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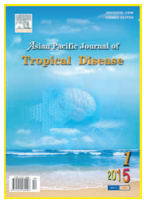

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Evaluation of haemato-biochemical and oxidative indices in naturally infected concomitant tick borne intracellular diseases in dogs

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PEER REVIEW

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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 3650 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 3 650 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 3 000 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