* spp. infected buffaloes. The concentration (mg/L) of SAA was non-significantly different between group II buffaloes (18.21±2.72) and group III buffaloes (12.71±1.83). However, SAA was significantly ($P<0.01$) higher in group I buffaloes infected with *Theileria* spp. (47.07±5.66) versus other buffaloes in group II and III (Fig 2A). Our observations are in agreement with the earlier findings of increase acute phase response proteins in *T. annulata* infected water buffaloes (El-Deeb and Iacob, 2012). Several inflammatory cytokines, including TNF-alpha, play important role in disease progression. TNF family along with IL-1 and IL-6 are considered important inducers of acute phase response in the liver (Baumann and Gauldie, 1994). The TNF-alpha was highest (18.73±1.43 ng/L) in *Theileria* spp. infected buffaloes of group I followed by group II (13.08±0.87 ng/L) and least in group III buffaloes (4.86±0.76 ng/L) and these values varied significantly ($P\leq0.01$) with each other (Fig 2B). In our study, a higher concentration of TNF alpha in TBDs positive and more in *T. annulata* infected buffaloes also suggest a possible mechanism for acute phase response and subsequent elevation in the serum SAA level in the circulation. However, logistic regression analysis showed a non-significant coefficient between SAA, TNF alpha and TBDs infected buffaloes.

Therapeutic management

The animal welfare ethic is to provide treatment for ailing animals apart from research. The overall response of oxytetracycline long-acting along with antipyretic was positive in 38.31 per cent buffaloes sampled, on day two of treatment. There was an improvement in appetite and rectal temperature. The response of initial treatment with oxytetracycline long-acting and antipyretic is attributed to its known efficacy against *A. marginale* (Swift and Thomas, 1983; Sarli *et al.* 2020) and the response rate was less as they were infected with other non-responsive pathogens. The overall case-fatality rate was 5.88% in TBDs infected buffaloes with the highest (20.00%) recorded in buffaloes infected with *Theileria* spp. The deaths recorded were within 48 hours of blood sampling. The majority (80.0%) of the *Theileria* spp. positive buffaloes responded within 7 days of treatment administered module-I and IV of therapeutic management. The majority of buffaloes (95.50%) co-infected with *Theileria* spp. and *A. marginale* given a combination of module-I, II and IV responded favourably after 10 days of treatment and the case fatality rate was 4.50% observed within 7 days of treatment. Similar to our findings, observation of mortality in adult cattle and calf infected with TBDs even after specific treatment has been reported by Kohli *et al.* (2014). The possible reasons for mortality are anemia, respiratory distress, persistent inappetence, anoxia, disseminated intravenous coagulopathy and toxemia (Nazifi *et al.* 2008; Godara *et al.* 2010; Underwood *et al.* 2015; Boes and Durham, 2017). All other TBDs infected buffaloes responded favourably with respective module combination within 7 days of treatment. Imidocarb is highly effective against *A. maginale* infection has been previously reported (Ashuma *et al.* 2013). Similar to our regimen, McHardy and Simpson (1974) have reported the efficacy of Imidocarb dipropionate @ 3 mg/kg capable of rapidly curing *A. marginale* by single subcutaneous injection. Supportive therapy by multivitamins, hematinic and anti-oxidants though cannot decrease the level of parasitemia yet influence recovery by improving hematocrit values (Nayak *et al.* 2018). However, Altug *et al.* (2014) reported that vitamin B is the most efficient supportive therapy for the treatment of *T. annulata* infection in cattle. Positive response after 7 days of treatment has been reported similar to our findings (Ganga *et al.* 2010; Altug *et al.* 2014). However, milk yield could not be restored in these buffaloes even after 15 days post-treatment. This may be attributed to slow response in optimizing the compromised haemato-biochemical profile after specific treatment controlling parasitemia. Slow response in the improvement of milk production in *T. annulata* infected cattle treated with buvarvaqunone has been reported (Ayadi *et al.* 2016). Tick control was achieved in all the buffaloes after 3 consecutive application of module-V at weekly intervals. This indicates ticks were sensitive to common acaricidal drugs used by the farmers and application of the same simultaneously to the environment and shared animals.

CONCLUSION

Future efforts should focus on the diagnosis of TBDs in buffaloes by molecular and serological methods due to low parasitemia, milder signs observed and co-infections. Efforts should also focus to validate the species of haemo-parasites circulating in the riverine buffaloes.

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