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Evaluation of haemato-biochemical and oxidative indices in naturally infected concomitant tick borne intracellular diseases in dogs

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PEER REVIEW

Peer reviewer

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Comments

The authors have evaluated concomitant tick borne hemoprotozoan diseases in dogs with all it's subsequent pathologic effects that seen during the disease process.Evaluation of oxidative stress indices during concomitant intracellular tick borne diseases in dogs is an additional information to small animal practitioners.
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ABSTRACT

Objective: To explore haemato-biochemical and oxidative stress indices due to concomitant tick borne intracellular diseases in dogs presented at Referral Veterinary Polyclinic, Indian Veterinary Research Institute, Bareilly during May 2010 to May 2012.

Methods: Microscopy of Giemsa blood smear and ELISA test (SNAP 4Dx) were carried out in suspected cases to confirm haemo-parasitic infection. Blood and serum samples were analyzed for oxidative stress indices and haemato-biochemical changes. All the ailing conditions were recorded to investigate the clinical pattern of concomitant tick borne diseases. Ultrasonographic study was carried out to obtain the hepatic involvement.

Results: Examination of 3 650 dogs revealed that 2.77% dog were positive for various tick borne diseases, out of which 21.78% were with concomitant infection. Clinical symptoms were noted with overall mean clinical score of 9.95±0.30. Ultrasonographic examination revealed hepatomegaly, distension of gall bladder, and ascites. Haemato-biochemical evaluation confirmed anaemia, leucopenia, thrombocytopenia, hypoproteinemia, hypoalbuminemia, hyperglobulinemia and hyperbilirubinemia with increased serum alanine amino transferase, alkaline phosphatase and gamma-glutamyl transpeptidase in concomitant infected dogs. The lipid peroxidation level of concomitant infection was significantly higher ($P<0.05$) than healthy group whereas superoxide dismutase, glutathione-reduced and catalase activity in concomitant infected group were decreased.

Conclusions: The severity of infection was more pronounced in dogs harboring *Ehrlichia*, *Babesia* and *Hepatozoon* and the oxidative stress may have a pathophysiological role in concomitant infection in dogs.

KEYWORDS

Concomitant infection, Heamato-biochemical, Oxidative stress, Dog

1. Introduction

Worldwide importance of tick born diseases (TBDs) in dogs has been accepted due to its high morbidity and mortality. The disease gets transmitted by the brown dog tick, *Rhipicephalus sanguineus*, which acts as a vector of several agents such as *Anaplasma platys*, *Babesia canis vogeli*, *Babesia gibsoni*, *Ehrlichia canis* (*E. canis*), spotted fever group *Rickettsia* spp. and *Hpatozoone canis* (*H. canis*) [1-3]. Interactions among these various parasitic agents unquestionably affect the organisms individually and alter their effects on the host. Concomitant tick borne infections are very common in endemic areas[4,5], but clinical reports are scarce[6-9]. Clinical findings ranged from incidental hematological changes to severe life-threatening illness due to synergistic pathological effects between the etiological

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agents. These factors complicate diagnosis, treatment and can adversely influence prognosis if the practitioner fails to suspect, document and treat each concomitant infection[5]. So, the present study was carried out to obain the prevalence, haemato-biochemical and oxidative stress alteration due to the concomitant tick borne infection.

2. Materials and methods

2.1. Study area

The present study was conducted on dogs presented in Referral Veterinary Polyclinic, Indian Veterinary Research Institute, Izatnagar, and Bareilly (UP) from May 2010 to May 2012.

2.2. Clinical study

During the study period, 650 ailing dogs with the history of tick infestation, erratic fever, chronic or prolonged illness and unresponsive to routine treatment were targeted out of 3650 cases of dogs presented in the clinics. The dogs were subjected to peripheral blood and buffy coat examination for intracellular blood parasite, and serological test for detection of circulating antibody of *E. canis* and *Anaplasma phagocytophilum* by using SNAP 4D× kit (IDEXX Laboratories, USA). Dogs positive with intracellular blood borne parasite were included in the present study. Six apparently healthy dogs of different age, sex and breeds, brought for either health checkup or for vaccination were used for comparison. In the study dogs were divided into four groups, *viz.* Group 1: *Ehrlichia* and *Babesia* infected (n=12), Group 2: *Ehrlichia* and *Anaplasma* infected (n=7), Group 3: *Ehrlichia*, *Babesia* and *Hepatozoon* infected (n=3), Group 4: Healthy (n=6).

2.3. Clinical examination

Each dog was subjected to detailed clinical examination as per standard procedure[10]. Presence of symptoms/signs/involvement of different body systems and systemic states were recorded. A clinical score of each ailing dog was worked out based on 17-points scale[10].

2.4. Ultrasonographic examination

To know the hepatic involvement in concomitant TBDs, ultrasonographic study was carried out on 22 concomitant infected dogs as per the standard procedures with Scanner 200 vet (Pie Medical, Netherland) or Sonosite model 600M and a 5.0 MHz AAS transducer[11].

2.5. Collection of blood

Blood samples were collected from saphenous/cephalic vein in clean dry sterilized vials with ethylene diamine tetracetate for hematological analysis. For serum separation, 5 mL blood without anticoagulant was collected and centrifuged at 3000 r/min for 5 min and were stored in deep freeze at −20 °C for further biochemical and enzymatic estimations.

2.6. Cytological examination

Smear from blood and buffy coat were examined with standard procedure for confirmation of tick borne intracellular organism *viz. Babesia, Ehrlichia, Anaplasma* and *Hepatozoon* organism. At least 200 leukocytes in each blood smear and up to 100 oil immersion fields in each buffy coat were screened for the presence of pathogen in white blood cell.

2.7. Parameters of study

Hematological parameters *viz.* hemoglobin (Hb), packed cell volume (PCV), total erythrocyte count (TEC), total leukocyte count (TLC), differential leukocytic count, platelets count, clotting time, red blood cell indices were analyzed as per the standard techniques[12].

Total protein and albumin (biuret method), creatinine (alkaline picrate method) and total bilirubin (modified Jendrasik and Grof method) were estimated with the help of a commercial kit (Span diagnostic kit, Span Diagnostic Limited, Surat, India).

Serum enzyme profile *viz.* serum alanine aminotransferase (ALT), asparate aminotransaminase (AST/SGOT), alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT) were measured by standard diagnostic kits (Span diagnostic kit, Span Diagnostic Limited, Surat, India).

Oxidative stress indices including lipid peroxidase (LPO), catalase, superoxide dismutase (SOD) and glutathione-reduced (GSH) were analysed by calorimetrically using commercial kit (Randox reagent, Randox Laboratories Ltd.) [13-16].

2.8. Statistical analysis of data

All the data were analyzed by using ANOVA test by Statistical Package SPSS 15 (SPSS, Science, Chicago, USA). The results were expressed in mean±SE. A value of P<0.05 was considered as significant.

3. Results

3.1. Prevalence of concomitant tick borne intracellular diseases (TBICDs) in dogs

During the study period, initially 650 dogs were suspected for TBICDs and later on 101 (15.54%) dogs were confirmed

for TBICDs of parasite origin. Out of 101 positive cases, the prevalence of concomitant TBICD was 21.78% (22/101). Of which 54.55% (12/22) dogs with ehrlichiosis and babesiosis (Group 1), 31.8% (7/22) with ehrlichiosis and anaplasmosis (Group 2) and 13.6% (3/22) with ehrlichiosis, babesiosis and hepatozoonosis (Group 3) were recorded. Concomitant infections were determined based on the result of Dot ELISA (SNAP 4D×) test (Figure 1) and microscopic examination.

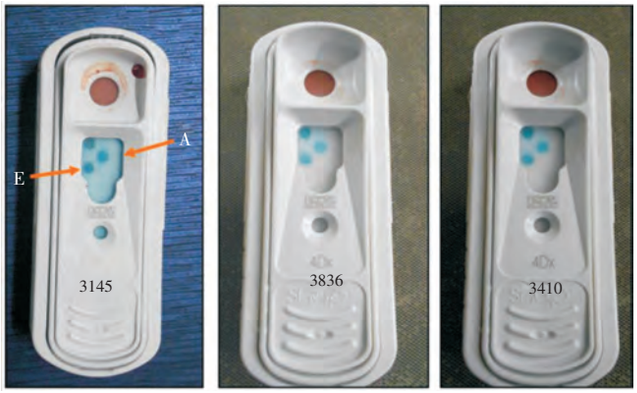

Figure 1. SNAP 4D× kit showing positive for concomitant infection of *E. canis* and *Anaplasma phagocytophilum*.
E: *E. canis*, A: *Anaplasma phagocytophilum*.

3.2. Clinical observations

Clinical manifestations of concomitant infection in dogs under the study are shown in the Table 1. Presence of ticks, pale mucous membrane and lymph node enlargement in most of the dogs (100.00%) with a lowest manifestation of abdominal distention (4.54%) were recorded. The mean clinical score in concomitant infection was 9.95±0.30 with an individual score varying from 6 to 13.

3.3. Ultrasonographic observation

Ultrasonography examination revealed 54.55% (12/22) cases with hyper echogenicity of liver, 21.73% (5/22) hepato-splenomegaly, 18.88% (4/22) splenomegaly and 4.54% (1/22) ascites in concomitant infections (Table 2 and Figure 2). Again out of 54.55% cases of hyper echogenicity, 33.33% (4/12) had gall bladder distension. In this study, it was observed that hyper echogenicity of liver was more prominent in concomitant infection.

Table 1
Pattern of clinical observation recorded in concomitant TBDs in dogs.

Clinical parameters	Mixed infection (*n*=22)	Percentage (%)
Tick	22	100.00
Lymph node enlargement	22	100.00
Pale mucous membrane	22	100.00
Staggering gait	19	86.36
Anorexia	17	77.27
Temperature	15	68.18
Vomiting	15	68.18
Diarrhoea	14	63.64
Respiratory	13	59.09
Nervous sign	13	59.09
Petechial hemorrhage	12	54.55
Muscular skeletal	10	45.45
Ocular sign	9	40.91
Malena	7	31.82
Inappetance	5	22.73
Epistaxis	2	9.09
Abdominal distension	1	4.54

Table 2
Ultrasonographic changes of liver and spleen in concomitant TBDs in dogs.

Organ		Concomitant infection (*n*=22)	Percentage (%)
Liver	Liver hyper echogenicity	12	54.55
	Gall bladder distention*	4/12	33.33
Hepatosplenomegaly		5	21.73
Splenomegaly		4	18.18
Ascites		1	4.54

*Gall bladder distention noted in 4 dogs out of 12 dogs showing hyper echogenicity of liver.

3.4. Hematological profile

The mean±SE values of hematological parameters of dogs suffering from concomitant TBICDs are shown in Table 3. There was significant (P<0.05) decrease in the Hb, TEC,

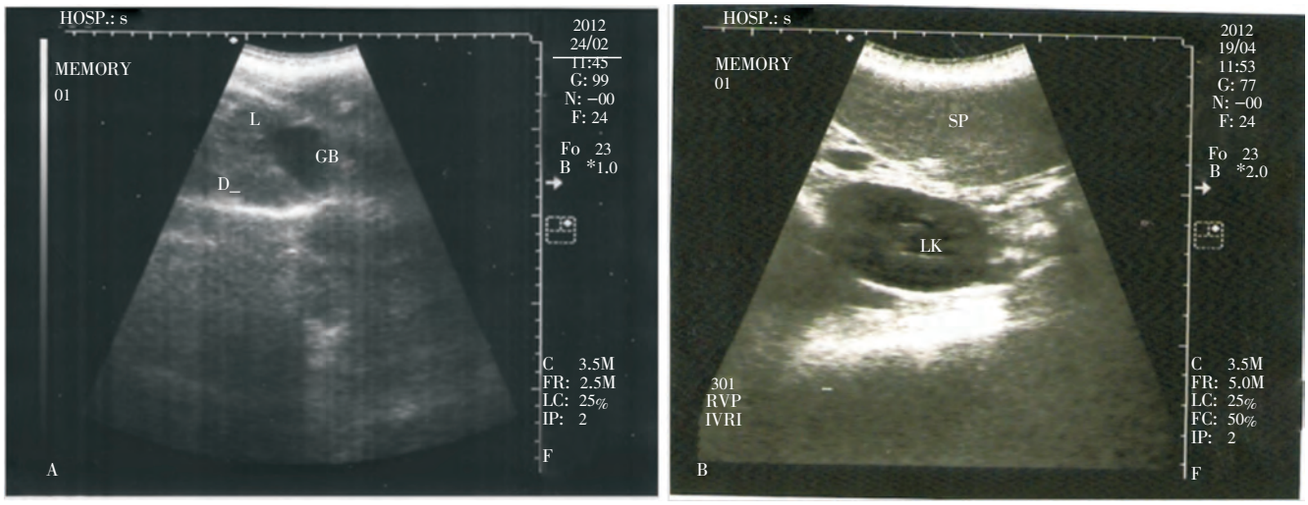

Figure 2. Distended GB with hyper echoic liver and splenomegaly.
A: hyper echoic liver, B: splenomegaly.

TLC and platelet count level in concomitant infection in comparison with healthy group. But Hb and PCV level of Group 3 were lower (P<0.05) among the other three groups. There was no significant variance of mean corpuscular volume, and mean corpuscular hemoglobin values of both infected and healthy groups but mean corpuscular hemoglobin concentration values decreased (P<0.05) in infected group in comparison with healthy group. It means the characteristic of anaemia was hypo chromic normocytic. Highest monocyte values (P<0.05) was observed in all three infected group when compared with healthy group (Group 4).

Table 3
Hematological profile of dogs with concomitant TBDs.

Parameters	Group 1 (n=12)	Group 2 (n=7)	Group 3 (n=3)	Group 4 (n=6)
Hb (g/dL)	7.71±0.59^a	7.98±0.65^a	5.34±0.34^b	11.95±0.26^c
PCV (%)	29.00±0.94^a	28.12±0.76^a	19.78±0.88^b	34.80±0.70^a
TEC ($\times10^6$μL)	3.29±0.24^a	3.12±0.33 ^{a}	3.01±0.67 ^{a}	5.10±0.06^b
TLC ($\times10^3$μL)	9.92±1.41^a	9.23±1.23^a	9.87±0.34^a	10.38±0.46^b
MCV (fL)	93.57±4.36	90.13±2.23	65.71±4.09	68.16±1.52
MCH (pg)	23.70±1.15	25.57±1.45	17.74±1.02	23.40±0.65
MCHC (%)	26.09±1.45^a	28.38±1.21^a	26.99±1.22^a	34.42±1.29^b
Platelets ($\times10^5$)	0.90±0.10^a	0.78±0.32^b	0.89±0.78^a	2.50±0.20^c
Clotting time (min)	6.93±0.47^a	7.02±0.34^a	8.12±0.56^a	3.48±0.08^b
Nutrophil (%)	71.77±2.10	73.77±2.10	71.77±2.10	76.50±1.48
Lymphocyte (%)	20.82±2.28	21.82±2.28	20.82±2.28	21.00±1.70
Monocyte (%)	5.73±0.61^a	6.65±0.61^a	5.23±0.61^a	1.00±0.40^b
Eosinophil (%)	1.41±0.26	1.43±0.26	1.47±0.26	1.00±0.00
Basophil (%)	0.24±0.11	0.22±0.11	0.21±0.11	0.00±0.00

Values are mean±SE. Values in the different column with the different superscripts are significantly different at P<0.05. MCV: mean corpuscular volume, MCH: mean corpuscular hemoglobin, MCHC: mean corpuscular hemoglobin concentration.

3.5. Serum biochemical profile

The total protein and albumin values of concomitant infected dogs were decreased (P<0.05) whereas serum globulin and creatinine levels were increased (P<0.05) (Table 4) when compared to healthy group. The values of albumin/globulin ratio of concomitant infected groups were lower (P<0.05). There was no significant difference of serum blood urea nitrogen levels but creatine levels were higher (P<0.05) in all the infected groups when compared with healthy group. Significantly higher bilirubin level (P<0.05) in mixed infected groups were recorded as compared to healthy group and Group 3 showed highest level.

Mean serum ALT, ALP and GGT activity in dogs infected with concomitant infections are shown in Table 4. The ALT activity was higher (P<0.05) in Group 3 when compared with other infected groups. Mean activity of ALP (IU/L) and GGT in infected groups were higher (P<0.05) than healthy group.

Levels of LPO, reduced GSH, activities of SOD and catalase in erythrocytes are summarized in Table 4. Erythrocytic lipid peroxides levels were higher (P<0.05) in Group 1, Group 2 and Group 3 in comparison to Group 4. Significant differences (P<0.05) in LPO levels was also observed among the concomitant infected groups with higher level in the Group 3 followed by Group 1, Group 2 and Group 4. However, SOD activities showed significantly (P<0.05) lower in Group 3 followed by Group 1, Group 2 and Group 4. In case of GSH levels of all the three infected group differed significantly (P<0.05) from healthy group with the value lowest in Group 1 followed by Group 2 and Group 3. Similar trend was observed in catalase activity which was minimum in Group 3, followed by Group 1, Group 2 and Group 4 and these values differed significantly (P<0.05) from each other.

Table 4
Serum biochemical profile and oxidative indices of dogs with concomitant TBDs.

Parameters	Group 1 (n=12)	Group 2 (n=7)	Group 3 (n=3)	Group 4 (n=6)
Protein (g/dL)	4.09±0.23^a	3.69±0.28^a	4.45±0.33^a	6.57±0.13^b
Albumin (g/dL)	1.21±0.12^a	1.28±0.12^a	1.31±0.12^a	4.56±0.07^b
Globulin (g/dL)	2.88±0.15^a	2.41±0.18^a	3.14±0.15^a	2.01±0.15^b
A:G ratio	0.42±0.08^a	0.53±0.11^a	0.74±0.18^a	2.26±0.03^b
Blood urea nitrogen (mg/dL)	35.88±2.42	33.88±2.32	38.88±2.15	34.45±8.36
Creatine (mg/dL)	1.16±0.04^a	1.19±0.04^a	1.17±0.04^a	0.62±0.15^b
Total bilirubin (mg/dL)	1.54±0.06^a	1.84±0.06^a	2.04±0.06^a	0.68±0.21^b
ALT (IU/L)	247.16±17.26^a	277.16±17.26^a	347.16±17.26^b	43.79±8.76^c
ALP (IU/L)	224.55±18.26^a	214.55±18.26^a	294.55±18.26^a	42.49±9.40^b
GGT (IU/L)	20.92±2.86^a	21.92±2.06^a	18.92±1.86^a	7.45±1.04^b
LPO (nmol MDA/mg Hb)	4.70±0.15^a	3.10±0.19^b	6.70±0.34^c	1.22±0.08^d
GSH (μmol/mg Hb)	0.33±0.02^a	0.39±0.02^a	0.53±0.02^a	2.14±0.02^b
SOD (U/mg Hb)	0.72±0.04^a	0.96±0.23^b	0.48±0.34^c	2.86±0.02^d
Catalase (U/mg Hb)	0.51±0.02^a	0.61±0.12^a	0.38±0.45^b	2.10±0.01^c

Values are mean±SE. Values in the different column with the different superscripts are significantly different at P<0.05.

4. Discussion

Incidences of TBICDs in dogs have been earlier reported from India[17-19]. Similarly, concomitant infection of *E. canis* with *H. canis* and *E. canis* with *Babesia* spp. were also earlier documented[20,21]. Tick borne infections such as ehrlichiosis, hepatozoonosis, anaplasmosis, rickettsiosis, lyme disease, babesiosis *etc.* are frequently seen not as independent, but as co-infections because the same vector is incriminated for transmission[22].

Clinical signs and symptoms help clinician to predict type of disease and help for confirmatory diagnosis. The wide variation in clinical picture may be due to many factors such as age, breed, immune competence of dogs, clinical phase of the diseases, variation in virulence between different strains *etc*. Lymphadenomegaly was observed mostly in acute condition due to accumulation of large number of white blood cells to the lymph nodes present around the infection to fight it. Pale mucous membrane and hemorrhages may be attributed to a combination of mild thrombocytopenia and vacuities[23]. Clinical signs in concomitant infections seen in the present study were similar with the result of De Tommasi *et al*[4]. In the present study, wide variation of clinical signs in concomitant infection have resulted in a syndrome, wherein each organism has potentiated the development of the other, resulting in a fulminating attack of canine ehrlichiosis and canine babesiosis and hepatozoonosis.

Ultrasonographic findings observed in various concomitant infections in the present study are in full agreement with earlier reporter[24-26]. Hepato-splenomegaly might have been due to multiplication of organism within circulating mononuclear cells and mononuclear phagocytic tissues of liver, spleen and lymph node[27]. Splenomegaly is because of reactive lymphoid hyperplasia and concurrent extramedullary hematopoiesis[28]. The sonographic changes in gall bladder with distention in the present study might be due to anorexia.

Anaemia, thrombocytopenia, monocytosis and increased clotting time were the main abnormalities in the present study similar to other reports[26]. Anaemia might be due to both intravascular and extra vascular haemolysis. The most consistent laboratory abnormality of thrombocytopenia in ehrlichiosis and babesiosis infected dogs, anaplasmosis and hepatozoonosis were in agreement with the present study[26,29,30].

Hypoproteinemia along with hypoalbuminemia, hyperglobulinemia and hyperbilirubinemia in concomitant TBDs in dogs were in agreement with previous observation and these might be due to a chronic inflammatory disease, anorexia or decreased protein intake[31]. The hyperbilirubinemia in the concomitant infected group might be due to liver damage caused by *E. canis* infection and *H. canis* multiplication and/or a hemolytic process due to *Babesia* spp. and/or ineffective erythropoiesis. Higher levels of ALT, ALP and GGT in dogs with all the infected group of the present study are in agreement with previous observations[30]. The ALT activity was higher ($P<0.05$) in Group 3 might be due to synergistic effect of babesiosis, ehrlichiosis and hepatozoonosis on hepatic function. Elevated levels of ALP may be related to the chronic disease. Furthermore, elevated alkaline phosphatase activity could result from higher osteoblastic activity or liver necrosis, or even more likely, from cholestasis caused by synergistic activity of babesiosis, ehrlichiosis and hepatozoonosis[32,33].

Free radicals have been implicated as playing an important role in tissue damage in a variety of pathological processes[34]. Overproduction of free radicals cause damage to nucleic acids, protein, lipids and other cellular components resulting in enhanced lipid peroxidation[35]. But antioxidants such as superoxide dismutase, catalase, GSH *etc*. counteract against adverse effect of free radicals. So, estimation of antioxidant enzymes activities and level of endogenous antioxidant in blood are reliable methods for assessment of oxidative stress[36]. It is clearly evident from the present study that the values of LPO in diseased groups were higher ($P<0.05$) than those of clinically healthy dogs whereas GSH, SOD and catalase were significantly decreased. There is no such report about oxidative stress indices in concomitant TBDs. Various researchers had given reports about oxidative stress in individual tick borne haemo-parasitic infection such as babesiosis, ehrlichiosis, and hepatozoonosis[20,37,38]. In the present study, increased levels of lipid peroxidation were detected in dogs infected with concomitant infection may be considered as an indication of cell injury. Higher LPO levels in Group 3 in comparison to other three groups suggested enhanced oxidative damage to erythrocytes either by synergistic effect of *Ehrlichia*, *Babesia* and *Hepatozoon*, or due to excess production of free radicals which suppress the antioxidant defense mechanism.

According to our data, erythrocytic SOD activity in concomitant infected dogs was significantly lower than the healthy group. Similar findings had been reported in bovine, ovine theileriosis, trypanosoma infection in humans, camels and schistosomiasis infection in humans[39-43]. However, Chaudhuri *et al*. and Nazifi *et al*. reported significant increases in erythrocytic superoxide dismutase activity in babesiosis in dogs and anaplasmosis in cattle respectively[37,44]. Decreased activity of SOD in concomitant infected group might be due to degradation by reactive oxygen species during the detoxifying process. SOD activity significantly ($P<0.05$) decreased in Group 3 in comparison to other infected group might be due to higher involvement of SOD activity to neutralize the overproduction of free radicals as a defense mechanism.

Similarly, GSH and CAT activity in concomitant infected group were also significantly decreased as compared to healthy group. The significant reduction in GSH and CAT activity was also reported by El-Deeb and Younis[39]. They reported a significant reduction in the levels of glutathione in *Theileria annulata* infected buffaloes compared with healthy buffaloes. It was reported in case of babesiosis in sheep and horse and trypanosomiasis in camel, thus supporting the findings of present study[42,45,46]. Decrease in CAT and GSH activity might be due to increased specific activity of these enzymes as an indirect compensatory response to increased oxidant challenge.

Based on the results of the present study, it was concluded that the severity of infection was more pronounced in ehrlichiosis associated with babesiosis and hepatozoonosis. Concomitant infections in dogs were manifested by wide range of clinical signs and symptom with overall mean clinical score of 9.95±0.30. Disturbed antioxidant mechanisms of erythrocytes, accompanied by a significant rise in lipid peroxidation of erythrocytes, implied that the oxidative stress may have a pathophysiological role in concomitant infection with ehrlichiosis, babesiosis and hepatozoonosis in dogs.

Conflict of interest statement

We declare that we have no conflict of interest.

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Comments

Background

TBDs in dogs have been an important issue due to its high morbidity and mortality. The disease gets transmitted by the brown dog tick, *Rhipicephalus sanguineus*, which acts as a vector of several agents such as *Anaplasma platys, Babesia canis vogeli, Babesia gibsoni, E. canis*, spotted fever group *Rickettsia* spp. and *H. canis*. So, the present study was carried out to obain the prevalence, haemato-biochemical and oxidative stress alteration due to the concomitant tick borne infection.

Research frontiers

Through this paper, the authors have projected the status of concomitant tick borne haemoprotozoan infection in dogs and biochemical changes takes place in their host/s thereof.

Related reports

From India, reports on epidemiological aspect of haemoprotozoan diseases are scarce. The present findings on the occurrence of co-infection of TBDs in dogs are found to be a comprehensive study, though the sample sizes were taken small. Assessment of hematological profiles enlightened the pathogenicity of etiological agent/s.

Innovations & breakthroughs

The findings of the present paper have re-validated the previous reports on the endemicity of TBICDs in dogs in India. The oxidative indices that resulted during the disease provocation is important.

Applications

Informations obtained through this piece of research findings shall throw light on the knowledge of TBICDs in dog and also possible biochemical changes that take place during the disease process.

Peer review

The authors have evaluated concomitant tick borne hamoprotozoan diseases in dogs with all it's subsequent pathologic effects that seen during the disease process. Evaluation of oxidative stress indices during concomitant intracellular TBDs in dogs is an additional information to small animal practitioners.

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