

## LATEST CLINICAL MANIFESTATIONS OF CANINE EHRLICHIOSIS: "THE PUP KILLER" - A PRELIMINARY COMMUNICATION

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Canine ehrlichiosis is a tick borne rickettsial infection that affects dogs worldwide. The objective of this study was to explore the involvement of *Ehrlichia canis* in the fatal acute respiratory distress observed in 23 pups; mainly purebred and mixed German shepherds, and Labrador retrievers, aged less than 6 months. The pups were presented with acute and severe dyspnoea, inappetance, inactivity, depression, wheezing, normal to weak pulse, tachycardia, tachypnoea and hypoxemia. Some had 2/6 to 3/6 grade right or bilateral systolic apical cardiac murmurs, splenomegaly, lymphadenomegaly and mild to moderate ascites. Hematological and biochemical analysis indicated regenerative anemia, thrombocytopenia, leukocytosis and hypoalbuminemia with blood smear positive for *Ehrlichia canis*. The coagulation profile of prothrombin time (PT), activated partial thromboplastin time (APTT) and fibrinogen levels were elevated. Thoracic radiography indicated interstitial pulmonary infiltrates, right sided cardiomegaly, and both pulmonary arterial and venous congestion. Echocardiography proved the pups had pulmonary arterial hypertension, and right-sided congestive heart failure (RHF). It was suspected that, PH and RHF observed were caused by cor pulmonale secondary to pulmonary pathology associated with canine ehrlichiosis. The treatment protocol comprised of anti-rickettsial agent, phosphodiesterase-5 inhibitor, bronchodilators, diuretics, antioxidants, vitamin B and amino acid supplements, and in some, plasma transfusion and vitamin K supplementation. The mortality rate was high with 15 dying from the total number.

Postmortem of dead pups revealed, pulmonary interstitial infiltrates of lymphocytes and macrophages, pulmonary hemorrhages, right sided cardiomegaly. The unique findings of this study were; the association of acute canine ehrlichiosis in pups with PH and RHF, utilization of wheezing and dyspnoea as a screening criterion to identify acute canine ehrlichiosis in pups and association of acute liver impairment mediated dysfunction of secondary hemostasis and the pulmonary hemorrhages observed in pups with canine ehrlichiosis.

**Key words:** *Ehrlichia canis*, pulmonary, hypertension, cardiac

Canine ehrlichiosis is a tick borne rickettsial infection in dogs that has a worldwide distribution. The infection is caused by *Ehrlichia canis* (*E. canis*) and the vector mainly responsible for the transmission of this organism is the brown dog tick, *Rhipicephalus sanguineus* (Groves, et al. 1975). The infective stages of *E. canis* or 'morulae' are often found in monocytes, hence, the disease is commonly referred to as canine monocytotropic ehrlichiosis. Lymphocytes are another type of agranulocytes where *E. canis* morulae are found (Neer et al., 2002; Mylonakis et al. 2003). In Sri Lanka, *E. canis* morulae are predominantly found in lymphocytes (Jayathilake et al. 2002). No age or sex predilection has been observed in affected dogs so far (Harrus S. and Waner T. 2011). However, there is an established breed predilection observed in German shepherds being highly susceptible (Nyindo et al., 1980).

Classical clinical signs of acute ehrlichiosis consist of fever, depression, anorexia, slight weight loss, lethargy, splenomegaly and lymphadenomegaly (Harrus and Waner, 2011). Bleeding tendencies that have been attributed to thrombocytopenia and thrombocytopathia, are manifested as petechiation, epistaxis and hematoma (Woody et al., 1991, Kodikara et al., 1996; Neer et al., 1998, Skotarczak, 2003). Hematological and biochemical abnormalities associated primarily are thrombocytopenia, leukopenia, mild anemia and hypergammaglobulinemia (Skotarczak, 2003). Presence of mild to moderate, generalized pulmonary interstitial infiltrates was also observed in some (Frank and Breitschwerdt 1999).

Diagnosis of acute ehrlichiosis is straightforward when the classic clinical signs are present. However, when presenting clinical signs deviated from the norm, diagnosis can be extremely challenging. In a clinical scenario, the diagnosis of canine ehrlichiosis is made by the history of tick exposure, clinical signs, presence of the organism in agranulocytes in a peripheral blood smear, complete blood count demonstrating thrombocytopenia, mild non regenerative anemia and biochemical panel showing hypoalbuminemia, hyperglobulinemia and hypergamaglobulinemia (Frank and Breitschwerdt 1999, Harrus S. and Waner T., 2011). Definitive diagnosis however, is achieved only by a positive indirect immuno fluorescence antibody test (IFA) or detecting the *E. canis* DNA by polymerase chain reaction (PCR).

### Case History

We have recently come across a form of lymphocytic ehrlichiosis, which causes severe acute disease. We were able to identify 3 susceptible patient groups; young pups less than 6 month old (mainly 2 to 3 months of age), old, obese dogs and dogs with already existing cardiac disease. The latter 2 groups of patients showed similar cardiopulmonary abnormalities observed in the young pups, but with less severity. The clinical presentation of the pups less than 6 months is particularly severe and acute with

high mortality rate. This short communication will discuss the disease observed in the pups.

Twenty six pups - mainly German shepherds, German shepherd cross bred and Labrador retrievers, with varying severity of a similar panel of clinical signs, presented to the Veterinary Teaching Hospital (VTH), Faculty of Veterinary Medicine & Animal Science, University of Peradeniya, Sri Lanka, were identified. Pups aged less than 6 months, mainly pups of age 2 to 4 months were presented with complaints of rapidly worsening acute dyspnoea, mildly distended abdomen, inappetence, inactivity and depression. The clinical signs reportedly started approximately 2 - 4 days before the presentation. Most of the pups were given symptomatic treatments for the condition by a veterinary surgeon, without any improvement. Some pups had a history of diarrhoea or vomiting, before developing severe dyspnoea, inactivity and the rest of the signs. During general clinical examination (GCE), wheezing, increased lung sounds on auscultation, normal to weak pulse, tachycardia, tachypnoea, mild to moderate ascites and presence of 2/6 to 3/6 grade right or bilateral systolic apical cardiac murmurs were discovered. Some of them had mild splenomegaly and lymphadenomegaly. They were normothermic, normotensive, with capillary refilling time (CRT) varying between less than 2 seconds to 3 seconds. The peripheral oxygen saturation (SPO<sub>2</sub>) levels were mildly or moderately reduced. The severity of dyspnoea, tachypnoea, wheezing, weak pulse and hypoxemia, was proportional to the length of time elapsed after the development of initial clinical signs to the time of presentation. Most of the pups were incompletely vaccinated. All the pups had a history of tick exposure, one time or the other. However, most of them were virtually tick free at the time of presentation.

The tentative diagnosis arrived at this stage was that the pups had pulmonary parenchymal disease with possible secondary pulmonary arterial hypertension and congestive right sided heart failure.

**Table 1.** Hematology and biochemical value ranges.

Parameter	Observed ranges	Median	Reference ranges
WBC	22.22 - 50.5	34.03	4.50 - 20.00 x10 <sup>3</sup> /μl
RBC	2.56 - 4.97	3.67	3.00 - 8.00 x10 <sup>6</sup> /μl
Hct	15.40 - 30.2	25.20	26.00 - 52.00 %
Hb	5.00 - 11.3	10.02	6.20 - 18.60 g/dl
MCHC	32.70 - 45.5	39.70	28.00 - 41.00 %
MCV	60.00 - 69.4	65.10	51.00 - 94.00 fl
Thrombocyte	71.00 - 316	166	200 - 500 x10 <sup>3</sup> /μl
TP	3.01 - 6.68	4.41	5.00 - 8.80 g/dl
Alb	1.41 - 2.59	2.04	2.60 - 3.30 g/dl
Glb	1.60 - 4.26	2.51	2.70 - 4.40 g/dl
ALT	9.00 - 87.6	25	21.00 - 102.00 U/dl
BUN	10.31 - 22.42	17.12	10.00 - 28.00 mg/l
PT	11.40 - 21	10.31	<10 seconds
APTT	27.10 - 29.9	21	<11 seconds
D-dimer	<0.2	<0.2	<0.2 mg/l
Fibrinogen	660 - 1200	560	200 - 400 mg/dl
Na <sup>+</sup>	138- 142	139	141.00 -152.00 mEq/l
Ca <sup>+2</sup>	9.42 - 9.89	9.42	8.00 -12.00 mg/dl
K <sup>+</sup>	4.50 - 4.8	4.50	3.60 -5.80 mEq/l

### Biochemical and Hematological Findings

Because of the severity of the clinical signs, the pups were treated as intensive care patients and immediate oxygen therapy and bronchodilators were given before subjecting them to further diagnostic tests. Complete blood count (CBC), examination of blood drops for microfilariae (MF), examination of peripheral blood smears stained with Leishman, and basic biochemical analysis consisting, blood urea nitrogen (BUN), total protein (TP) and albumin (Alb) and alanine aminotransferase (ALT) and urinalysis of voided samples were done by the standard methods used in the VTH reference laboratory<sup>a</sup>. Measuring serum electrolyte concentrations of calcium (Ca<sup>+2</sup>), sodium (Na<sup>+</sup>) and potassium (K<sup>+</sup>) was done by the standard methods used by a private laboratory<sup>b</sup>. Noninvasive blood pressure (NIBP) values and peripheral oxygen saturation (SPO<sub>2</sub>) percentage were measured using a PM-9000Vet, Mindray patient monitor<sup>c</sup>. Right lateral (RL) and dorsoventral (DV) thoracic radiographs<sup>d</sup> and ECG<sup>e</sup> were obtained by standard methods. 2-dimensional (2D), M-mode and colour and spectral Doppler echocardiography examinations were done on right parasternal short axis (RPSA), right parasternal long

axis (RPLA), left apical, and left parasternal (LP) cranial views, using a MyLab30Vet ultrasound scanning machine<sup>f</sup>, according to the guidelines given by American Society of Echocardiography (Thomas et al., 1993). The coagulation times; prothrombin time (PT)<sup>a</sup>, activated partial thromboplastin time (APTT)<sup>a</sup> and fibrinogen levels<sup>a</sup> and D - dimer levels (in 2 pups)<sup>b</sup> were also measured. Baermann test was performed to identify dog lung worm larvae (*Angiostrongylus vasorum*), using fecal samples. Nasal swabs were submitted for bacterial cultures and antibiotic sensitivity test (ABST)<sup>g</sup>.

CBC revealed normal to elevated white blood cell (WBC) counts, normal to reduced red blood cell (RBC) counts, normal to reduced hematocrit value (Hct), normal to reduced hemoglobin (Hb) levels, normal to elevated mean corpuscular hemoglobin concentration (MCHC), mean corpuscular volume (MCV) at lower normal margins and normal to moderately reduced thrombocyte counts (table 1). The normal CBC counts were observed in pups which were presented as soon as the clinical signs were developed or clinical signs identified during routine GCE. Peripheral blood smear showed; activated mature lymphocytes, prominent,

**Table 2.** Tricuspid valve velocity and pressure gradient ranges obtained from 13 pups, indicated ranges of mild to severe PH. Median values indicated moderate PH\* (Kellihan and Stepien, 2010).

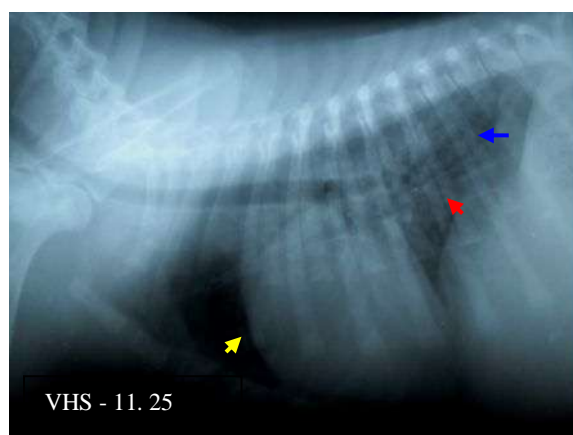
Parameter	Observed ranges	Median	Reference ranges for PH*
Velocity m/s	3.15 - 6.7	3.92	Mild $\geq 2.8$ to $< 3.5$ , moderate 3.5 - 4.3, severe $> 4.3$
Pressure Gradient mmHg	39.70 - 179.60	61.50	Mild $\geq 31.4$ to $< 50$ , moderate 50–75, severe $> 75$

large and round *E. canis* morulae, often multiple (5-6) in a single lymphocyte. The percentage of the infected lymphocytes was around 1% - 17%, average being about 8%. Some pups were additionally, mildly positive for *B. gibsoni*. Polychromic macrocytes (PCM), ghost cells, spherocytes, microcytes, hypochromacia, presence or absence of microagglutination, target cells (codocytes), acanthocytes, megathrombocytes, toxic neutrophils, band neutrophils, reactive monocytes, presence or absence of nucleated red blood cells (nRBC) were also observed in the blood smear. All the pups were concluded having acute canine ehrlichiosis based on the presence of morulae in lymphocytes.

Biochemical profile showed lower normal to reduced TP, mildly reduced Alb and normal to reduced globulin (Glb) levels. Normal ALT, lower normal levels of BUN, mildly prolonged PT (more than 10 seconds) and APTT (more than 11 seconds), and increased fibrinogen levels (more than 200 - 400 mg/dl). D-dimer levels were at normal levels (less than 0.2 mg/l) (Spangler and Russel, 2003).  $\text{Na}^+$ ,  $\text{Ca}^{+2}$  and  $\text{K}^+$  levels were not significantly changed (table 1). Urinalysis revealed absent to trace proteinuria.

Baermann tests were negative for lung worm larvae in all the tested pups. RL and DV radiographs showed, mild to moderate bronchiolar and moderate generalized interstitial patterns, cranial lobar and caudal lobar pulmonary arterial dilatation and venous congestion (figure 1 and figure 2). Right sided cardiac enlargement was observed in most of the pups (figure 2). Pups with evidence of right sided cardiac enlargement in thoracic radiographs showed

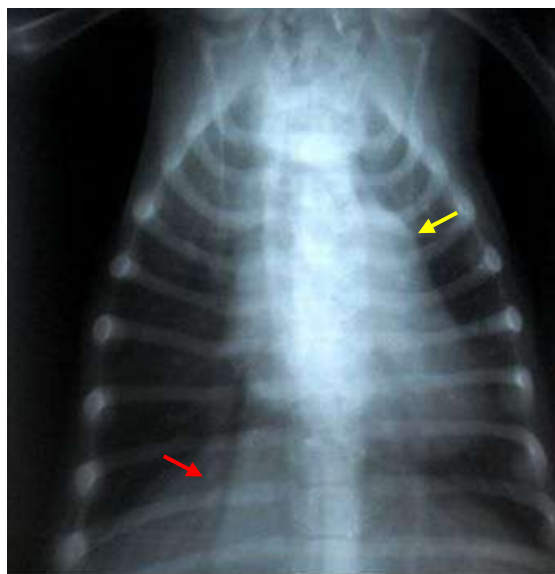
deep Q waves in ECG, but their mean electrical axis (MEA) was normal.



**Figure 1.** Right lateral thoracic radiograph. Diffused interstitial pulmonary pattern appearing as a coarse 'mesh' around the caudo-dorsal lung field (blue arrow). Bronchiolar pattern visible as small 'doughnut' shaped rings (red arrow). Right sided cardiomegaly (VHS - 11.25) with the silhouette of the heart showed enlargement of the right side of the heart (yellow arrow). VHS - vertebral heart score.

Echocardiography revealed a vast array of abnormalities. All the pups with severe clinical signs had severe PH and right-sided congestive heart failure (RHF). Pulmonary artery (PA) diameter was increased when compared to the diameter of the base of the aorta in RPSA heart base view (figure 3). Dilatation of the right ventricle (RV) and right atrium (RA), flattening of the inter ventricular septum (IVS) with paradoxical septal motion, reduced left ventricular (LV) chamber size and pseudohypertrophy of the LV wall were observed in 2D, RPSA and RPLA 4 chamber views (figure 4 a. and b.). In M-mode examination, the ejection fraction (EF) and fractional shortening (FS)

were severely elevated and LV was hyperdynamic figure 4. c.). There was tricuspid valve (TV) insufficiency observed in all the pups who had right systolic apical murmurs (figure 5) and (table 2). None of the pups had any congenital heart diseases identified during echocardiography.



**Figure 2.** Dorsoventral thoracic radiograph. Dilatation of the main pulmonary trunk appearing as a bulge at 1 O' clock position, indicating possible pulmonary hypertension (yellow arrow). Dilatation of the right caudal lobar artery at the level of 9<sup>th</sup> rib (red arrow).



**Figure 3.** 2D echocardiography at the base of the heart in right parasternal short axis view. Increased diameter of the main pulmonary artery (24.1 mm), when compared with the aortic root diameter (18.0 mm).



**Figure 4. a.** 2D echocardiography of the 4 chamber view in the right parasternal long axis view. Dilatation of the right ventricle (yellow arrow), flattened interventricular septum, reduced left ventricle chamber size, pseudohypertrophy of the left ventricle wall and interventricular septum.

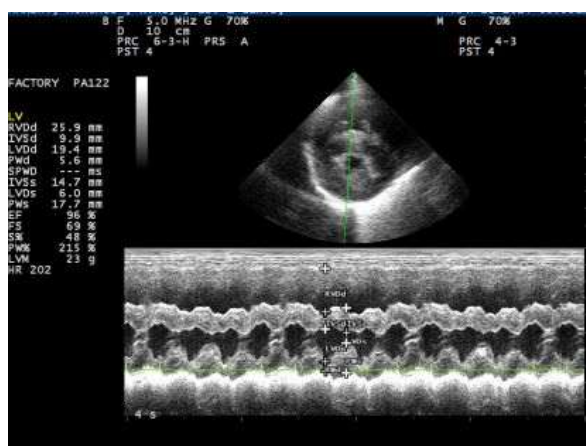


**Figure 4. b.** 2D echocardiography view of the right parasternal short axis view at the level of papillary muscles. Paradoxical septal motion, reduced left ventricle chamber size and pseudohypertrophy of the left ventricle wall and interventricular septum.

### Treatment Protocol

The initial therapeutic regimen observed for all the patients was similar, with certain modifications applied for the individual patients; oxygen therapy, bronchodilators (salbutamol<sup>j</sup> inhaler at 1-2 puffs, q8h, aminophylline<sup>k</sup> at 10 mg/kg, q12h), diuretics (furosemide<sup>l</sup> at 2-4 mg/kg q8h) dose depending on the degree of severity of pulmonary congestion, doxycycline<sup>m</sup>, as the antirickettsial against *E. canis*, at 10 mg/kg, q24h, either ciprofloxacin<sup>h</sup> at 10 mg/kg, q24h or clavulanated amoxicillin<sup>i</sup> at 20

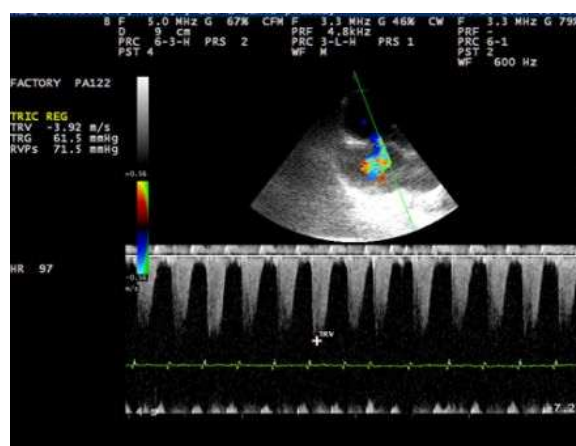
mg/kg, q8h (decided by the results of ABST), given as bactericidal antibiotic against possible concurrently existing bacterial lung infection, spironolactone<sup>n</sup> at 2 mg/kg, q24h given as a treatment for congestive heart failure, silymarin containing antioxidant<sup>o</sup>, given as a liver protectant, against oxidative liver damage, vitamin B<sup>p</sup> was given to aid in erythropoiesis and an amino acid supplement<sup>q</sup> was also given to facilitate protein synthesis. The decision of giving sildenafil<sup>r</sup> as a phosphodiesterase-5 (PDE-5) inhibitor was determined by the degree of PAH, as well as the degree of dyspnoea and hypoxemia indirectly detected by SPO<sub>2</sub> levels. The decision of plasma transfusion was based on PT and APTT; if a pup had prolonged PT and APTT, a single fresh plasma transfusion at 10 ml/kg rate was performed after minor cross matching. Implementing vitamin K<sup>s</sup> supplementation was based on PT.



**Figure 4. c.** M-mode tracing of the left ventricle at the level of papillary muscles. Hyperdynamic wall of the left ventricle with exaggerated fractional shortening (69%) and ejection fraction (96%).

Despite of the management therapy and doxycycline<sup>m</sup> given as the specific treatment against *E. canis*, the progression of the clinical signs followed a similar pattern in all the pups during the subsequent 18 - 24 hours. The severity of dyspnoea and ascites were not reduced in all the pups. SPO<sub>2</sub> levels were reduced to approximately 70%, NIBP gradually reduced from normal to hypotension, average systolic NIBP been around 80 mmHg. These values gradually improved by the day 2 in survivors, but

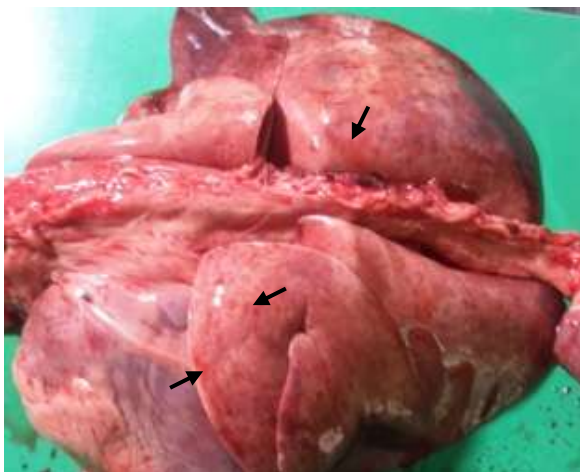
gotten further worsened in the ones who had succumbed. *E. canis* infected lymphocyte percentages were not much altered in pups presented with severe clinical signs. In contrast, the pups who had minimum initial clinical signs (only wheezing), and with one or two infected lymphocytes, showed an increased number of infected lymphocyte percentage during the first 18 - 24 hours despite of treatment. Also, the number of RBC and WBC with abnormal morphologies was further worsened. All the pups developed moderate to mild leukocytosis with a left shift. After 18 - 24 hours, the pups that had normal thrombocyte counts, showed a downward trend in the platelet number with or without being thrombocytopenic. In contrast, the pups that had severe clinical signs, but survived, started showing an upward trend in the thrombocyte count. Irrespective of the management therapy, the pups that had severe clinical signs and succumbed to the disease, showed downward trend in their clinical manifestations and vital parameters; dyspnoea and pulmonary infiltrates had gotten worsened. SPO<sub>2</sub> and NIBP values had gradually fallen down, pulse had become gradually weak and rapid, with few having severe tachycardia.



**Figure 5.** Continuous wave Doppler tracing of the tricuspid valve insufficiency in left parasternal cranial view. Regurgitation jet appearing as a negative tracing from the baseline during systole. The velocity of the regurgitation jet - 3.92 m/s, the pressure difference between the right atrium and the right ventricle - 61.5 mmHg, the systolic pressure in the pulmonary artery - 71.5

mmHg - indicating moderate pulmonary hypertension.

Out of the total number, only 8 survived and from the survivors, 2 were treated before showing overt clinical signs of congestive right heart failure (diagnosis was made by detecting the presence wheezing, pulmonary congestion, *E. canis* morulae in lymphocytes and reactive lymphocytes in the blood smear). Three, responded to the addition of sildenafil<sup>r</sup> and survived. Three were given plasma transfusions within 12 hours of presentation. All non survivors were the ones who were previously symptomatically treated for the presenting condition and were delayed in presenting to the VTH. Mortality rate was 69%, however, it could be higher, as these observations were based only on the patients directly examined by the main author. The pups who had recovered showed gradual reversal of PH, correction of flattening of IVS, disappearance of paradoxical septal motion, reduction of the RV and RA dilatation, normalizing of LV chamber size and LV wall thickness, complete resolution of RHF signs, with the reversal of TV valve insufficiency.

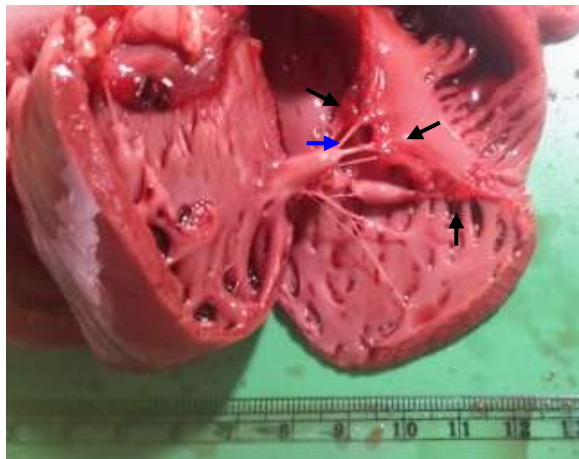


**Figure 6.** Postmortem image of pulmonary hemorrhages (black arrows). Extensive scattered multi-focal, occasionally coalescing, dark red foci of echimotic hemorrhages (black arrows).

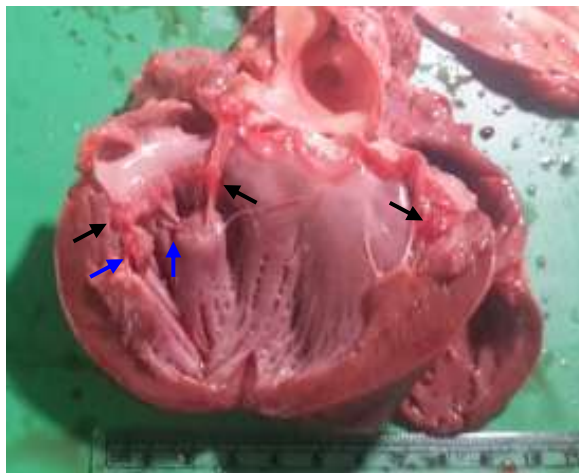
### Postmortem Findings

Gross and histopathology of the postmortem (PM) of the dead pups showed various degrees of generalized interstitial pulmonary edema, with infiltrates of lymphocyte and

macrophages with and mild to extensive pulmonary hemorrhages (figure 6). Most showed pleural and pericardial hemorrhages, with few showing, hemorrhages in lymph nodes, kidneys, bladder and pancreas. PM of all the succumbed pups showed mild to moderate peritoneal fluid (modified transudate). Pulmonary congestion, PH and RHF, identified during ante-mortem were confirmed by the PM results. Interestingly, most of these pups had tricuspid or mitral valve dysplasia (figure 7 and 8). None of them had atrial or ventricular septal defects. Hepatic congestion and splenic hyperplasia were also identified in some pups. No lungworms or heartworms (*Dirofilaria immitis*) were detected during the PM.



**Figure 7.** Postmortem image of tricuspid valve dysplasia. Smoothly thickened and glistening valvular leaflets (black arrows) and short thick chordae tendinae (blue arrow).



**Figure 8.** Postmortem image of mitral valve dysplasia. Smoothly thickened valvular leaflets (black arrows), shortened chordae tendinae (blue arrows).

## DISCUSSION

Because of the presence of non-classical clinical signs, and the absence of some classical clinical signs related to canine ehrlichiosis, it was challenging at first to identify the nature of the disease. Some findings of this study were compatible with what were reported by previous investigations on canine ehrlichiosis. More importantly, several findings were previously unreported and novel. In previous studies, a breed prevalence was demonstrated by showing German shepherds being more susceptible to canine ehrlichiosis (Nyindo et al., 1980). This study also confirmed that and, in addition to German shepherds, Labrador retrievers were also seemed to be susceptible. Previously, no age predilection was established. In this study, pups of age less than 6 months, mainly 2 - 3 month old pups, were predominantly affected. Nonspecific clinical signs; reduced appetite, inactivity and depression, observed in all the pups were previously recorded (Woody et al., 1991, Neer et al., 1998, Skotarczak, 2003). Fever, which was an often found clinical sign, was absent in all the pups during the presentation (Troy et al., 1980; Skotarczak, 2003). Splenomegaly and lymphadenomegaly were classical signs previously recorded that were also found in some of the pups of this study. Most prominent and consistent clinical sign in these pups was wheezing, which was not recorded anywhere as a clinical sign related to canine ehrlichiosis. Also, they showed severe dyspnoea, which is not a classical clinical sign in canine ehrlichiosis, though, adult respiratory distress syndrome has been observed in humans affected with *Ehrlichia* species (Modi et al., 1999; Patel et al., 1999; Weaver et al., 1999). In these pups the consistency of the presence of wheezing and diagnosing of the condition as canine ehrlichiosis was very high, that the authors utilized the correlation as a diagnostic tool for identifying the disease in subsequent pups, presented without other related clinical signs.

Presence of ascites was not recorded as a classical clinical manifestation in dogs with naturally infected canine ehrlichiosis, previously, except by Locatelli et al. in

2012. Unver et al. has reported ascites in a dog experimentally infected with a virulent strain of *E. canis* (Unver et al., 2009). Ascites could be due to RHF and vasculitis, since, the Alb levels were not sufficiently reduced in the pups in the study to cause ascites. Presence of cardiac murmurs was again, not previously reported as associated with canine ehrlichiosis, except by Locatelli et al. (Locatelli et al., 2012). Murmurs could be attributed to 2 factors; tricuspid valve (TV) insufficiency secondary to annular separation of the TV leaflets due to dilatation of RA and RV or congenital AV valve dysplasia affecting both or either TV or mitral valve (MV).

Changes observed in the hematological parameters were also interesting. The increased number of PCM, indicated regenerative anemia. Instead of classically reported normocytic, normochromic, non-regenerative anemia, we have found anisocytosis with a large number of PCM indicating regenerative anemia, in most of the pups. Examination of the reticulocyte index would have given more information supporting this finding. Presence of microcytosis and hypochromacia indicated iron deficiency, which was not previously reported as associated with canine ehrlichiosis. Presence of ghost cells, spherocytes, and in some, micro agglutination indicated immune mediated hemolytic anemia (IMHA), with both intravascular and extravascular hemolysis, which is recognized as a trigger factor for disseminated intravascular coagulation (DIC) (Scott - Moncrieff et al., 2001). Presence of acanthocytes and target cells were not reportedly linked with canine ehrlichiosis. Acanthocytes are present when there is an elevated level of cholesterol, compared to the phospholipids, in the lipid bilayer of RBC membranes (Cooper et al., 1980). Acanthocytes are reportedly found in dogs with liver disease, which causes alteration of the plasma lipid and cholesterol composition (Rebar et al., 1981, Weiss et al., 1993, Christopher and Lee, 1994). Target cells are often found in non-regenerative anemia (Harvey, 2001).

Most of the pups, had later developed leukocytosis with a left shift, indicating



severe ongoing inflammation. Presence of toxic neutrophils again, is something that was not stated in previous literature in relation with canine ehrlichiosis. Various agents, including bacterial infections can cause toxic effects on neutrophils at the bone marrow stage and be manifested as toxic neutrophils.

Presence of megathrombocytes was not an unusual finding in canine ehrlichiosis since, concurrent consumption or destruction of platelets due to ongoing hemorrhage, DIC or formation of antiplatelet antibodies could be present even with the thrombocyte levels within normal range (Russel, 2010). Pups in this study were normal to moderately thrombocytopenic; patients with such thrombocyte levels would not have spontaneous hemorrhages, therefore, pulmonary hemorrhages seen in PM cannot be explained by their thrombocyte levels alone.

Finding of mild hypoalbuminemia in biochemical analysis is also a non-classical finding reported in few studies previously (Frank and Breitschwerdt, 1999; Mylonakis et al., 2010; Locatelli et al., 2012). Hypoalbuminemia indicated impaired production of Alb by the liver, Alb loss by vasculitis and third space sequestration as, none of the pups had either glomerulonephritis (absent to trace proteinuria in urinalysis of a voided sample is insignificant) or protein losing enteropathy (Codner and Maslin, 1992). Consistent changes in TP or Glb were not identified in this study, as opposed to what were reported by previous studies (Frank and Breitschwerdt, 1999).

Elevation in the coagulation times; PT and APTT and fibrinogen levels, were unusual findings that were not recorded previously as associated with canine ehrlichiosis, to the authors knowledge. In a retrospective study on canine ehrlichiosis conducted by Frank and Breitschwerdt, no elevation of PT, APTT or fibrinogen was observed (Frank and Breitschwerdt et al., 1999). Mild elevations of PT and APTT in the pups, indicated the impairment of intrinsic, extrinsic and common pathways of secondary hemostasis. Reduced synthesis of coagulation factors by the liver or

coagulation factor depletion by DIC, can lead to elevated PT and APTT (Lisman et al., 2002, Senzolo et al., 2006). Elevated D - dimer level is a reliable indicator of DIC (Bick, 2003). Normal D - dimer levels (also absence of schistocytes in blood smears) observed in the pups, ruled out DIC. There were indications from hematological and biochemical parameters, that the liver function could have been impaired in those pups. Therefore, the mild elevations of PT and APTT, could be related to impaired coagulation factor synthesis by the liver. The impaired secondary hemostasis could have contributed to pulmonary hemorrhage presented in the pups. All the pups who were transfused with plasma within 12 -18 hours, being survived, indicated that, supplying coagulation factors, corrected the abnormalities of secondary hemostasis, temporarily during the acute crisis. However, often multiple plasma transfusions are needed to be given at 12 hour intervals, to correct the coagulation factor defects (Littlewood, 2000). The PT and APTT levels were normalized in the survived pups tested after the clinical recovery. Fibrinogen, an acute phase protein being elevated, indicated an ongoing inflammation, possibly in the liver or lungs. Elevation of fibrinogen ruled out DIC as a possible cause for hemorrhage (Stokol, 2010).

From this study, the authors propose that in the form of acute canine ehrlichiosis found in these pups, the abnormalities of secondary hemostasis due to reduced coagulation factor synthesis by the liver, is one of the possible contributory factors for the pulmonary hemorrhages; therefore, for the severity of the condition.

Thoracic radiography findings of diffuse interstitial pattern were observed in previous studies of canine ehrlichiosis related with interstitial pulmonary edema or hemorrhage (Locatelli et al., 2012). Bronchiolar pattern observed could be due to peri-bronchiolar cuffing of the bronchiolar wall, by cellular infiltrate or fluid, leading to the thickening of the bronchiolar wall, which was manifested as wheezing during GCE (Locatelli et al., 2012; Lamb, 2002).

Dilatation of the main PA trunk at the 1 O' clock position of the cardiac silhouette in the

DV view, collaborated the finding of PH. Etiology of PH can be multifactorial. However, causes for PH observed in the pups in this study could be narrowed down to secondary complication of pulmonary disease and hypoxia (Kelliham and Stepien, 2010). Pulmonary edema caused by vasculitis associated with canine ehrlichiosis can lead to pulmonary hypoxia (Reardon and Pierce, 1981, 2004, Unver et al., 2009). Pulmonary arteries respond to pulmonary hypoxia by reactive pulmonary artery vasoconstriction, thus causing PH (Kelliham and Stepien, 2010). Additionally, interstitial edema causes resistance to blood flow through the lung parenchyma, increasing the PA pressure. Finding PH and secondary RHF in these pups was an estimation of the severity of the ongoing acute lung injury.

Association of PH and secondary RHF with canine ehrlichiosis is not a classic clinical sign. Locatelli et al. in the previously mentioned publication, reported a case study of an adult dog diagnosed with chronic canine ehrlichiosis diagnosed with PH (Locatelli et al., 2012). The case study did not specify which type of agranulocyte was infected with *E. canis*, but the paper generally talked about canine monocytic ehrlichiosis. In that light, this is the first publication reporting canine lymphocytic ehrlichiosis causing PH and RHF in dogs. Furthermore, no reports are available on pups with acute canine ehrlichiosis manifesting PH and secondary RHF as a complication.

PH creates a considerable elevation of the RV afterload (Kelliham and Stepien, 2010; Johnson et al., 1999). Since, onset of PH was acute in these pups, the secondary remodeling of the RV by concentric hypertrophy, had not occurred, as in chronic and gradual onset of PH. Instead, the RV dilatation by eccentric hypertrophy occurred, causing the right side of the heart to become both volume and pressure overloaded, thus leading the RHF.

Small LV chamber size and pseudohypertrophy of the LV wall indicated reduced LV volume due to RHF. Reduction of LV volume explained the gradual decline of the NIBP and weakening of pulse observed in later stages of the disease.

Complete reversal of PH and TV insufficiency observed in survived pups. Therefore, the TV valve insufficiencies observed during the clinical stage of the recovered pups, could be attributed to the annular separation and abnormal apposition of the valvular leaflets due to secondary remodeling of the heart.

However, in some of the succumbed pups, during PM, either MV or TV dysplasia or both were evident, therefore, in those particular cases, congenital valvular dysplasia may have contributed to the severity of the condition by exacerbating the cardiac failure that had already existed and ultimately to the death.

PM findings of pulmonary hemorrhages and interstitial edema were also consistent in all the succumbed pups. Pulmonary hemorrhages as discussed earlier, could be related coagulation factor defects or to thrombocytopathia caused by *E. canis*, although, in most pups, platelet aggregations were visible in the blood smear, indicating normal platelet function (Harrus et al., 1996, Green 1997, Valera et al., 1997, Lovering et al., 1980). The pups in the study had normal to moderate reduction of thrombocyte levels, which were not sufficiently low to cause hemorrhages (Russel, 2010). This finding collaborated with the findings of the retrospective study done by Frank and Breitschwerdt, where, hemorrhages were manifested as epistaxis with the dogs having normal platelet counts (Frank and Breitschwerdt, 1999). There were plenty of evidences gained by PM of the pups to say that severe pulmonary infiltrates and pulmonary hemorrhages causing severe hypoxia could have been the cause of death. Since, the disease progression of those pups was very rapid, most of the time, a detailed investigations could not be performed. The diagnosis was made on the results of minimum data base. Rationalization of the tests performed was based considering minimum distress to the patients and the tests that were only deemed essential were performed. Performing blood gas analysis would have given direct values of hypoxemia however, that facility was not available. Broncho-alveolar lavage - BAL, was not performed considering the critical

situation of the patient and the risk of anesthesia. Testing for *Dirofilaria immitis* was not considered necessary as the pups were less than 7 months old (Nelson et al., 2005). Furthermore, in Sri Lanka, no evidence of presence of canine heartworm disease was ever reported.

## CONCLUSION

This is the first time an association of acute canine ehrlichiosis with severe pulmonary parenchymal changes and secondary pulmonary hypertension was reported in pups, to the author's knowledge. Furthermore, no records were found that, one of the causes for pulmonary hemorrhages associated with acute canine ehrlichiosis being the impaired secondary hemostasis due to hepatic impairment. Elevation of fibrinogen, PT and APTT in the acute canine ehrlichiosis affected pups are distinct findings as well. No age predilection was ever identified with acute canine ehrlichiosis previously, yet in this study we have found pups of less than 6 months showed more susceptibility and severity in manifesting this condition. Presence of microcytosis, hypochromacia, acanthocytes and target cells with acute canine ehrlichiosis was also unique findings. Moreover, utilizing the presence of wheezing as a screening criteria for pulmonary interstitial edema associated with acute canine ehrlichiosis was never recorded anywhere. Therefore, authors conclude that this study reports several unique and novel manifestations of a severe form of acute canine ehrlichiosis, further studies of which, would give more insight into the pathophysiology of the exact nature of this form of the disease.

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