

COMPLEX CLINICAL PRESENTATIONS OF TICK-BORNE DISEASES IN DOGS IN SRI LANKA

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Vector-borne diseases (VBD) which were mostly seen in the tropics, in locations with limited resources and surveillance (Gubler, 2009), have now reached high or medium occurrence in all continents, except Australia [CVBD (Companion Vector Borne Diseases) website].

A global re-emergence of VBDs, with a high frequency of transmission has been recorded due to multiple environmental and social factors, such as, increased human and animal bonding, mobility across borders, animal trade, global warming, resistance to pesticides, changes in agricultural practices, deforestation and reforestation and loss of biodiversity (WHO, Vector-borne diseases, February 2016). Pets are not considered a direct source of infection to people, but direct transmission from an infected animal can occur via accidental inoculation of contaminated blood or tissue.

This re-emergence of VBDs in humans and animals, has presented a 'One Health' issue highlighting the requirement for interaction between veterinary and human medical sectors for the benefit of animal and human health and the global environment (Braks *et al.*, 2007; Day, 2011; Gubler, 2009). There are many challenges in achieving the above goals: (1) promoting detection of these diseases in animals and humans, (2) undertaking comparative and translational research (3) developing robust diagnostic tests and surveillance systems (4) studying the connection between pet animals and wildlife reservoirs (6) promoting awareness of pet owners on the importance of regular ectoparasite control, and (7) developing strategies to minimize the risk involved in pet animal mobility with regard to zoonotic diseases. This paper intends to highlight the complex clinical presentations associated with tick-borne diseases (TBD) based on scientific information and personal experiences on such diseases in dogs.

The organisms responsible for TBDs in South-East Asia and the Indian Subcontinent are: *Anaplasma* (phagocytophilum and platys), *Babesia* (vogeli, gibsoni and canis), *Ehrlichia* (canis, chaffeensis), *Hepatozoon canis*, and *Rickettsia* (felis, japonica, conorii, typhi & orientia tsutsugamushi) (CVBD). Vector-borne hemoparasites in dogs in Sri Lanka are *Anaplasma platys*, *Babesia gibsoni*, *Babesia canis*, *Ehrlichia canis* and *Hepatozoon canis*. Presence of Rickettsial parasites (*R. conorii*, *R. typhi*, *Orientia tsutsugamushi*) also had been detected serologically (Nanayakkara *et al.*, 2013).

Furthermore, intermittent, persistent infections or co-infections caused by *Babesia*, *Anaplasma*, *Ehrlichia*, *Hepatozoon*, *Rickettsia* and *Mycoplasma haemocanis* (*Haemobartonella*) are commonly encountered by practitioners. Co-infections of *Ehrlichia*, *Babesia* and *Hepatozoon* can occur particularly in endemic areas since the same tick species can transmit several pathogens. Such infections probably explain the variations in clinical presentation, pathogenicity and response to therapy (Shaw *et al.*, 2001).

Babesia organisms are broadly divided by their size in to small and large. The large babesia which were previously known as *Babesia canis* are reclassified as *B. canis*, *B. vogeli*, *B. rossi* and *Babesia sp* (Coco). The small Babesia previously known as *Babesia gibsoni* are reclassified as *B. gibsoni* and *B. conradae*; *Babesia microti* as *Theileria annae*; and *Babesia equi* as *Theileria equi* (Irwin, 2009). Babesiosis in cats is less common and is manifested as an afebrile, chronic, low-grade disease, with anorexia, lethargy, anemia, depression, and occasionally icterus. Feline Babesiosis is caused by *Babesia felis*, *Babesia cati* (India), *B. canis* (Europe), *B. canis presentii* (Israel), *B. vogeli* (Thailand).

Ehrlichia and *Anaplasma* spp. are closely related with four distinct clades: *Anaplasma*, *Ehrlichia*, *Wolbachia* and *Neorickettsia* genogroups (Dumler *et al.*, 2001; Ferla *et al.*, 2013).

Ehrlichia gene group- *Ehrlichia canis*, *E. chaffeensis* and *E. ewingii*

Anaplasma gene groups- *E. Phagocytophilum* (formerly known as *E. equi*) and *E. platys* (synonym *Anaplasma platys*) *Ehrlichia Phagocytophilum* (*Anaplasma*) infection in neutrophils has become increasingly significant in human, canine and feline populations causing granulocytic ehrlichiosis.

Neorickettsia gene groups - *E. sennetui*, *E. risticii* and *Neorickettsia helminthoeca*

Ehrlichia chaffeensis cause human monocytic ehrlichiosis, *Ehrlichia ewingii* causes human granulocytic ehrlichiosis, and *Anaplasma phagocytophilum* cause human anaplasmosis (formerly known as human granulocytic ehrlichiosis, or HGE).

Transmission

The transmission of TBD pathogens can occur in different ways. In biological transmission, the pathogen

undergoes some biological development in the body of the arthropod vector in order to complete its life cycle. Mechanical transmission is a simple transfer of the organism on contaminated mouth parts or other body parts, without multiplication or developmental change of the pathogen in the arthropod (Gubler, 2009). In addition to biological and mechanical transmission, direct transmission of TBD can occur through dog bites, contaminated equipment, transplacentally and via blood transfusions. Although *Ehrlichia canis* and *Hepatozoon canis* are transmitted by ticks, *E. canis* is transmitted through the saliva of ticks during a blood meal, while *H. canis* is transmitted by ingestion of a tick containing mature sporozoites (Baneth *et al.*, 2007; Harrus and Waner, 2011).

Hemotrophic mycoplasmas (hemoplasmas), formerly classified as *Haemobartonella* and *Eperythrozoon* species, with a single circular chromosome are transmitted by *Rhipicephalus sanguineus* and *Dermacentor reticulatus* ticks, from a blood transfusion and also directly through saliva during a fight (Willi *et al.*, 2006; Nascimento *et al.*, 2012). They adhere to the surface of the erythrocytes and cause extravascular hemolysis. *Mycoplasma hemofelis*, *M. hemocanis* and *Candidatus* are closely related (Sykes and Tasker 2016; Braga *et al.*, 2012). Hemoplasmas in dogs are *M. haemocanis*, *Candidatus M. haematoparvum* (Nascimento *et al.*, 2012). *Mycoplasma haemofelis*, *Candidatus Mycoplasma haemominutum*, *Candidatus Mycoplasma turicensis* cause feline infectious anemia in the presence of retrovirus-induced immunosuppression or interaction with blood progenitor cells.

Phagolysosome

Extrinsic incubation period (time required to develop inside the arthropod and to progress to the infective stage) is generally 7 to 14 days (Gubler, 2009). Mitochondria are important for the division of *Babesia* by binary fission (10 h *in vitro*) which depends on nucleic acid synthesis and energy metabolism via aerobic and anaerobic glycolysis, converting glucose to lactate (Rajapakshage *et al.*, 2012). The mitochondrial DNA (mtDNA) consists of three functional genes essential for energy metabolism.

cytochrome c oxidase subunit I (COXI) - electron transfer in respiratory chain

cytochrome c oxidase subunit III (COXIII) - assembly and stabilization of the entire cytochrome complex

cytochrome b (CYTb) - subunit of COXIII - electron transfer

Ehrlichia organisms survive and multiply in the infected cell by their ability to inhibit the fusion of the phagosome with lysosome (Park and Rikihisa, 1991; Wells and Rikihisa, 1988).

Immunity

The cell-mediated immunity (CMI), the primary but not exclusive, is critical for the outcome of an infection (Waner *et al.*, 2001). Macrophages play a leading role in CMI by phagocytosing and killing of organisms by O₂-

dependent and O₂-independent mechanisms, and in initiating an acute phase response by releasing cytokine leading to a humoral immune response.

Cytokines contribute to both host pathology and host immunity (Hemmer *et al.*, 2000). The T-cell-induced immunity and proinflammatory cytokine, Interferon gamma (IFN γ) secretion are predominant mechanisms for recovery from and immunity to ehrlichial infections (Waner *et al.*, 2001). The cytokine IFN γ released from Th1 cells, macrophages and natural killer (NK) cells, enhance Tumor necrosis factor alpha (TNF α) expression which up-regulate the expression of adhesion molecules in endothelia, promoting sequestration of organisms to endothelia (Hemmer *et al.*, 2000; Irwin, 2009; Tajima and Rikihisa, 2005). Furthermore, the recruit of leukocytes by TNF α lead to changes in vascular epithelium resulting in increased vascular permeability, oedema and tissue injury. Therefore, TNF α production is detrimental to the host as it enhances inflammation, leading to abnormal perfusion, tissue hypoxia, sepsis and systemic inflammatory response (SIRS). Increased expression of cytokines, IL-10 and IL-4 is important for resolution of parasitemia (Hemmer *et al.*, 2000).

The immunologic responses among monocytophilic *Ehrlichiae* of the family Anaplasmataceae (*E. canis*, *E. chaffeensis*, *A. platys* and *A. phagocytophilum*) are similar (Brouqui and Dumler, 2002). Polyclonal hypergammaglobulinemia comprising IgM and IgA appear in 2-7 days, and shift to IgG by two weeks post-infection (Iqbal *et al.*, 1994; Weisiger *et al.*, 1975). A titre of =1:160 in 2-4 weeks can rise to 1:2560 - 1:20,480 in >4 weeks. Declining titres may indicate recovery while very high gammaglobulinemia can pose a risk of hyperviscosity causing nervous signs. The CD4:CD8 ratio is a deciding factor for pathogen elimination or survival. The cytokines from Th1 cells (IFN γ & TNF α) result in elevation in IgG₂, which is responsible for pathogen elimination (Nair *et al.*, 2016). An infection causes immunodysregulation reducing lymphocyte blastogenesis and decreasing CD4:CD8 ratio yielding low Th1-cytokines which will result in elevation in IgG₁, which is poor in pathogen specific, and therefore cause severe infection. Quantitative ELISA has shown a predominant elevation in IgG₂ in *E. canis* infection when compared with *E. chaffeensis*, *A. platys* and *A. phagocytophilum* infections. A dog with clinical signs and hematological parameters suggestive of Chronic Monocytic Ehrlichiosis (CME) with a single serum IgG IFA titer of 1:256 can be defined as a “definitive” case, while a “probable” case would be one with clinical signs and symptoms and a single IgG IFA titer of 1:64-1:128 (Waner *et al.*, 2001). However, hypogammaglobulinemia has been seen in pancytopenic dogs (Waner *et al.*, 2001). Marked pancytopenia due to bone marrow hypoplasia is a hallmark of chronic severe Ehrlichiosis.

Cross-reactions between apicomplexan parasites and operator subjectivity may limit the benefits of serology. Cross reactivity between ehrlichiosis, anaplasmosis, or

neorickettsiosis infections can be distinguished by western immunoblotting (Harrus and Waner, 2011). There is no serological cross reactivity between *E. canis* and *Anaplasma platys*, and little cross reactivity, if any, with *Rickettsia rickettsii*, of Rocky Mountain Spotted Fever (Harrus and Waner, 2011).

Persistent titres may occur in persistent infections, reinfection, treatment failure, and an abnormal immune response. It has been speculated that dogs infected with vector-transmitted pathogens may develop persistent infection within systemic circulation and therefore may be more prone to develop autoimmune anti-nuclear antibodies (ANA) (Smith *et al.*, 2004). It has been suggested that in persistent VBD infections, prolonged antigenic stimulation due to ineffective apoptosis or defective removal of cell debris by neutrophils and macrophages might lead to production of perinuclear antineutrophil cytoplasmic autoantibodies (pANCA) (Karagianni *et al.*, 2012).

Detection of parasites in blood and tissues

Detection of piroplasms can be done morphologically, serologically or using molecular techniques. Cell culture isolation is expensive and time consuming. Serology may not differentiate acute from chronic phases, and testing need to be done in experienced diagnostic labs with stringent quality control measures. Cytological examination of thin blood films by light microscopy lacks both sensitivity and specificity and depends on the degree of parasitaemia. It might be difficult to detect *Babesia* through light microscopy when hemolytic anaemia or thrombocytopenia is present. However, the probability of detection of *Babesia* is high in smears made from capillary beds (ear tip, toe nail) or from cells beneath the buffy coat. Detection in carrier dogs can be unrewarding due to very low, often intermittent parasitaemias (Irwin, 2009).

The morulae of *Ehrlichia* and *Anaplasma* can be detected either in mononuclear cells, neutrophils or platelets, depending on the organism. The detection of morulae could be optimized by examining buffy coat smears (1000 oil immersion fields) (Harrus and Waner, 2011). Precautions must be taken not to confuse ehrlichial inclusions with platelets, lymphocytic azurophilic granules, and phagocytosed nuclear material when examining blood smears. Cases may be erroneously diagnosed as lymphocytic leukemia as a large proportion of lymphocytes can show prominent azurophilic granules, cleaved or indented cell nuclei with mature nuclear chromatin, consistent with large granular lymphocytes (Heeb *et al.*, 2003).

Although the detection limit of light microscopy is approximately 0.001% parasitaemia for *Babesia* (Böse *et al.*, 1995) and only 4% of blood smears for *E. canis* (Woody and Hoskins, 1991), Polymerase Chain Reaction (PCR) has a high sensitivity and specificity in parasite detection when there are approximately 50 organisms/ml (Birkenheuer *et al.*, 2003) and 9 parasites/ μ l (Matsuu *et al.*, 2005). However, fluctuating parasitemia can limit parasite detection by PCR testing. A one-step multiplex

PCR has been developed for simultaneous detection of *Ehrlichia canis*, *Babesia* spp and *Hepatozoon canis*, from blood samples in a single reaction (Kledmanee *et al.*, 2009). The primers used are specific to *E. canis* VirB9, *Babesia* spp 16S rRNA and *H. canis* 16S rRNA genes. The sensitivity in detecting *H. canis* in buffy coat, blood and bone marrow is high with PCR compared with cytology using the same tissue (Otranto *et al.*, 2011).

General Clinical findings

Critical determinants of infection severity are virulence of the organism, age, gender and the immune status of the patient. The records of the Veterinary Teaching Hospital (VTH) reveal that 34% of the dogs with TBD were less than one year old, and 52% of them were less than 6 months old. Males were at higher risk of infection with M:F ratio of 1.5. Approximately 28% of males was critically anemic (HCT <10%), compared with 14% females.

Rottweilers, Labradors, Boxers and cross bred dogs of any age were more susceptible for Babesiosis and Ehrlichiosis than other breeds. However, mostly young adults of German shepherds, Dobermann, and Pomeranians showed higher susceptibility. It was assumed that the cross breeds were at a higher risk probably because the probability of getting infected is high as they were mostly free ranging and also more exposed to dog bites during fights.

The degree of parasitemia depends on the immune status of susceptible dogs. When infected with the KR-1 strain of *B. microti*, the SCID (Severe Combined Immunodeficiency) mice and TCR knockouts (T cell receptor-beta-deficient mice) had shown to sustain severe parasitemia; IFN- γ deficient mice had developed a less severe parasitemia (Clawson *et al.*, 2002). In contrast, the levels of parasitemia in JHD-null mice which lack B-lymphocytes and antibodies were indistinguishable from the wild-type animals (Clawson *et al.*, 2002). These data indicate that cellular immunity is critical for the clearance of *B. microti*.

Clinical signs and hematological changes, such as, hyperthermia, severe/critical anemia, thrombocytopenia, hypoalbuminemia, lymphadenopathy, hepatomegaly, splenomegaly and evidence of internal bleeding are not specific to a single tick-borne pathogen. The overall clinical and haematological findings propose a multi-system disease complex, and therefore, the findings should be carefully interpreted to avoid incorrect diagnosis and inappropriate treatment. Common clinical signs seen in TBDs are:

- Hyperthermia 104-106 °F (40-41°C), Sometimes hypothermia in Babesiosis (98.7°F)
- Anemia severe/critical, Evan's syndrome (IMHA/IMTP)
- Leukocytosis/Leucopenia
- Thrombocytopenia with or without clinical bleeding such as, epistaxis, hematuria
- Hypoalbuminemia

- Tachycardia (up to 80/min), elevated CRT
 - Tachypnoea (up to 80/min), dyspnoea, pulmonary oedema
 - Lymphadenopathy, splenomegaly, hepatomegaly, liver enzyme, jaundice, bilirubinuria
- Occasional signs seen in Ehrlichiosis are;
- Neurologic (ataxia, seizures, paraparesis/tetraparesis [upper/lower motor-neuron deficits], stupor, vestibular disease)
 - Polyarthrititis (stiffness, swollen joints, reluctance to move [*E. ewingii* and *A. Phagocytophilum*])
 - Ocular lesions (chorioretinitis, retinal detachment, uveitis)
 - Vomiting, diarrhea

Systemic Findings

The vector-borne parasites cause severe damage to various organs in the host. The highest pathological grading have been seen in *E. canis* infected dogs followed by *E. chaffeensis*, *A. platys* and *A. phagocytophilum*, respectively (Nair *et al.*, 2016). Chronic ehrlichiosis is associated with irreversible bone marrow destruction (Skotarczak, 2003). The infection status of *E. canis* and *E. chaffeensis* can be detected better with PCR testing of spleen and lymphnodes compared to *A. platys* and *A. phagocytophilum* (Nair *et al.*, 2016). Detection of Ehrlichia DNA in tissues may not necessarily correlate with the presence of viable organisms, as Ehrlichial DNA can be found in blood or haemolymphatic tissues in dogs in which the infections are at sub-clinical level or has contributed to other disease conditions and masked by the presenting condition (Gal *et al.*, 2008). It has been shown experimentally that Ehrlichial DNA could not be detected from blood or spleen after 9 and 60 days of treatment with doxycycline, respectively (Harrus *et al.*, 2004). Therefore, it can be presumed that DNA from dead *E. canis* organisms gets probably cleared up in dogs and does not persist in the tissues for a long time after the elimination of infection by successful treatment or by an effective host immune response.

Anemia and Fever

Anemia caused by hemolysis, internal hemorrhage, or poor erythropoiesis could be critical leading to hypoxemia. The hemolysis could be intravascular and/or extravascular and either immune-mediated (IMHA) or non-immune mediated. Both intra and extravascular hemolysis lead to fever, icterus with elevated bilirubin. Ghost cells, autoagglutination and hemoglobinemia (cell-free plasma hemoglobin indicated by an elevated mean corpuscular hemolysis, MCHC), and hemoglobinuria are evidences of immune-mediated intravascular hemolysis. Erythrophagocytosis and spherocytes, indicating complete and partial phagocytosis, respectively, are evidence of immune-mediated extravascular hemolysis where hemolysis occurs in the mononuclear phagocytic system (MPS). Hemolysis due to non-immune mediation occurs as a result of oxidative damage, intensive lipid peroxidation due to generation of reactive oxygen species

(ROS) molecules in parasitized erythrocytes, increased membrane permeability and decreased membrane potential. Malondialdehyde (MDA), an end product of ROS excreted in the urine, blood, and body fluids indicates oxidative stress and non-immune mediated hemolysis in *B. gibsoni*, *B. canis* and in experimental mixed infection of *Ehrlichia canis* and *B. gibsoni* (DVM360.com). Elevated MDA is a prognostic marker of disease severity and outcome.

In most occasions, a marked drop in HCT could be seen before parasitemia is detected in peripheral blood. This happens when erythrocyte destruction is greater than the degree of parasitemia indicating lysis of both parasitized and non-parasitized erythrocytes. The compensatory response to anemia by the bone marrow is evident by polychromasia indicating the presence of reticulocytes. The bone marrow response is classified as non-regenerative, or decompensatory, when nucleated erythrocytes (NRBC) are evident in peripheral blood, indicating the need to elevate the RBC volume by stimulating erythropoiesis or transfusing erythrocytes in order to alleviate hypoxemia. However, blood transfusions may not be possible in IMHA due to the presence of anti-erythrocytic antibodies which would lyse the transfused cells. Of the rickettsial organisms, *E. canis* cause the most significant drop in the HCT when compared with *E. chaffeensis*, *A. phagocytophilum* and *A. platys* (Nair *et al.*, 2016). Samples with autoagglutination may record an elevated red cell distribution width (RDW).

The immune driven hypoproliferative anemia (Anemia of Inflammatory Disease, AID) could be another finding in Babesiosis and Ehrlichiosis. In AID, the availability of iron get reduced as a result of cytokine activation which inhibit duodenal Fe absorption, increase Fe uptake and retention in macrophages, reducing erythropoietin production in the kidneys, and directly inhibiting erythroid progenitor cells (Weiss and Goodnough, 2005). Bone marrow erythropoiesis can get impaired due to cytokine-mediated apoptosis, down-regulation of erythropoietin receptor expression on erythroid progenitor cells, poor expression of other pro-haematopoietic factors, and low Fe availability for hemoglobin synthesis (Weiss and Goodnough, 2005). Hypoferremia despite adequate Fe stores is a host response to limit microbial access to Fe. This inhibition of pathogens due to limited access of iron, has been demonstrated in *Ehrlichia* (Park and Rikihisa, 1991).

When comparing the pathogens of the family Anaplasmataceae, pronounced anemia and reduction in PCV was seen in *E. canis* infections, and severe thrombocytopenia was manifested with the platelet-trophic *A. platys* (Nair *et al.*, 2016).

Vasculopathy, Hemoconcentration and Hypercoagulability

Following intravascular hemolysis, the decompartmentalized hemoglobin or cell-free plasma oxyhemoglobin causes irreversible oxidation of endothelial NO resulting in vasomotor instability and

vasculopathy (Minnecci *et al.*, 2005; Palmer *et al.*, 1988; Rother *et al.*, 2005). The NO regulates homeostatic vascular functions such as vasodilation, inhibition of platelet activation and thrombosis, inhibition of endothelial adhesion molecule and endothelin expression, and modulation of intimal and smooth muscle proliferation (Furchgott and Zawadzki 1980).

High mortality may occur as a result of loss of intravascular fluid due to increased capillary permeability, causing the extravascular protein-rich fluid compartment to raise leading to hemoconcentration and hyperviscosity, known as “red biliary”. This state will be clinically evident either as an absolute elevation of HCT from severe or critical anemia to over 30% or a normal HCT that is inappropriate for the degree of hemolysis (relative elevation of HCT) (Pardini, 2000).

Haemoglobin-mediated oxidative damage of endothelia during intravascular haemolysis is generally prevented by Hemopexin in plasma (Kuleš *et al.*, 2014). Endothelial damage can also occur by reactive oxygen intermediates (ROS), activation of the complement cascade and cytokine activation on endothelia, as a result of autoagglutination, circulatory stasis and sequestration of parasitized cells. Endothelial phospholipase damage activates the coagulation cascade leading to the hypercoagulable state of disseminated intravascular coagulation (DIC) and formation of micro-thrombi. Thromboelastography (TEG) and thromboelastometry (ROTEM) can detect hypercoagulable states (Wiinberg *et al.*, 2005). The TEG gives a graphical representation of clot formation and lysis, evaluating precoagulation, coagulation, and fibrinolysis.

The micro thrombi formed from DIC can lead to venous thrombosis, obstruction of vessels and tissue ischemia, particularly in the brain and lungs, thus producing clinical signs associated with pulmonary thromboembolism (PTE) and nervous system. The coagulation panel will reveal thrombocytopenia and hyperfibrinogenemia, increased circulating D-dimers, shortened PT or aPTT (Song *et al.*, 2016). The consumption coagulopathy resulting from DIC will exacerbate hemorrhage caused by direct vasculopathy and thrombocytopenia, thereby increasing the risk of mortality due to hemorrhages. Plasma may reveal hypofibrinogenemia, elevated APTT, PT, FDP, D-dimer, and buccal mucosal bleeding time. The hemorrhaging will be clinically evident as epistaxis, hematuria, petechia, and melena as single or multiple manifestations. The survival for dogs with aortic thrombosis (ATh) or aortic thromboembolism (ATE) would be between 50% and 60%, and those with chronic clinical signs have a better prognosis than those acutely or severely affected (Williams *et al.*, 2016). The prevalence of PTE in dogs is underestimated in necropsies, since thrombi in dogs lyse more rapidly than in humans (within 3 hours of death) due to greater plasminogen activator activity, greater platelet lytic activity, and secretion of plasminogen activator by the pulmonary endothelium (Goggs *et al.*, 2009).

Thrombocytopenia

Severe thrombocytopenia is a consistent finding in the acute phase of Babesiosis. Thrombocytopenia, either constant or cyclic, is an almost consistent finding in Rickettsial infections, but it cannot distinguish Ehrlichial/anaplasma infections (Nair *et al.*, 2016). Thrombocytopenia can be critical or severe, with or without clinical bleeding. Significant persistent thrombocytopenia is observed with platelet-trophic *A. platys*, and also *E. canis* and *A. phagocytophilum* infections even though cell tropisms for the latter two pathogens are monocytes and granulocytes, respectively (Nair *et al.*, 2016). Thrombocytopenia from *A. platys* infection is a result of pathogen antigens, whereas thrombocytopenia in Ehrlichiosis is due to anti-platelet antibodies, but not due to anti-ehrlichial antibodies (Nair *et al.*, 2016), because *E. canis* alters the immune system to overproduce natural anti-platelet antibodies with high affinity (Harrus *et al.*, 1998; Harrus *et al.*, 1996). Decreasing platelet counts correlate well with increasing anti-thrombocyte IFA titres and PCR positivity for *E. canis* DNA 16S rRNA, highlighting the importance of evaluating the platelet counts in suspected dogs (Harrus and Waner, 2011; Harrus *et al.*, 1998; Harrus *et al.*, 1996; Köster *et al.*, 2015). It is important to rule out platelet agglutination in blood smears when evaluating the actual platelet count. Immune mediated thrombocytopenia (IMTP) occurring together with immune mediated hemolytic anemia (IMHA) is identified as the Evans Syndrome.

Evidence of bleeding without severe thrombocytopenia could be due to thrombocytopathia causing reduced platelet aggregation, reduced platelet adhesiveness, Interference in PF3 release and platelet migration inhibition causing reduced pseudopod formation.

Hypoxemia and Hypoperfusion

The pathophysiological sequelae induced by PTE result in hypoxemia, hyperventilation, and dyspnea. The arterial hypoxemia secondary to multiple abnormal ventilation: perfusion (V:Q) ratios in the affected lungs, can get complicated from interstitial and alveolar edema (congestive atelectasis) causing diffusion impairment, airway closure and alveolar collapse. An occlusion of >60% of the pulmonary vasculature will increase pulmonary vascular resistance (PVR) which will reduce the pulmonary arterial flow (Ebert *et al.*, 1967). Reflex vasoconstriction secondary to alveolar hypoxia may also contribute to elevated PVR leading to pulmonary arterial hypertension (PAH) and increased right ventricular (RV) afterload, RV dilatation and dysfunction (Goggs *et al.*, 2009). The reduction in left ventricular filling as a consequence of RV dilatation will decrease cardiac output leading to signs of forward failure (hypotension, cardiogenic shock). If the patient survives an acute crisis the residual pulmonary hypertension will lead to a long term backward failure resulting in hepatomegaly, ascites, and pleural effusion.

The increased vascular permeability due to vasculopathy will cause tissue oedema and O₂ extraction deficit leading to severe hypoxemia and hypovolemia. These mechanisms finally lead to severe hypoperfusion, clinically evident as elevated capillary refilling time (CRT). The resulting hypoperfusion will demand a change in the distribution of the cardiac output (Q) in order to maintain perfusion to vital organs and tissues, namely heart, lungs, brain, diaphragm, and intercostal muscles (Adachi *et al.*, 1976). Such changes in the presence of restricted coronary blood flow and coronary sinus PO₂ could be achieved by increasing myocardial O₂ consumption and efficiency, and the cardiac work. The hemodynamic and metabolic changes in such patients would be bradycardia, hypotension, reduced cardiac index [CI = LV Q/ BSA (mL/min/m²)], reduced shock index (i.Shock =HR/SBP), mild changes in pulmonary capillary pressure (PCP) and central venous pressure (CVP), reduced mixed venous saturation (SvO₂, percent O₂ bound to HB in right atrium), reduced venous O₂ pressure (PvO₂), reduced O₂ transport, while increasing O₂ consumption (VO₂), O₂ extraction (TEO₂), and serum lactate. The prognosis of such patients would be grave due to generalized edema and pulmonary edema (greater mortality), ultimately leading to cardiac failure and multiple organ dysfunction. Such patients may show evidence of hepatic dysfunction (hypoalbuminemia, markedly elevated ALT, elevated bilirubin, icterus and bilirubinuria), impaired gas exchange & respiratory dysfunction (dyspnea, tachypnoea up to 80/m), pulmonary edema, acute respiratory distress syndrome (ARDS), and renal azotemia which will drastically increase the risk of mortality (Harison *et al.*, 2012). The systemic vasoconstriction resulting from vasculopathy and hypovolemia will further impair renal function leading to renal failure.

Clinical Biomarkers of tick-borne diseases

Clinical biomarkers signify multisystemic pathologies (Diniz *et al.*, 2008; Köster *et al.*, 2015; Koutinas *et al.*, 2012).

Blood Profile- IMHA. Hemolytic anemia, Thrombocytopenia, Hemoconcentration
Coagulation profile- Thrombocytopenia, consumption coagulopathy (APTT, PT, D-dimer)
Hemochemical abnormalities - metabolic acidosis & respiratory alkalosis, Hyperlactatemia,

Hyperbilirubinemia of >170 µmol/L, elevated ALT

Cardiac- Elevated cardiac troponin I (cTnI) concentrations. The ECG abnormalities were not associated with disease severity, outcome, or plasma cTnI concentrations, other than the ventricular premature complexes associated with high cTnI.

Respiratory -Pulmonary edema.

For acute and chronic diseases of Ehrlichia and anaplasma, the carriers can be categorized according to results of multiple tests, as shown in Table below. Cross-reactions are common in serological tests such as IFA, dot-ELISA ("Immunocomb"). Nested PCR (nPCR) by 16S rRNA amplification, detect DNA earlier than serology, and cross-reactions are uncommon. A p30-based nested PCR assay has been developed for detection of *E. canis* in both dog and ticks (Stich *et al.*, 2002). The coombs' test cannot diagnose IMHA because it lacks sensitivity.

Most dogs recover from clinical signs with treatment but no single drug had been successful in completely eliminating the organisms, and thus requiring adjunctive treatment, including O₂ therapy, immunosuppressants, antithrombotics, antimicrobials and fluid. Antibesiacidal drugs act by many different mechanisms. Atovaquone (ubiquinone analog) block mitochondrial electron transfer leading to reduced energy production for parasites. Berenil (Diminazene aceturate) interferes in aerobic glycolysis (reduce energy production) and multiplication (interact with DNA minor groove). The Berenil resistant genes (CYTb) can transfer during pathogen multiplication, and the proliferation potential and the degree of parasitemia is low in Berenil resistant *Babesia* strains. Berenil toxicity can occur from overdosing, repeated dosing, and even with recommended doses on *Babesia* negative animals. The anticholinergic anthelmintic, Imidocarb (carbanilide) can be used in most tick-borne diseases. Drug combinations have shown to be either effective or suppress parasitemia below the limit of detection, thereby reducing the transmission of protozoa through vectors.

Ehrlichia organisms survive and multiply in the infected phagocyte by their ability to inhibit the fusion of the phagosome lysosome (Park and Rikihisa, 1991; Wells and Rikihisa, 1988). Treatment of Ehrlichiosis with doxycycline, an antibiotic which inhibits prokaryote protein synthesis, restores phagosome lysosome fusion (Wells and Rikihisa, 1988).

Table 1. Interpretation of laboratory findings in Ehrlichiosis

Serology	nPCR	WBC	Interpretation
Positive	Positive	Leukocytosis	Acute disease (Morulae)
Negative	Positive	Leukocytosis	Acute disease
Positive	Negative	Pancytopenic	Chronic disease
Positive	Negative		Persistent titres (Carriers/ Treated)

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