CARDIAC HISTOPATHOLOGY AND ELECTROCARDIOGRAPHIC CHANGES IN CANINE BABESIOSIS

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SUMMARY

Electrocardiographic (ECG) changes have never been reported in canine babesiosis. Based on the metabolic, electrolyte, and myocardial alterations described for the disease, such changes are to be expected. The purpose of this study was to describe ECG changes in canine babesiosis, and to correlate those changes to clinical severity, outcome and cardiac histopathological changes.

Four groups of dogs with babesiosis were studied: mild to moderate anaemia (n=40), severe anaemia (n=35), concurrent immune-mediated haemolytic anaemia (n=18) and complicated (n=28). Lead II ECG was recorded at admission for 1 minute in all dogs, and repeated after 24 hours in admitted dogs (groups II – IV). Six lead ECG was recorded in 88 dogs. Full necropsy was performed between 30-60 minutes after death on 16 dogs (5 died on arrival, 11 had ECG recording). Gross cardiac pathology was recorded and histopathology of myocardial sections from ventricles, atria, apex and interventricular septa was evaluated, using a scoring system for haemorrhages, necrosis, inflammatory infiltrate and fibrin microthrombi.

The following ECG changes were recorded: sinoatrial (7%) and atrioventricular blocks (4%), ventricular premature complexes (7%), low R-amplitude (23%), prominent Q (33%), axis deviations (40%), prolonged QRS (32%), ST depression and coving (28%), large T (42%), and notched R (28%). Differences between groups were minor and inconsistent. Gross pathological changes were pericardial effusion (25%) and subepicardial (56%) and subendocardial haemorrhages (63%). Histological changes were haemorrhages (69%), necrosis (50%), inflammation (63%) and fibrin microthrombi (75%). The only correlation between pathology and ECG was low R-amplitude and pericardial effusion. There was a significantly higher prevalence of sinus bradycardia and irregular sinus rhythm in the non-survivors.

Both ECG and pathological changes were non-specific, but there were similarities to the pattern of changes that have been described for myocarditis and myocardial ischaemia.

Antiarrhythmic treatment was only required in 1 dog. Thus, the clinical application of the ECG changes found in this study was limited. It was concluded that the heart suffers from the same pathological processes described in other organs in canine babesiosis, namely inflammation and hypoxia. Cardiovascular management, if necessary, should be based on functional monitoring rather than ECG.

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CHAPTER 1

LITERATURE REVIEW

1.1 General Introduction

Canine babesiosis is a tick-borne disease caused by the haemoprotozoan parasite *Babesia canis.* The causative subspecies in Southern Africa is *B. canis rossi.*[1] Babesiosis primarily affects erythrocytes causing intra-erythrocytic parasitaemia, which results in both intravascular and extravascular haemolysis. This may result in regenerative anaemia, anaemic hypoxia, anaerobic metabolism and metabolic acidosis. Parasitaemia, erythrolysis, and parasite destruction are assumed to cause activation of various inflammatory mediators and result in pyrexia.[2,3]

Babesiosis is classified clinically as uncomplicated or complicated.[2,3] Uncomplicated babesiosis presents with signs related to parasitaemia and haemolysis with no other organ involvement.[3] Signs detected in uncomplicated babesiosis are anaemia, pale mucous membranes, water-hammer pulse, anorexia, depression and splenomegaly.[3] Uncomplicated babesiosis is further subclassified as mild or moderate with a packed cell volume of above 15%, and severe with packed cell volume of below 15%. Complicated babesiosis is associated with other organ involvement with specific clinical signs for each organ failure.[3]

Babesiosis can thus involve multiple organs, probably as a result of an excessive inflammatory response rather than from the parasite itself.[2] Organs that are known to be involved in canine babesiosis are the kidney, brain, liver, lungs and heart.[3] The excessive inflammatory response is an endotoxic shock-like state,[3] resulting in the systemic inflammatory response syndrome (SIRS).[4] The end result of this inflammatory process in various organs can predispose to the multiple organ dysfunction syndrome (MODS).[4]

1.2 Heart lesions in canine babesiosis

Heart lesions appear to be rare in canine babesiosis,[2,3] however, the prevalence has never been reported. Gross lesions that have been identified are subepicardial and subendocardial haemorrhages,[5] hydropericardium, and haemopericardium,[5-7] and, rarely, focal necrosis and infarctions. (unpublished necropsy reports, Department of Pathology, Faculty of Veterinary Science, University of Pretoria). Histologically, microthrombi have been described in the myocardium,[5] which may lead to infarction and necrosis.[7] Multifocal necrosis with secondary macrophage and neutrophil infiltration, are infrequently reported (Unpublished necropsy reports, Department of Pathology, Faculty of Veterinary Science, Onderstepoort). Dilated and congested cardiac blood vessels with considerable numbers of infected erythrocytes and free parasites have been also reported,[6,8] as well as haemorrhages.[7] In cattle and horses, ecchymotic haemorrhages in the epicardium, endocardium and myocardium have been described, but they are non-specific agonal lesions in these species.[9] However, in cattle infected with *B. bovis*, blood vessels in the myocardium had distinctly higher parasitaemia than the jugular blood, and degeneration of the myocardium and myocardial haemorrhages have been described.[10]

1.3 Electrocardiographic changes in myocarditis and ischaemia

In babesiosis, cardiac lesions can develop as a result of the 2 mechanisms postulated for tissue damage, namely an overwhelming inflammatory response and anaemic-hypoxic mechanisms.[11] Ischaemia and myocarditis can cause a decrease in resting potential of the involved cells leading to a conduction block and resulting in arrhythmias.[12] Ischaemia, leading to myocardial injury and ending with necrosis is well documented in human cardiology, especially in myocardial infarction, and is assumed to be applicable to other myocardial diseases.[13] These changes are accompanied by typical electrocardiographic (ECG) changes.

In humans, ischaemia can result in alterations of the T wave (increased amplitude and inversion), ST segment (depression or elevation) and the QRS wave (increased Q-amplitude, increased or decreased R-amplitude, and prolonged QRS-duration).[13] In a

study on myocardial infarction in the dog and cat, common histopathological changes reported were myocardial necrosis and inflammatory infiltrate, and ECG changes were ventricular tachycardia (VT), atrial fibrillation, and ventricular premature complexes (VPCs).[14] Although the aetiology and the multifocal nature of heart involvement in babesiosis is different from the infarcts described in humans and dogs and cats in the above-mentioned studies, identical lesions that have been described in babesiosis, such as necrosis and inflammation, may give the same ECG changes. Some of the infarcts described in the dog and the cat were thrombo-emboli resulting from immune-mediated haemolytic anaemia (IMHA),[14] which is a common complication of babesiosis.[2,3]

Myocarditis might cause slow conduction resulting in prolonged QRS, complete atrioventricular (AV) block, VPCs, and VT.[15,16] The ECG changes in viral myocarditis in man can mimic those of myocardial infarction, namely ST elevation, pathologic Q, and abnormal T.[17] In Chagas' disease, a disease characterised by parasitic myocarditis, reported ECG changes in man were bradycardia, prolonged PR and QRS, low amplitude R-wave, notching and slurring of the QRS-complex, ST and T-wave abnormalities, various AV blocks, bundle branch block, and VPCs.[18,19] The conduction disturbances were more severe in the chronic form of the disease.[19] The myocardial changes were diffuse or multifocal inflammation and necrosis leading to residual, but not progressive, chronic fibrosis and fatty infiltration that also involved the conduction system {sinoatrial (SA) and the AV nodes and the bundle of His}.[19,20] In dogs with Chagas' diseases, the same findings have been reported, but the acute and chronic stages were less defined.[20] Histological and ECG changes did not necessarily correlate.[20]

1.4 Haematological, metabolic and electrolyte abnormalities in canine babesiosis that may induce electrocardiographic changes

Systemic and metabolic disorders that may induce ECG changes and that have been described in canine babesiosis include anaemia,[2] acute respiratory distress syndrome,[2] hypotension,[21] hypokalaemia,[3,22] acidosis[22] and acute renal failure.[3]

Anaemia might lead to myocardial hypoxia and ischaemia, and may result in conduction abnormalities and ECG changes as described above. Acute respiratory distress syndrome and hypotension can aggravate the hypoxia. Hypoxaemia can depress conduction and cause AV block.[2,23] Hypokalaemia can cause tachycardia,[24] depression of the ST-segment, decreased T-amplitude, U-wave, prolonged QRS and ventricular arrhythmias.[2,15,25] Metabolic acidosis suppresses conduction and may cause AV blocks and induce re-entrant arrhythmias.[23] The end result may be VPCs that can develop into VT, fibrillation or asystole.[24] Acute renal failure can cause hyperkalaemia and uraemia.[26] Hyperkalaemia can cause bradycardia, high narrow T wave, wide QRS, decreased or eliminated P wave, elevated ST, AV conduction defect and VPCs that may develop to VT, fibrillation or asystole.[15,27] Uraemia can also cause VPCs.[24] However, in a study done on malaria patients with azotaemia, no correlation with the ECG changes were recorded.[28] These metabolic influences might reduce the specificity of the ECG in detecting myocardial lesions *per se*.

1.5 The heart in sepsis and endotoxic shock

According to the redefinition of sepsis in human literature, which includes systemic response to any infection (bacterial, viral, fungal and protozoal),[4] babesiosis is a septic condition. The synergistic combination of tumour necrosis factor-α and interleukin-1 β has been identified as playing an important rule in the mechanism of cardiac myocyte depression in septic shock.[29] These mediators play an important role in malaria infection[30] and have been proposed to play the same role in babesiosis.[3] An older explanation for myocardial depression in septic shock is ischaemia,[31] which is part of the proposed pathophysiology of complicated babesiosis.[11] Various ECG changes have been described for sepsis. Ventricular premature contractions are a common ECG change detected in sepsis or endotoxic-like states in the dog,[24,32-36] and can develop to VT, and multifocal VPCs that can result in asystole and death. Other changes recorded in dogs and cattle with sepsis are premature atrial depolarization, atrial fibrillation,[34] first or third degree AV block, ST segment slurring, and left bundle branch block.[37,38] Tachycardia is a common sign of sepsis,[31] and in man, a heart rate below 106 beats per minute (bpm) at

presentation and below 95, 24 hours after the onset of septic shock, have been found to be good prognostic indicators.[39]

Light microscopic examination of hearts from dogs with induced sepsis showed minimal myocardial neutrophilic infiltrate and no necrosis, but electron microscopy showed endothelial cell oedema, capillary intraluminal fibrin deposition, focal myofibrillar loss, mitochondrial loss, mitochondrial swelling and degenerative myelin.[40] In a study on dogs with gastric dilatation and volvulus, 9/13 dogs had myocardial degeneration and necrosis and 5 of them had concurrent inflammatory infiltrate. An attempt to correlate these lesions with cardiac arrhythmias was unsuccessful.[36]

1.6 The heart in malaria

The acute phase in human malaria has many similarities to canine babesiosis.[3,8,41] Gross cardiac changes that have been described in malaria include general dilatation[42] and pericardial effusion.[43] Histological changes that have been reported include myocarditis, local haemorrhages in the endocardium and epicardium,[42,44] fragmentation of myocardial fibres,[45] necrosis, and ischaemia.[46] Changes associated with myocarditis included foci of inflammatory infiltrates consisting of histioid cells, young fibroblasts, round cells and plasma cells.[47] Somewhat similar infiltrate was also noted in the pericardium.[47] Fatty degeneration of the myocardium following vascular occlusion has also been described.[42,44,45] Myocardial degeneration and necrosis can be extensive and resemble myocardial infarction in rare cases.[42,44] Capillaries within the myocardium showed microthrombi, dilatation, congestion, and sequestration of parasitised red blood cells with resultant capillary and coronary vascular occlusion.[47,48] The endothelial wall contained parasites, and capillary endothelium showed cytoplasmic changes with hyalinosis of the interstitial collagen tissue especially near the epicardium and beyond the muscular tissue of both ventricles.[47] In a study comparing the prevalence of parasitised erythrocyte sequestration in various organs, the heart had the second highest prevalence after the brain and before the liver, lung and kidneys. However, in this study the vessels did not appear tightly packed, and there were no signs of endothelial damage.[49] The cardiac

lesions are most commonly acute, but endomyocardial fibrosis as a chronic process due to malaria has been rarely reported.[44,50] There is no precise data about prevalence of cardiac pathology in malaria. However, it was described by some authors as rare,[45] and by others as occasional.[44]

Electrocardiographic changes that have been detected in malaria are: ST-T anomalies[43] and flattened and negative T,[51] which are non-specific changes.[28] Elongation of the QT segment was a common finding in a few studies, but it was probably a side effect of quinine therapy.[28] The described changes are classified as of ventricular origin, and postulated to be a result of myocardial anoxia.[51] Relative sinus tachycardia[51] and bradycardia are often recorded but were not considered as an ECG abnormality by some authors.[46] Other ECG changes recorded in falciparum malaria include supraventricular and ventricular premature contractions, slight right axis deviation,[45] paroxysmal VT, and 2-3 seconds of conduction pauses.[46] Changes recorded a few seconds prior to death are tachy- or bradyarrhythmias followed by idioventricular rhythm and then asystole.[46]

Electrocardiographic changes are much more common in acute compare to chronic malaria,[43,48,52] involving 23% - 36% of cases.[28,43] Arrhythmias have been reported in 11%, and ST-interval and T-wave abnormalities in 22% of cases. In chronic cases ECG changes have been described very rarely.[50] There was no difference in the prevalence of ECG changes when the ECG recording was done within the initial 48 hour of hospitalisation or after,[28] and some of the changes remained and were even aggravated after 21 days.[51] The ECG abnormalities usually normalised within a few months.[43] In 1 study, the few ECG changes recorded in critical malaria patients were not found to account for death. The authors, based on the ECG findings and their clinical experience, found the heart function surprisingly good, and related the ECG changes to metabolic and electrolyte disturbances associated with complicated malaria and not to the disease process within the myocardium.[46]

1.7 Conclusion

Electrocardiographic changes have never been reported in canine babesiosis. Based on the heart lesions and metabolic disorders observed in babesiosis and the similarities to other diseases such as malaria and sepsis, in which ECG changes have been described, ECG changes may have been overlooked in the past.

CHAPTER 2

OBJECTIVES

1. To investigate ECG changes in canine babesiosis.

2. To investigate cardiac histopathological changes in dogs that die from babesiosis.

3. To relate cardiac histopathological findings to the ECG changes.

CHAPTER 3

RESEARCH QUESTIONS

1. Are there ECG changes in canine babesiosis?

2. If there are changes:
   a. Are they related to clinical severity?
   b. Is there a pattern?
   c. Is there a change over time (24hr sampling)?
   d. Do specific ECG changes have a prognostic value?
   e. Are the ECG changes correlated to plasma potassium concentration?

4. Are there cardiac histopathological changes in dogs that die from babesiosis?

5. If there are ECG changes and histopathological changes, are these changes correlated?

CHAPTER 4

MATERIALS AND METHODS

4.1 Electrocardiographic study

4.1.1 Study design

A prospective study was performed on 121 dogs with canine babesiosis. All the dogs were client-owned dogs presented at the Outpatient clinic of the Onderstepoort Veterinary Academic Hospital (OVAH). Informed consent of the owner was required to enroll a patient in the study. The study was approved by the Ethics and Research Committee of the Faculty of Veterinary Science, University of Pretoria.

Inclusion criteria were:

- Babesiosis diagnosed on a thin capillary blood smear, stained with Cams Quick stain[a], on light-microscopy.
- Any breed or sex.
- Body weight of more than 3kg.
- No cardiac disease previously diagnosed or treated, or suspected at presentation on full clinical examination.

Exclusion criteria were:

- Any concurrent medication started before presentation.
- Clinical signs that have not been previously described for babesiosis.
- Evidence of chronic heart problem.
- Concurrent *Ehrlichia canis* or *E. platys* identified on peripheral blood smear.

The dogs were classified into four groups, based on a clinical classification system proposed by Jacobson and Clark[3]:

[a] CA Milsch, Krugersdorp, RSA.

- Group 1 - Uncomplicated cases with mild to moderate anaemia. These cases had packed cell volume (PCV) ≥ 15% and did not require blood transfusion.
- Group 2 - Uncomplicated cases with severe anaemia. These cases had PCV < 15%, and/or required blood transfusion and responded to antibabesial treatment and blood transfusion within 24 hours.
- Group 3 – Immune-mediated haemolytic anaemia (IMHA).
- Group 4 – Complicated cases. Complicated babesiosis cases had 1 or more of the following complications: oliguria, cerebral babesiosis, coagulopathy, severe icterus, gastrointestinal involvement, respiratory distress or haemoconcentration ("red biliary").

The complications were diagnosed clinically to make "bed-side" classification possible in an outpatient setting; however, this classification was aided by further diagnostic tests in most cases that were hospitalised and by necropsy in those dogs that succumbed to the disease. The complications were defined as follows:

- Oliguria: urine production <1ml/kg/hour that was not resolved by rehydration fluid therapy.
- Cerebral babesiosis: central neurological signs not attributable to any other cause.
- Coagulopathy: bleeding tendency presenting as petechiation and/or other haemorrhage.
- Severe icterus: visual assessment of severe icterus.
- Gastrointestinal involvement: vomiting and/or bloody diarrhoea.
- Respiratory distress: expiratory dyspnoea that did not resolve with blood transfusion, characterised by abnormal lung sounds and/or nasal or oral, frothy discharge.
- Haemoconcentration: haematocrit ≥35% in conjunction with state of collapse, grossly visible haemoglobinaemia and/or haemoglobinuria.

Immune-mediated haemolytic anaemia was diagnosed based on positive "in saline agglutination test (ISA)". In saline agglutination test was performed by mixing a drop of heparinised blood with 5 drops of saline and looking for microscopic agglutination of red blood cells. The reason to have IMHA as an individual group was because it is a common

complication that had the potential to dominate the complicated babesiosis group, and it may play an important role in myocardial infarction.[14]

Therapy of all cases was tailored to individual requirements and not standardised. Post treatment, survival data was collected.

4.1.2 Data collection

At the time of admission, the following data was collected for each dog: owner's details, signalment (breed, age, sex, and weight), and date and time of admission. Full clinical examination and capillary blood smear were performed, followed by blood collection for PCV determination and ISA test, using a syringe flushed with heparin[b]. Plasma potassium concentration was determined in 68 dogs on admission, using the heparinised blood and a semi-automated blood-gas analyser[c].

Electrocardiographic recording was made using a 1-channel 12 lead standard ECG recorder[d] with the dog in a (standard body position)[53]: restrained in right lateral recumbency on a rubber mat, legs positioned parallel to each other and vertical to the long axis of the body, and keeping the head and neck flat on the table. Alligator clips, placed on the lead wires, were attached to the skin proximal to the olecranon and the patella. The jaws of the clips were slightly flattened to relieve the discomfort of pinching. Sedation was not used, except in 1 dog that was treated with pentobarbital for convulsions secondary to cerebral babesiosis before his second ECG. Clipping or shaving of the hair was not necessary, and the hair was flattened with alcohol and moistened with ECG paste[e]. A 1-minute lead II ECG was recorded in all dogs. If the dog moved during the recording, the ECG was stopped until adequate restraint was achieved, and 1 minute recording was then completed. The monitor was set for paper[f] speed of 50mm/min, and amplitude calibration of 1mV = 1cm.

[b] Balanced (Lithium) heparin, Instrumentation Laboratory, Italy
[c] Chiron Diagnostics, Rapidlab 348 pH Blood Gas Analyzer, Essex, England
[d] Nihon Kohden 12-lead ECG monitor, model 6551/F, Japan
[e] Edelweiss Pharmaceutical, South Africa

Manual Hum filter (notch filter at 50Hz) and EMG filter (cutoff frequency of –3dB at 35Hz) were used in all dogs. To rule out a possible influence of the filters, in 88 dogs, a record of 10 complexes of lead II without filters was done. In these 88 dogs 6 lead auto-recording with filters ON (bipolar limb leads I, II, III, and augmented unipolar limb leads aVR, aVL, and aVF, 4 seconds per lead) was performed as well. The frontal plane mean electrical axis was calculated on all ECGs on which lead II did not give the most positive deflection.

The ECG was recorded from all dogs prior to antibabesial treatment. A second ECG was recorded 24 hours post-admission from dogs that were hospitalised and survived 24hr. If the dog died within an hour of the ECG recording, the recording was excluded from the data analysis to avoid agonal changes. All the ECG data was available to the duty clinician. Specific treatment, following these changes, was performed in only 1 dog, but an ECG recording after 24 hours was still performed.

The following parameters were evaluated from the ECG tracing on lead II:

- Arrhythmias, classified according to their origin, named in the standard way[54] and counted.
- Cardiac rhythm, classified either as sinus arrhythmia, regular, regular irregular, or irregular irregular.
- Presence of notch on the descending slope of the R-wave.
- Presence of ST coving.

Heart rate was calculated by counting the number of RR-intervals over 5 non-consecutive 3 second intervals multiplied by 4.

The following parameters were measured and averaged from 5 non-consecutive RR-intervals on lead II:

- P-wave amplitude and duration.
- PR-interval duration.

[f] Dykam MSL 50/3, Israel

- R-amplitude.
- QRS-duration.
- QT-interval duration.
- ST-segment depression or elevation.
- T-wave amplitude.

Duration and amplitude were measured with an ECG ruler to the nearest 0.5 mm (0.05 mV or 0.1 second). Rhythm was evaluated with a caliper.

4.1.3 Data analysis

For data analysis and comparisons between groups, presence of arrhythmia, R notches and ST-coving were noted as either present or absent and rhythm as regular (including sinus arrhythmia, regular and regular irregular rhythms) or irregular. The numerical parameters were compared to the standard dog ECG,[54] and noted as categorical variables. Categorical variables were used because the normal range of ECG parameters is so wide that the mean values of the study population and the groups were likely to fall in the normal range, hiding many dogs with abnormal values. The following parameters were categorised as normal or high: P-amplitude, P-duration, QRS-duration and ST depression or elevation, and the following parameters were categorised as low, normal or high: PR-interval, R-amplitude and QT-duration. T-amplitude was evaluated as a T/R ratio. A ratio of 0.25 or less was regarded as normal and a ratio above 0.25 as high. In those 88 dogs with records without filters, 10 consecutive R-amplitudes were measured and averaged. They were categorized as low, normal or high compared with the standard values. T/R ratio without filters was also calculated and analysed compared to the standard. The standard dog ECG is different for different ages and sizes of dogs. We defined puppies as ≤ 8 month of age, adult dogs as > 8 month, toy/small breeds ≤ 10kg, large breeds ≥ 25kg and giant breeds as ≥ 45kg.

The data was captured using Excel 97[TMg] and analysed by Dr PJ Becker (Medical Research Council, Pretoria) using standard computer statistical software (Stata version

[g] Microsoft, USA

6[h]). Separate analyses were done for the first and the second ECG, for the whole population, for each group and for survivors and non-survivors. Each ECG parameter was analysed for the percentage of abnormal (low and/or high) and normal observations and was tested against the expected normal distribution with the binomial test (95% within the normal range and 5% outside this range). The Fisher's exact test was used for comparison between the prevalence of abnormal observation among different groups. Two-sample t-test with equal variance was used to compare T-amplitude, ST deviation, R-amplitude and QRS-duration categories (low, high or normal) with respect to potassium concentration**.** To evaluate if the change between the first and the second ECG was consistent in the population, the paired t-test was used for the numerical parameters (Wilcoxon matched pairs signed-ranks test was used for comparison if less than 15 observations) and the McNemar's test for symmetry was used for the categorical parameters. To evaluate the difference between groups with respect to the change over 24hr in each parameter, one- way analysis of variance was used. In all tests the level of significance was $P < 0.05$.

4.2 Histopathological study of myocardial lesions

4.2.1 Study design

Any dog that fulfilled the inclusion criteria for the ECG study and subsequently died had gross pathology and histopathological assessment of the heart done. Dogs that showed lesions typical for pre-existing heart disease, such as moderate and severe endocardiosis and chamber dilatation or hypertrophy, were excluded from both the ECG and the pathologic studies.

4.2.2 Data collection

To avoid post mortal changes, which could mimic ischaemia and necrosis, samples were collected 30 - 60 minutes after death. The pericardium was examined for the amount of fluid and , if present, its colour. The heart was opened in standard fashion, and evaluated for changes such as dilatation, gross valvular and jet lesions. Both the endocardium and

[h] Stata corporation, College station, Texas, USA

the epicardium were evaluated macroscopically for haemorrhages (size and location) and gross infarcts. Samples were taken for histological examination from the following sites: papillary muscles of the left and right ventricles, interventricular (IV) septum, left and right auricle of the atrium, and apex. Full thickness (from epi - to endocardium), 5 - 7mm thick sections were taken and placed in marked containers with 10% buffered formalin. Additional samples were taken from haemorrhages seen macroscopically. All macroscopic examinations were done by the investigator.

Samples were wax embedded, sectioned at 5µm, and stained with haematoxylin and eosin and modified Martius Scarlet blue, specifically for fibrin. Sections were evaluated by light microscopy. All sections were evaluated by Dr J Pearson (Department of Pathology, Faculty of Veterinary Science, University of Pretoria), who was blind to the ECG results.

Twenty 400x, adjacent, but not overlapping fields, 10 from the subendocardium and 10 from the subepicardium of each site, were examined. The following was noted and scored separately for each site: necrosis, inflammatory cell infiltration, haemorrhage, and fibrin.

Necrosis of myocardial fibres was recognised by increased cytoplasmic eosinophilia, loss of striation, fragmentation and nuclear pycnosis. Scoring was done as follows:

0: No necrotic cells found in any 1 of the 20 fields.

1: Five or less necrotic fibres in any 1 of the 20 fields.

2: Six to twenty necrotic fibres in any 1 of the 20 fields.

3: More than 20 necrotic fibres in any 1 of the 20 fields.

Other parameters such as location within the myocardium (subepicardial, mid-myocardial or subendocardial) and the distribution (focal, multifocal or diffuse) were also noted.

Interstitial myocardial infiltration was examined for the cell type, location and distribution, and was scored as follows:

0: No inflammatory cells in any 1 of the 20 fields.

1: Five or less cells in any 1 of the 20 fields.

2: Six to 20 inflammatory cells in any 1 of the 20 fields.

3: More than 20 inflammatory cells in any 1 of the 20 fields.

Haemorrhages and fibrin microthrombi were examined for their location. Both haemorrhage and fibrin were scored 0 if they were not present in any field and 1 if present.

4.2.3 Data analysis

For statistical analysis, scores 2 and 3 for necrosis or inflammatory infiltration, as well as the presence of haemorrhage or fibrin microthrombi, were categorised as abnormal. The association between the presence of any abnormality within a specific site and the ECG categorical parameters was assessed by the 1 sided t-test. The same test was used to assess the association between specific abnormalities and the ECG parameters. Level of significance was $P < 0.05$.

CHAPTER 5

RESULTS

5.1 Electrocardiographic study

Of the 128 dogs enrolled in the study, 7 were excluded from the following reasons; death within an hour (2 dogs), concurrent bacterial pneumonia, concurrent parvovirus, aggressiveness, valvular endocardiosis or pulmonic stenosis (1 dog each). Electrocardiographic recordings from 121 dogs were included. Group 1 (mild – moderate anaemia, uncomplicated) had 40 dogs, group 2 (severe anaemia, uncomplicated) 35, group 3 (IMHA) 18, and group 4 (complicated) 28. Eleven of the 121 dogs died, 3 were euthanised and 8 died naturally. The 8 that died naturally were classified as non-survivors (all from the complicated group). The dogs that were euthanised were from the complicated group (2 dogs), and from the IMHA group (1 dog). They were not categorised as non-survivors since they were euthanised from a financial reason and their prognosis could not be predicted (because of their various complications). One dog died at home, after the owners declined required hospitalisation. The rest 109 dogs were discharged, and defined as survivors. The signalment of the study population is summarised in Table 1. In respect to gender proportions and age, there was no statistical difference between the study groups. The breeds' prevalence was too spare for a statistical analysis. The weight was the only parameter in which the study groups were significantly different.

In the complicated group, the 28 dogs had 1 or more of the following complications; severe icterus (18 dogs), respiratory distress (10), cerebral babesiosis (8), coagulopathy (4), oliguria (4), gastrointestinal disturbances (4) and haemoconcentration (3).

Further diagnostic tests and necropsy that aided the diagnosis of the complications were:

- Four of the 18 dogs with severe icterus died and all of them had liver changes on necropsy, namely bile stasis, leukostasis, hepatocyte injury or necrosis. One of the 4 dogs had serum chemistry that revealed moderately elevated serum alanine

transaminase. Of the 14 icteric dogs that survived, 2 had serum chemistry that revealed moderately elevated alanine transaminase, alkaline phosphatase, total bilirubin or bile acids.

- Five of the 10 dogs with respiratory distress died and all of them had necropsy findings of acute interstitial pneumonia. One of these dogs had blood gas analysis showing severe hypoxaemia (pO_2 = 40 mmHg, reference range 75-100). Four of the 5 dogs with respiratory distress that survived had thoracic radiographs, all of them showed an interstitial lung pattern. Blood gas analysis in 1, showed mild hypoxaemia (pO_2 = 65 mmHg).
- Seven of the 8 dogs with cerebral signs died and 6 of the 7 had necropsy findings of cerebral haemorrhage, malacia or neutrophilic infiltration.
- All 4 dogs with oliguria died and 2 of them had kidney changes on necropsy, such as haemoglobin pigmentation. The other 2 did not have kidney changes on necropsy, but both of them had potassium concentration measured, revealing severe hyperkalaemia in 1 (9.58 mmol/L), and mild hyperkalaemia (5.35 mmol/L) in the other. The latter had also urinalysis, showing high numbers of cellular casts.
- Three of the 4 dogs with coagulopathy died and all had necropsy signs consistent with disseminated intravascular coagulation (DIC), such as scattered haemorrhages in various organs.

Measurable data derived from the lead II first ECG of the 121 dogs are summarised in Table 2. The means of all the measurable parameters were within the normal range for both the first and second ECG (Table 3). Heart rate among the whole study population did not differ from the normal, but all 3 dogs with bradycardia on the first ECG were in the complicated group. Two of these did not survive (Fig. 1), and the third was euthanised, making the distribution in these 2 groups significantly different from normal (Table 4). There was a significant difference in the prevalence of bradycardia and tachycardia between non-survivors and survivors. Among the survivors, 3/109 (2.75%) had tachycardia and none had bradycardia, while the non-survivors showed no tachycardia but 2/8 (25%) had bradycardia. This phenomenon was also expressed by high QT in 1 of the non-survivors and short QT in 1 of the survivors. However the QT

difference was not significant, probably due to the small numbers of non-survivors (p = 0.073). There was a trend towards a significant difference in the abnormalities in heart rate between the 4 groups (p= 0.063), with only tachycardia in both the uncomplicated anaemic groups and bradycardia only in the complicated group.

The prevalence of abnormalities in the measurable ECG parameters for the whole population, each group, survivors, and non-survivors on the first ECG are summarised in Table 4. Abnormal P- amplitude, P-duration, PR-duration and QT-duration were found in prevalence expected for a normal population (not significantly more than 5% of dogs were out of the reference range).

Low-voltage R-amplitude (<0.5 mV) was seen in 28/121 (23%) dogs on the first ECG when recorded with filters and in 9/88 (10%) dogs, when recorded without filters (Fig. 2), both significantly more frequent that the expected normal distribution (Table 4). Of the 28 dogs with low R when recorded with filters, 25 had a 6 lead ECG recorded, and on 23 of them the low R-amplitude was also evident on all other leads. In 1 of these 25 dogs the R-amplitude was higher than 0.5 mV in lead I, and in another dog in was higher in lead aVR (both measured 0.55 mV). Electrical alternans together with low-voltage R-amplitude was observed in 2 dogs on the first ECG, and in 2 dogs on the second ECG. One dog had electrical alternans but normal R-amplitude. There was no difference in the prevalence of low R between the study groups, but when measured with filters the non-survivors had 50% prevalence compared with 21.3% in the survivors, with a borderline significance (p = 0.08). However, this difference disappeared when measured without filters. Plasma potassium concentrations were similar in dogs with low and normal R-amplitude.

Prolonged QRS-duration was observed across the whole study population, in each group and among the survivors in a significant higher prevalence, compared with the expected normal distribution (Table 4). However, it was not significant among the non-survivors. Increased ST deviation (depression of more than 0.2 mV or elevation of more than 0.15 mV from the base line) was seen in significant percentage of the whole population, in all

groups and in the survivors and non-survivors. Ninety percent of the dogs with ST deviation had depression, and it was very often accompanied by ST coving. There was a significant difference in the prevalence of abnormal ST on the first ECG between the groups: the highest was in the IMHA group, then in the mild/moderate anaemia group, then in the complicated group and the lowest prevalence in the severe anaemia group. This difference disappeared in the second ECG. T-amplitude was high (higher than R-amplitude/4) in 42% of the whole population when the R-amplitude was recorded with filters, and in 18% when the R-amplitude was recorded without filters, both significantly higher than the expected normal distribution. The percentage of dogs with high T was significantly higher than the expected normal distribution in all groups when recorded with filters. When recorded without filters it was significant in the severe anaemia, IMHA, complicated and survivor groups. There was no correlation between the plasma potassium concentration and the ST deviation, or the T-wave amplitude, either with or without filters.

The percentages of ECG abnormalities in the categorical parameters are summarised in Table 5. Fifteen dogs (12.4%) had arrhythmias on the first ECG, 3 in both recordings and 7 only on the second ECG. The types of arrhythmia were as follows (some dogs had more than 1 type):

- Sinoatrial block: 9 dogs, 5 on the first ECG and 4 different dogs on the second ECG.
- Ventricular premature complexes: 9 dogs, 7 on the first ECG (1 had also SA block) and 2 different dogs on the second ECG.
- Ventricular tachycardia: 2 dogs; 1 on the first ECG, and the other developed VT on the second ECG after VPCs on the first ECG.
- First degree AV block: 2 dogs both on first and second ECG (same dogs).
- Second degree AV block: 3 dogs; 2 on first ECG (1 had also SA block), and another dog only on the second ECG.

Three dogs with SA block had also temporary atrial standstill with junctional escape rhythm (normal QRS with no P). One of these dogs also had ventricular escape rhythm (10 VPCs / min) (Fig. 3). Apart from 2 dogs, all VPCs originated in the left ventricle. In 1

dog they originated from the right ventricle, and in the other they were multiform and originated from both right and left ventricles. The number of VPCs ranged from 1 to 20 per minute. One dog that had 20 VPCs / min on the first ECG developed 96 VPCs / min on the second ECG that was defined as VT (1 of the 2). This was the only dog that received antiarrhythmic therapy (Mexiletine[i]), and the arrhythmia resolved the next day. The other VT had only uniform bizarre QRS at a rate of 180 / min that resolved the next day without treatment. The QRS was negative showing deep and wide S, suggestive of left ventricular origin and complete right bundle branch block. Second degree AV block was present in 3 dogs. Two dogs had Mobitz type II, 1 only on the first ECG together with a SA block, and the other was present only on the second ECG. One dog had Mobitz type I on the first ECG. The QRS configuration was normal in all 3 dogs (type A block).

On the first ECG, the complicated group had the highest prevalence of arrhythmias, 6/28 dogs (21%), followed by the IMHA group with 3/18 dogs (16%), the mild – moderate anaemia with 4/40 (10%) and the severe anaemia with 2/35 (6%) (Table 5). This difference, however, was not statistically significant On the second ECG 8/10 dogs with arrhythmia came from the severe anaemia group, with a borderline significance (p=0.06) compared to prevalence in the other groups.

Irregular rhythm was recorded in 7/121 dogs (6%). The prevalence was significantly higher in the non-survivors, 2/8 (25%) compared with 3/109 in the survivors (2.75%). The other 2 dogs with irregular rhythm were euthanised. Prevalence of irregularity was higher in the complicated group compared with the other groups (Table 5), but the significance was borderline (p=0.09).

Notched R is (step formation in the descending slope of the R wave) was seen in 34 (28%) dogs in the study on the first ECG. It was more clearly visualised in recordings without filters (Fig. 4). It was present in 6/40 (15%) dogs in the mild – moderate anaemia group, 9/35 (26%) in the severe anaemia, 9/18 (67%) in the IMHA and 7/28 (25%) in the complicated group (Table 5). The prevalence was significantly different between groups

[i] Mexitil, Boehringer Ingelheim, Randburg, RSA

and remained so in the second ECG as well. Notched Q was present in 2 dogs, and notched S in 1.

Forty dogs showed Q-amplitude of ½ of the R-amplitude voltage or higher in at least 1 of the ECG recordings. In 16 of these dogs the Q-amplitude was higher than the R. In these dogs the Q-amplitude ranged from 0.15 to 1.07 mV with filters, and 0.24 to 1.97 mV with no filters. Five dogs showed S-amplitude of ½ of the R-amplitude voltage or higher in at least 1 of the ECG recordings. Four of these dogs had S-amplitude higher than the R. In these 5 dogs the S-amplitude ranged from 0.16 to 0.5 mV with filters on, and 0.27 to 0.7 mV with no filters. One dog showed both Q and S of higher amplitude than the R-amplitude (QrS or W configuration). This was better visualised when recorded without filters, as with filters the Q and S tended to merge creating QS configuration. Seventeen dogs showed right axis deviation in at least 1 of the ECG recordings, ranging from 104º to -136º. Nineteen dogs showed left axis deviation in at least 1 of the ECG recordings, ranging from 30º to -102º.

The difference between the first and the second ECG for the measurable parameters is summarised in Table 6. All non-survivors died within 24 hours and did not have a second ECG, preventing comparison between survivors and non-survivors. The only significant changes between the first and second ECG were slowing of the heart rate, increase in the R-amplitude and prolongation of the QT interval. The change in the R-amplitude from the first to second ECG was significantly different between groups, with and without filters, showing a greater increase in the IMHA and complicated groups than the severe anaemic group.

5.2 Cardiac pathology

Sixteen dogs were included in the histopathological study. Eleven dogs had 1 valid ECG on admission prior to death. Although fulfilling the inclusion criteria for the ECG study the other 5 dogs were included only in the histophathological study, 2 that had an ECG but died within 1 hour of recording, and 3 others that died on arrival. Thirteen dogs died naturally and 3 were euthanised. Two additional dogs were excluded from both the

histopathological and ECG study due to moderate endocardiosis in 1 and pulmonic stenosis in the other. Fifteen of the 16 dogs were complicated cases and 1 was a dog with IMHA that was euthanised.

5.2.1 Macroscopic study of cardiac lesions

Twelve of the 16 dogs had macroscopic cardiac lesions including pericardial effusion and pericardial, epicardial and endocardial haemorrhages (Table 7). Four dogs had pericardial effusion, defined as more than 5ml of effusion; 3 of these had about 10 ml and 1 had more than 20ml. The fluid was red, clear, and watery resembling haemolysed serum. One dog had a large pericardial ecchymosis. Ten dogs had macroscopic epicardial haemorrhages (Fig. 5). These haemorrhages tended to be superficial and did not extend more than 3mm into the myocardium. They were multifocal in 6 dogs, focal in 2 and diffuse in 2. The diffuse haemorrhages extended to all chambers in 1 dog, and were limited to the ventricles in the other 1. The distinct epicardial haemorrhages were ≤ 5mm in diameter in 6/8 dogs, 20mm in 1 and 30mm in another. The most common site of the epicardial haemorrhages was the left ventricle (n= 8) especially around the coronary arteries, followed by right ventricle (n = 4), right atrium (n = 3) and left atrium (n = 1). Macroscopic endocardial haemorrhages were seen in 9 dogs, 5 multifocal, 3 focal and 1 extendeding diffusely through the left ventricle. All were ≤ 2mm deep. Seven out of 8 distinct ones were ≤ 5mm in diameter and 1 was 20mm. The most common site of the haemorrhages was the left ventricle (n = 6) especially at the papillary muscles, followed by right ventricle (n = 4), right atrium (n = 3) and left atrium (n = 1).

5.2.2 Histopathological study of the myocardial lesions (Table 7)

Fifteen of the dogs had at least 1 microscopic lesion. Necrosis (score ≥ 2) was seen in 8 dogs (Fig. 6 and 7), in the left ventricle (n = 2), IV septum (n = 2), left atrium (n = 3), right atrium (n = 1) and apex (n = 2). The necrosis was moderate (score 2) in 80% of the lesions, severe (score 3) in 20%, multifocal in 80% and focal in 20%. The location of the necrotic fibres ranged from subepicardial myocardium to subendocardial myocardium with no predilection site. The foci tended to be concentrated in 1 area of the cardiac muscle (seen in a particular area in 80% of lesions and scattered in 20%). Cell infiltrate

(score ≥ 2) was seen in 10 dogs, in the left ventricle (n = 6), right ventricle (n = 4), IV septum (n = 3), left atrium (n = 3), right atrium (n = 6), and apex (n = 3). The inflammatory infiltrate was moderate in 64% (score 2) and severe (score 3) in 36%. In 96% of the sites with inflammatory infiltrate, it was multifocal and diffuse in 4%. The cell infiltrate was composed of round cells (macrophages and lymphocytes) in 57% of the lesions, round cells and neutrophils in 36% and neutrophils in 7%. In 53% of the lesions the inflammatory foci were scattered in the myocardium, while in the rest of the lesions they were limited to 1 area mainly in the subendocardial myocardium (21%), or the subepicardial myocardium (11%).

Microscopic haemorrhages were seen in 11 dogs (Fig. 8), in the left ventricle (n = 8), right ventricle (n = 4), IV septum (n = 5), left atrium (n = 2), right atrium (n = 1) and apex (n = 6). The haemorrhages were multifocal in 81% and focal in 19%. The haemorrhages were located from subepicardial myocardium to subendocardial myocardium with no predilection site, tended to be concentrated in 1 area of the cardiac muscle (seen in a particular area in 84% of lesions and scattered in 16%).

Fibrin microthrombi were seen in 12 dogs (Fig. 9), in the left ventricle (n = 8), right ventricle (n = 3), IV septum (n = 5), left atrium (n = 4), right atrium (n = 4) and apex (n = 6). The fibrin microthrombi were multifocal in 94% and focal in 6%. The fibrin microthrombi tended to be concentrated in 1 area of the cardiac muscle (seen in 1 area in 71% of lesions and scattered in 29%), and the location ranged from subepicardium (26%) to mid-myocardium (19%) to subendocardium (13%).

In summary, myocardial necrosis, inflammation, haemorrhage or fibrin microthrombi occurred at similar rates in this study. Lesions tended to be multifocal, but were generally limited to 1 area within the myocardium. The ventricles were the most common site, especially the left ventricle including the apex.

5.3 Correlation between pathology and electrocardiography

Eleven dogs had valid ECG and necropsy. This is a small number and significant associations between histopathology and ECG were identified for only a few parameters. Low R (when taken with filters) and pericardial effusion fluid had a significant statistical association. This was not the case when records were done without filters, but 2 of the dogs with pericardial effusion did not have a record without filters. Low R (with filters) was relatively more common in dogs with subepicardial haemorrhages, but the association was not statistically significant ($p = 0.11$). There were 2 associations with borderline significance: T-wave (when analysed with R with filters) with necrosis at any site ($p = 0.07$) and T-wave (when analysed with R with filters) with haemorrhage at any site ($p = 0.06$). Those changes were not correlated when T-wave was analysed without filters, but the number of dogs with recording without filters was even smaller ($n = 8$).

5.4 Tables

Table 1: Signalment of the study population

	Whole population	Mild-moderate anaemia	Severe anaemia	IMHA	Complicated cases	Survivors	Non-survivors
Observations	121	40	35	18	28	109	8
Weight (kg)							
-mean	16.62	17.25	14.41	23.54	14.02	17.02	12.35
-range	3-50	3-50	3-43.5	3.3-45	3.1-40	3-50	3.5-29.6
Age (years)							
- mean	2.11	2.55	2	1.64	1.9	2.08	2.38
- range	0.17-12	0.17-12	0.17-9	0.5-5	0.17-10	0.17-2	0.25-8
Sex							
- males	68	26	17	12	13	61	6
- females	53	14	18	6	15	48	2
Breed							
- Mongrels	37	14	12	3	8	34	3
- Boerboel	11	2	4	4	1	11	-
- Fox Terrier	7	3	2	-	2	7	-
- German Shepherd	6	5	-	-	1	6	-
- Maltese	6	3	1	-	2	5	1
- Rottweiler	6	3	1	1	1	5	-
- Dachshund	5	2	2	1	-	5	-
- Spaniel	5	-	3	1	1	4	1
- Bull Terrier	4	1	1	1	1	3	-
- Border Collie	3	-	-	1	2	3	-
- Dalmatian	3	-	-	1	2	3	-
- Jack Russell	3	1	2	-	-	3	-
- Labrador	3	1	1	-	1	3	-
- Staffie	3	1	1	-	1	2	1
Other breeds With 1-2 dogs	19	4	5	5	5	15	2

Table 2: Electrocardiographic measurements (mean ± standard deviation) for the first recording.

	Reference values	Whole population	Mild-moderate anaemia	Severe anaemia	IMHA	Complicated	Survivors	Non-survivors
Observations		121	40	35	18	28	109	8
Heart rate (beats/min)	70-160 (adults) 60-140 (giant) ≤180 (toy) ≤220 (puppies)	137 ± 31	130 ± 30	149 ± 27	131± 30	137 ± 37	140 ± 29	114 ± 40
P-amplitude (mV)	≤ 0.4	0.12 ± 0.05	0.13 ± 0.05	0.12 ± 0.03	0.11± 0.04	0.11 ± 0.05	0.12 ± 0.04	0.08 ± 0.04
P-duration (sec)	≤ 0.04 ≤ 0.05 (giant)	0.04 ± 0	0.04 ± 0	0.04 ± 0.01	0.04 ± 0.01	0.04 ± 0	0.04 ± 0	0.04 ± 0
PR-duration (sec)	0.06-0.13	0.1± 0.02	0.1 ± 0.02	0.09 ± 0.02	0.09 ± 0.01	0.09 ± 0.02	0.1 ± 0.02	0.09 ± 0.02
R-amplitude with filters (mV)	0.5 - 3 (large) 0.5 - 2.5 (small)	0.78 ± 0.41	0.86 ± 0.42	0.7 ± 0.41	0.94 ± 0.37	0.67 ± 0.39	0.79 ± 0.41	0.63 ± 0.43
R-amplitude without filters (mV)	0.5 - 3 (large) 0.5 - 2.5 (small)	1.47 ± 0.65 (n=88)	1.65 ± 0.69 (n=27)	1.32 ± 0.62 (n=26)	1.71± 0.53 (n=15)	1.26 ± 0.62 (n=20)	1.5 ± 0.66 (n=79)	1.26 ± 0.72 (n=6)
QRS-duration (sec)	0.05 (small) 0.06 (large)	0.05 ± 0.01	0.05 ± 0.01	0.05 ± 0.01	0.06 ± 0.01	0.05 ± 0.01	0.05 ± 0.01	0.05 ± 0.01
QT-duration (sec)	0.15-0.25	0.19 ± 0.02	0.19 ± 0.02	0.18 ± 0.02	0.21 ± 0.02	0.2 ± 0.03	0.19 ± 0.02	0.21 ± 0.04
ST deviation (mV)	-0.2-0.15	-0.11 ± 0.16	-0.14 ± 0.13	-0.07 ± 0.12	-0.19 ± 0.19	-0.07 ± 0.21	-0.12 ± 0.16	0 ± 0.2
T amp (mV)	≤R/4	0.19 ± 0.14	0.2 ± 0.1	0.13 ± 0.1	0.27± 0.17	0.2 ± 0.18	0.19 ± 0.14	0.19 ± 0.14

Table 3: Electrocardiographic measurements (mean ± standard deviation) for the second recording.

	Standard	Whole population	Severe anaemia	IMHA	Complicated
Observations*		62	29	13	15
Heart rate (beats/min)	70-160 (adults) 60-140 (giant) ≤180 (toy) ≤220 (puppies)	124 ± 30	120 ± 32	113 ± 20	140 ± 29
P-amplitude (mV)	≤ 0.4	0.11 ± 0.04	0.1 ± 0.03	0.12 ± 0.04	0.12 ± 0.05
P-duration (sec)	≤ 0.04 ≤ 0.05 (giant)	0.04 ± 0	0.04 ± 0	0.04 ± 0	0.04 ± 0.01
PR-duration (sec)	0.06-0.13	0.1± 0.02	0.1 ± 0.02	0.1 ± 0.01	0.09 ± 0.02
R-amplitude with filters (mV)	0.5 – 3 (large) 0.5 – 2.5 (small)	0.88 ± 0.44	0.78 ± 0.48	1.19 ± 0.46	0.81 ± 0.41
R-amplitude without filters (mV)	0.5 – 3 (large) 0.5 – 2.5 (small)	1.7 ± 0.72 (n=46)	1.56 ± 0.69 (n=21)	2.16 ± 0.68 (n=12)	1.57 ± 0.72 (n=10)
QRS-duration (sec)	0.05 (small) 0.06 (large)	0.05 ± 0.01	0.05 ± 0.01	0.06 ± 0.01	0.05 ± 0.01
QT-duration (sec)	0.15 - 0.25	0.21 ± 0.03	0.21 ± 0.02	0.23± 0.02	0.2 ± 0.03
ST deviation (mV)	-0.2 - 0.15	-0.06 ± 0.15	-0.05 ± 0.1	-0.1± 0.17	-0.05± 0.21
T amp (mV)	≤R/4	0.2 ± 0.15	0.14 ± 0.09	0.29± 0.18	0.21± 0.18

∗Group 1, mild – moderate anaemia, had only 5 observations; therefore, these results were only included in the whole population group.

Table 4: Prevalence of abnormalities in the first ECG among measurable parameters*

	Whole population (n = 121)	Mild-moderate anaemia (n = 40)	Severe anaemia (n = 35)	IMHA (n = 18)	Complicated (n = 28)	Survivors (n = 109)	Non-survivors (n = 8)
Heart rate							
- high	2.48	2.5	5.71	0	0	2.75	0
- low	2.48	0	0	0	**10.71**	0	**25**
P-amplitude							
- high	0	0	0	0	0	0	0
P-duration							
- high	1.67	0	2.86	3.7	0	1.85	0
PR-duration							
- high	1.67	2.5	2.86	0	0	1.85	0
- low	0	0	0	0	0	0	0
R-amplitude with filters							
- high	0	0	0	0	0	0	0
- low	**23.33**	**20**	**28.57**	**11.11**	**29.63**	**21.3**	**50**
R-amplitude without filters	(n=88)	(n=27)	(n=26)	(n=15)	(n=20)	(n=79)	(n=6)
- high	1.14	0	0	6.67	0	1.27	0
- low	**10.23**	**7.41**	**15.38**	0	**15**	**10.13**	**16.67**
QRS-duration							
- high	**31.67**	**35**	**34.29**	**38.89**	**18.52**	**33.33**	12.5
QT-duration							
- high	0.83	0	0	0	3.7	0	12.5
- low	1.67	5	0	0	0	1.85	0
ST deviation - high (depressed or elevated)	**28.33**	**37.5**	**14.29**	**44.44**	**22.22**	**28.7**	**25**
T-amplitude with filters							
- high	**41.67**	**40**	**34.29**	**55.56**	**44.44**	**40.74**	**62.5**
T-amplitude without filters	(n=88)	(n=27)	(n=26)	(n=15)	(n=20)	(n=79)	(n=6)
- high	**18.39**	11.11	**20**	**20**	**26.67**	**19.23**	0

*Bold underlined values are significantly different from the expected normal distribution (significantly > 5% in parameters with only high and normal, and > 2.5% in parameters with low, normal and high).

Table 5: Percentage of abnormalities in the first ECG among categorical parameters

Group	Whole population (n = 121)	Mild – moderate anaemia (n = 40)	Severe anaemia (n = 35)	IMHA (n= 18)	Complicated (n = 28)	Survivors (n = 109)	Non – survivors (n=8)
Arrhythmia	12.4	10	5.71	16.67	21.43	10.09	25
Irregular rhythm	5.79	5	0	5.56	14.29	2.75	25
Notched R	28.33	15	25.71	66.67	25.93	29.63	25
ST coving	29.17	35	17.14	44.44	25	29.36	25

Table 6: Differences between the measurements of the first (Table 2) and the second ECG (Table 3).

Data is shown as the difference between the 2 recordings (second – first), taken 24hr apart. Significant values (bold and underlined) are those where the change from the first to the second recording was consistent in that specific group.

Group*	All population	Severe anaemia	IMHA	Complicated
Observations	62	29	13	15
Heart rate (beats/min)	**-21 ± 26**	**-29 ± 24**	**-19 ± 29**	**-15 ± 20**
R-amplitude with filters (mV)	**0.18 ± 0.19**	**0.13 ± 0.15**	**0.31 ± 0.17**	**0.22 ± 0.25**
R-amplitude without filters (mV)	**0.3 ± 0.36** (n = 46)	**0.18 ± 0.35** (n = 21)	**0.41 ± 0.35** (n = 12)	**0.48 ± 0.32** (n = 10)
QRS-duration (sec)	0	0	0 ± 0.01	0
QT-duration (sec)	**0.02 ± 0.02**	**0.03 ± 0.02**	**0.02 ± 0.03**	**0.02 ± 0.02**
ST deviation (sec)	-0.03 ± 0.14	0 ± 0.13	-0.09 ± 0.19	-0.01 ± 0.11
T amp (mV)	0.01 ± 0.11	0.01 ±0 .1	0.01 ± 0.12	0.01 ± 0.1

∗Group 1, mild – moderate anaemia, had only 5 observations; therefore, these results were only included in the whole population group.

Table 7: The distribution of the cardiac macro- and microscopic lesions in the pathological study

	Macroscopic epicardial haemorrhage	Macroscopic endocardial haemorrhage	Necrosis (score ≥ 2)	Cell infiltrate (score ≥ 2)	Microscopic haemorrhage	Fibrin micro-thrombi
	N = 10	N = 9	N = 8	N = 10	N = 11	N = 12
Left atrium	N = 1	N = 1	N = 3	N = 3	N = 2	N = 4
Right atrium	N = 3	N = 3	N = 1	N = 3	N = 1	N = 4
Left ventricle	N = 8	N = 6	N = 2	N = 6	N = 8	N = 8
Right ventricle	N = 4	N = 4	N = 0	N = 4	N = 4	N = 3
IV septum	N = 0	N = 0	N = 2	N = 3	N = 5	N = 5
Apex	N = 0	N = 0	N = 2	N = 3	N = 6	N = 6
Multifocal	60%	56%	80%	96%	81%	94%
Focal	20%	33%	20%	0%	19%	6%
Diffuse	20%	11%	0%	4%	0%	0%
Distinct area	100% (Superficial)	100% (Superficial)	80%	37%	84%	71%
Scattered	0%	0%	20%	53%	16%	29%

5.5 Figures

Figure 1: Electrocardiogram (lead II, 50mm/s, 1cm/1mV) from a 6-month-old, female, 4 kg, mongrel dog with sinus bradycardia as the only ECG abnormality. The dog died from cerebral babesiosis, 8 hours after this recording. Myocardial necrosis, haemorrhages and fibrin microthrombi were evident on histology (Figs. 6, 7, 8 and 9).

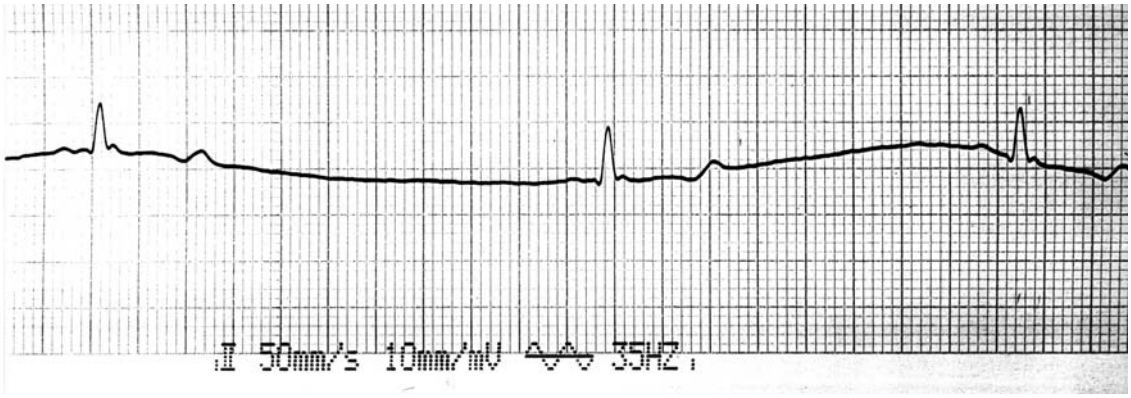


Figure 2: Electrocardiogram (lead II, 50mm/s, 1cm/1mV) from a 5-month-old, male, 7 kg, Chow Chow. The dog died 3 hours after this recording from severe lung oedema. Note the low voltage R-amplitude on both recordings without (2A) and with filters (2B). The dog had severe subepicardial haemorrhages and mild pericardial effusion on necropsy (Fig. 5).

Figure 2A:

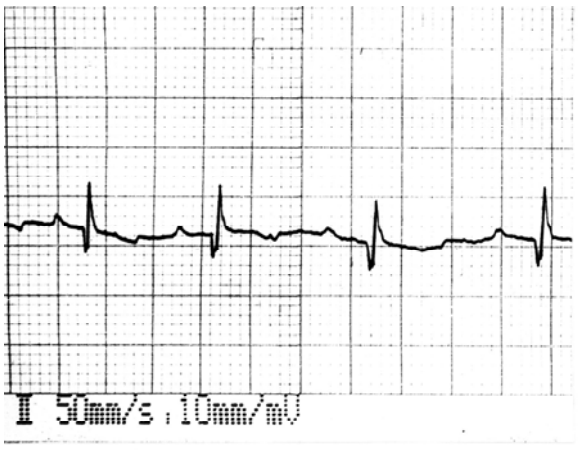


Figure 2B:

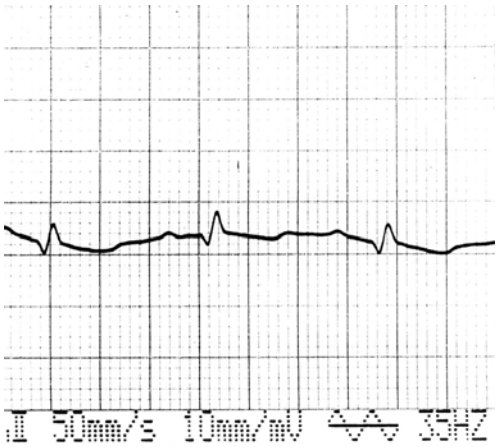


Figure 3: Electrocardiogram (lead II, 50mm/s, 1cm/1mV) from a 4-years-old, female, 15 kg, Bull Terrier with SA block and an escape VPC. The dog was euthanised after a diagnosis of complicated babesiosis (severe icterus). No macro- or microscopic cardiac lesions were found on histopathology.

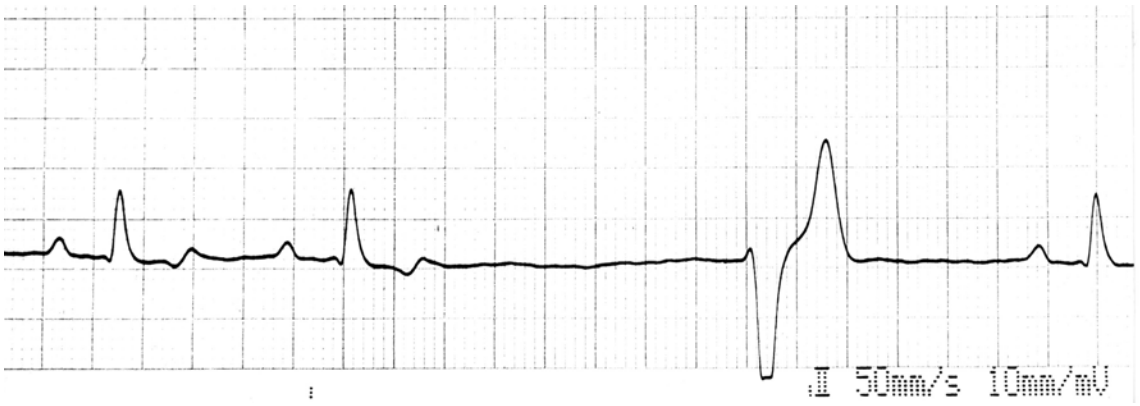


Figure 4: Electrocardiogram (lead II, 50mm/s, 1cm/1mV) from a 10-month-old, female, 17 kg, Pitbull Terrier with R notch and ST coving. The dog had severe anaemia (PCV = 12%) with no complications. Note the clear notch (step formation) on the descending slope of the R-wave when recorded with filters OFF, which is less clear when recorded with filters ON. Note also the ST coving and the large T present in both recordings.

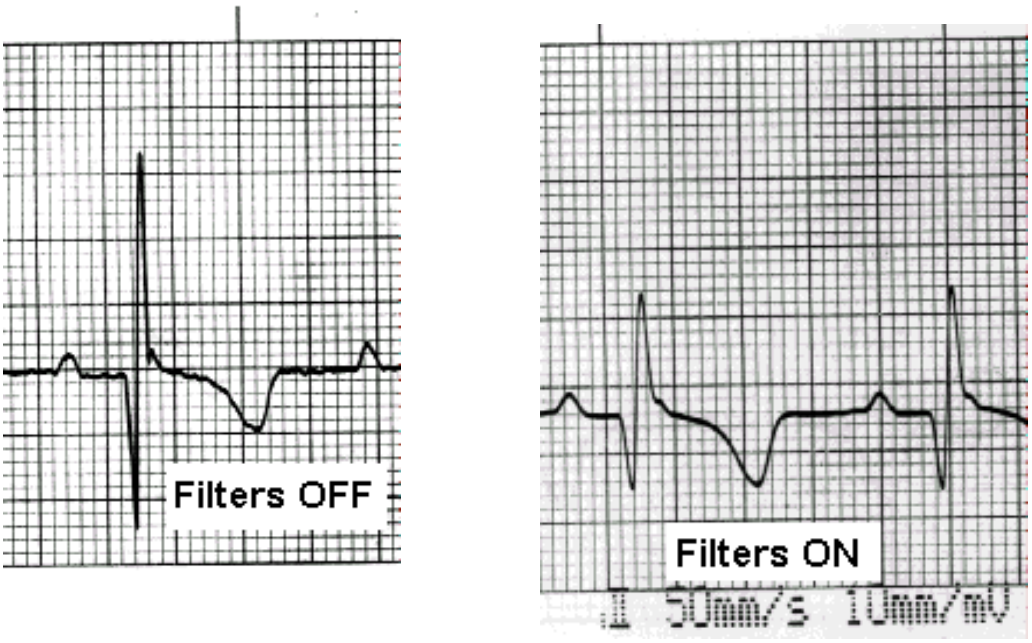


Figure 5: Macroscopic picture of the heart from the same dog as in Fig. 3, showing extensive multifocal haemorrhages of the subepicardium, mostly limited to the left ventricle (LV). Haemorrhages were also present in the brain. On histology myocardial inflammatory infiltrate, haemorrhages and fibrin microthrombi were present in 3 sites, but no necrosis.

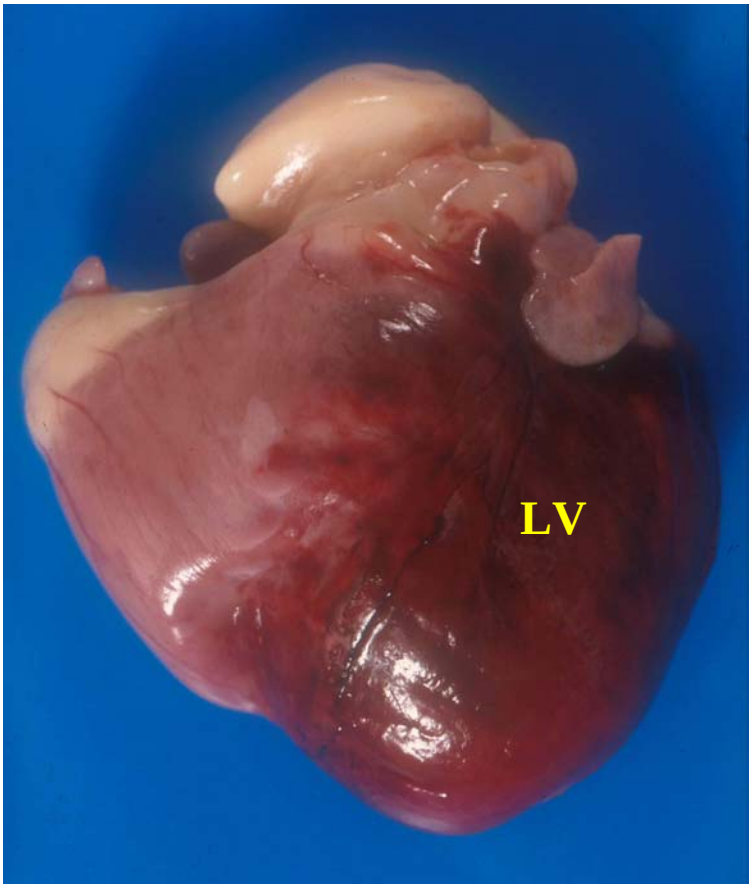


Figure 6: Histologic section of subepicardial myocardium (haematoxylin-eosin, x100) from the same dog as Fig. 1. At the top left corner, the whole area is showing early signs of severe (score 3) myocardial necrosis (MN). Note the more eosinophilic colour and the pycnotic nuclei of the necrotic fibres. There is a visible different between the necrotic area and the normal part, which is paler. Haemorrhage is also present (arrow).

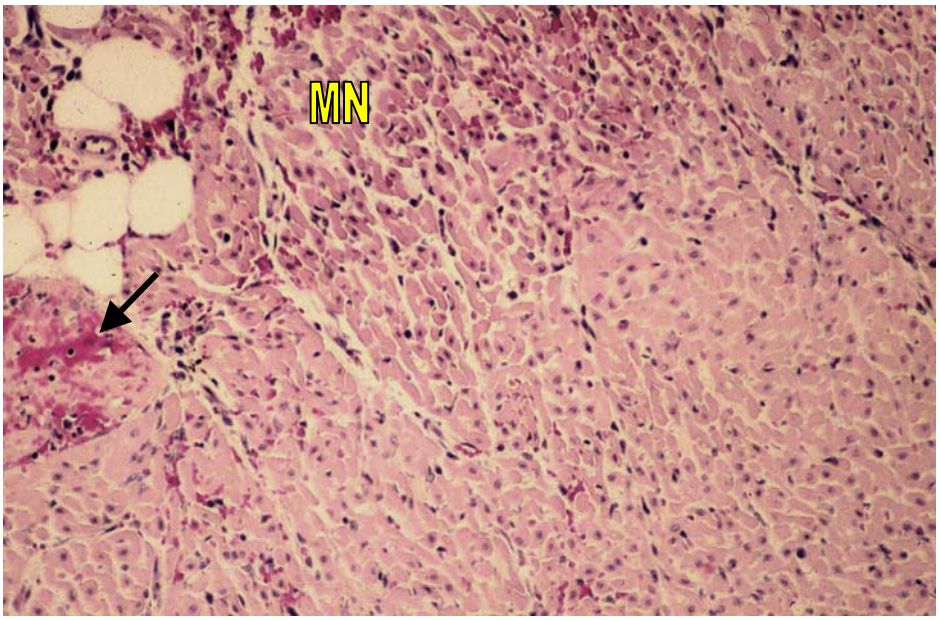


Figure 7: Histologic section of subepicardial myocardium (haematoxylin-eosin, x200) from the same dog on figures 1, and 6. On the right side of the picture there is blood vessel (BV) with a fibrin microthrombi, surrounded by severe necrotic myocardial necrosis (MN) and mixed inflammatory infiltrate composed of neutrophils (arrowhead) and macrophages (arrow). Note the hemorrhage at the bottom left corner.

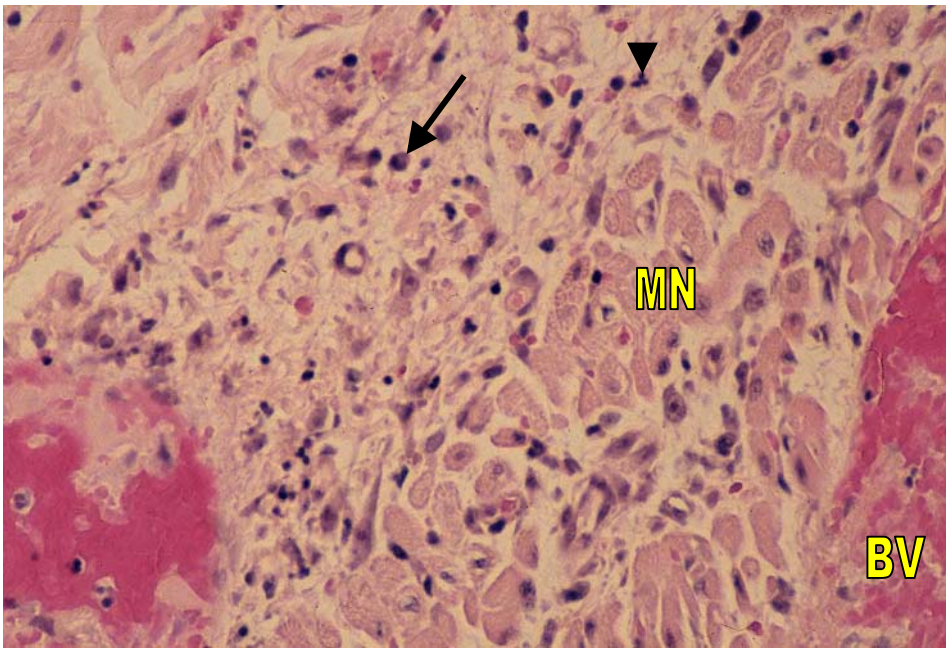


Figure 8: Histologic section of subepicardial myocardium (haematoxylin-eosin, x200) from the same dog as Figs. 1, 6 and 7. Myocardial haemorrhage is present throughout the section.

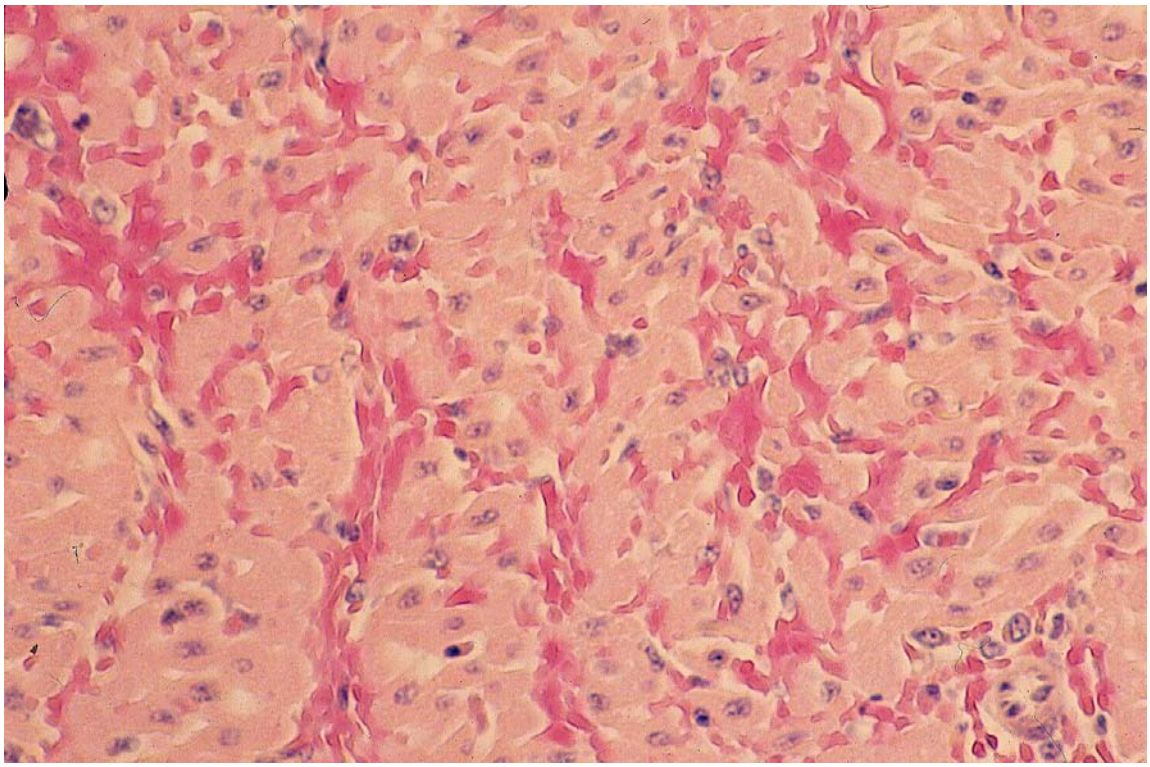


Figure 9: Histologic section of subepicardial fibrin thrombus from the same dog as Figs. 1, 6, 7 and 8. Figure 9A was done with a Martius-Scarlet-Blue stain (x100), which stains fibrin red, compared to figure 9B (haematoxylin-eosin, x100), where the microthrombus is pink, and it is difficult to differentiate fibrin from red blood cells.

Figure 9A:

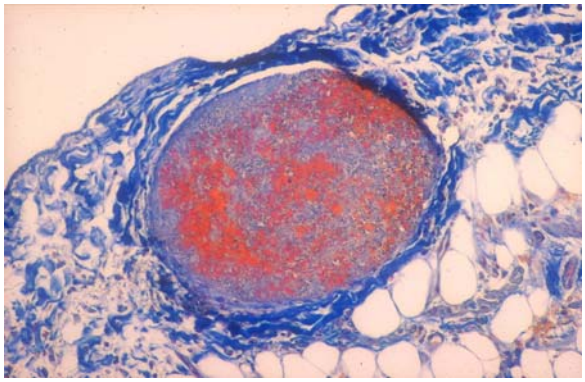
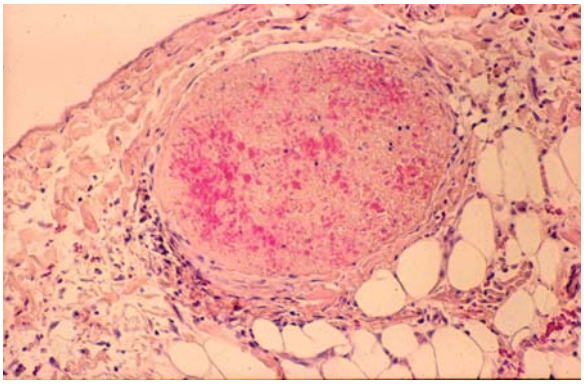


Figure 9B:



CHAPTER 6

DISCUSSION

6.1 Electrocardiographic changes

This study showed that ECG changes occur in canine babesiosis in up to 40% in some parameters. The more common changes were VPCs, SA block, low voltage R-amplitude, prolonged QRS, ST deviation and coving, and high T-amplitude. Irregular rhythm and bradycardia were only seen in the complicated and non-survivor groups.

The mean heart rate for the whole population was 137 (± 31) bpm, which is on the high end of the normal range, which was reported in septic conditions.[4] This rate results in increased cardiac output and enables the dog to compensate for the anaemia and metabolic effects of the disease.[55] The mean heart rate of the non-survivors was lower, 114 (± 40) bpm. Bradycardia was seen in 3 dogs, all of which were from the complicated group. Two of these dogs died naturally and 1 was euthanised, making the prevalence of bradycardia in the complicated and the non-survivor groups significantly higher than the expected normal population. The prevalence was significantly higher in the non-survivors compare with the survivors. Thus it would appear that bradycardia could be a poor prognostic indicator in canine babesiosis. Sinus bradycardia associated with syncope and sudden death has been reported in severe clinical cases of canine cardiomyopathy.[56] Bradyarrhythmia can have a negative clinical impact with even moderate physical activity, and a more severe physical challenge can cause cerebral hypoxia.[57] The same might occur with the energy requirements of a systemic disease such as complicated babesiosis, leading to the apparent poorer prognosis seen in this study. All the bradycardias in this study were sinus bradycardia, 2/3 had irregular sinus rhythm with SA block, and the third had a regular sinus rhythm. Sinus bradycardia may result from increased parasympathetic stimulation due to various causes such as gastrointestinal disturbances, respiratory disease, elevated intracranial pressure, hypothermia, hyperkalaemia, and various drugs. Alternatively primary pathology within the heart such as ischaemia or SA node disease can cause bradycardia.[58,59] All 3 dogs with bradycardia in this study had clinical and histological signs of cerebral babesiosis, which is associated

with poor prognosis.[60] As the conduction system was not examined in this study a primary involvement of the conduction system could not be ruled out. Conduction system involvement including the SA node has been reported in dogs with myocarditis.[19,20,61,62] All 3 dogs with bradycardia had cardiac abnormalities on necropsy including fibrin microthrombi in the left atrium in 1 dog, and macroscopic and microscopic haemorrhage, necrosis, inflammatory infiltrate and fibrin microthrombi in multiple sites in the 2 other dogs.

There was a significantly higher prevalence of irregular cardiac rhythm in the non-survivor group, which could be another poor prognostic indicator in canine babesiosis, as the haemodynamic consequences of irregular rhythm were associated with poor prognosis in canine dilated cardiomyopathy.[63] Eleven dogs in this study had irregular rhythm in 1 of the 2 ECGs. Nine of these dogs had arrhythmias: SA block in 7, second degree AV block in 1 and VPCs in 1. The other 2 dogs had irregular RR intervals with normal P-QRS-T configuration, which were not consistent with any classified arrhythmia.[64] Since the irregular rhythm had normal P-waves, it probably originated from irregular SA node stimulation. Therefore, it seems that the irregularities in this study resulted from conduction disturbances, especially the SA node. Of the dogs that had irregular rhythm 2 died and 2 were euthanised. Three of the 4 dogs had at least 1 abnormal histopathological finding. No correlation was found, however, between irregular rhythm and to any type of lesion or any specific site. Based on the ECG findings of conduction abnormalities associated with the irregular rhythm, the conduction system could have been involved in the inflammatory and necrosis observed in the myocardium.

Arrhythmia was diagnosed in 15/121 (12.4%) of the dogs on the first ECG, and in 10/63 (15.9%) on the second ECG. This is a higher prevalence compare to 0.6% that have been described for a group of 2710 dogs with no heart disease.[16] This prevalence is similar to what has been reported in severe falciparum malaria in man, but in that study, ECG was recorded over a 24 hours period.[46] In the latter study no death could be attributed to cardiac arrhythmia, which was similar to our study, as there was no significant differences in the prevalence of arrhythmia between survivors and non-survivors. The

only difference in the prevalence between the study groups was on the second ECG, were 8/10 dogs with arrhythmia cameg from the severe anaemia group (P=0.056, borderline significance). Reperfusion injury, is a possible cause for this difference.

Sinoatrial block and VPCs were the most common arrhythmias in this study seen in 9 dogs each. Sinoatrial block is a failure of the SA node to conduct a normal impulse, whereas sinus arrest is a failure to form an impulse. We did not differentiate between the 2 conditions and defined both of them as SA block, since the differences in their surface ECG features are inconclusive.[64] Sinoatrial block can be a normal incidental finding in the dog.[64,65] However, this is probably not the case in this study as: it was found in a relatively high percentage, the dogs were sick and stressed and thus expected to have high heart rate, and the SA blocks were detected during a relatively short recording time of 1 minute. Seven of the 9 dogs with SA block had normal P configuration, and 2/9 had an abnormal P mainly before an escape rhythm, suggestive of SA node pathology. Three of these dogs had necropsy: 1 showed no cardiac lesion, 1 had fibrin microthrombi in the left atrium, and the third had different types of lesions in multiple sites except the right atrium. Since the SA node is situated in the right atrium, and none of the dogs had right atrium pathology, these findings were inconclusive in terms of pathology associated with the SA block. However, there are a few reports in dogs with myocarditis associated with SA block,[66] sinus arrest,[62] and atrial standstill.[61] Sinoatrial node involvement has been described in Chagas' disease in dogs.[20] In man, concurrent ischaemia was commonly seen with sinus node dysfunction,[67] since the sinus node is particularly vulnerable to vascular occlusive disease.[68] With the high prevalence of myocarditis and necrosis in this study, similar sinus node pathology can not be ruled out, especially as all sings of sick sinus syndrome (sinus bradycardia, irregular sinus rhythm and SA block)[68] were seen in this study.

Sinoatrial block can be a result of extracardiac causes namely vagal irritation, drug toxicity or hyperkalaemia.[64] In this study 2 dogs with SA block had cerebral babesiosis and 1 had respiratory distress, both of which are potential causes of SA block due to vagal irritation, however, the rest of the dogs were uncomplicated cases (groups 1-3). In 3

dogs were plasma potassium concentration was measured, it was normal in all 3. It was therefore, not possible to implicate a specific cause to the SA block in this study.

Ventricular premature complexes, is a very non-specific arrhythmia that has been described in myocarditis from various causes,[16] myocardial infarcts and ischaemia,[14] septic conditions,[24,32-36] anaemia,[64] and acid base and electrolyte disturbances.[15,24] As all these conditions have been previously described in canine babesiosis, it was not surprising that the 10 dogs with VPCs or VT in this composed a very heterogeneous group: 4 were complicated, 3 severe anaemia, 2 IMHA, and 1 mild - moderate anaemia. In the complicated group all 4 had icterus, 2 had also concurrent respiratory distress and 1 had coagulopathy. One dog died and 1 was euthanised. The 1 that died had lesion at all sites including macro- and microscopic haemorrhages, fibrin microthrombi, multifocal necrosis and mixed inflammatory infiltrate limited to the left ventricle. The dog that was euthanised did not have any heart pathology. Six of the 10 dogs with VPCs or VT had plasma potassium concentration measured; 3 of which had low potassium and 3 were normal. Hypokalaemia has been reported to be associated with VPCs,[27] and had relatively high prevalence of association with VPCs in this study. It is a likely possibility that other electrolyte and acid base changes that occur in canine babesiosis may induce VPCs, however, as these parameters were not evaluated in this study, this question remains unanswered. It seems that the causes of VPCs and VT in this study are both cardiac and extracardiac.

Ventricular tachycardia was diagnosed in 2 dogs, of which only 1 was treated (with mexiletine). Both dogs showed apparent resolution of the arrhythmia after 24 hours. Ventricular tachycardia is defined as the most serious tachyarrhythmia,[24] and the fact that both dogs recovered so quickly supports the lack of correlation between arrhythmia and survival found in this study. This could indicate that, in canine babesiosis, the necessity of ECG for therapeutic considerations is limited. In an experimental study, VT with high survival rate occurred 20 – 24 hours post induction of right ventricular infarction.[69] Gross myocardial infarction was not found in this study but small multifocal areas of necrosis were found relatively common. There is a possibility that this type of necrosis could have

induced VT. All the above-mentioned causes for VPCs could also be associated with VT.[64]

Five dogs in this study had AV blocks: first degree in 2 and second degree in 3. These types of AV blocks can be an incidental findings in the normal dog,[65,70] but the dogs in the reported studies had a long time of acclimatization and the ECG recording was either long or frequent. This was not the case in this study, where the recording duration was relatively short, and the dogs were stressed and sick. A Mobitz type II AV block was seen in dogs, during an experimental study, after inducing ischaemic damage at the area of the proximal bundle of His and increased heart rate.[71] This condition could occur in babesiosis, but since all dogs with AV block survived and thus did not have necropsy, this explanation could not be challenged. Atrioventricular blocks (first and second) have been reported in acute and chronic Chagas' disease in association with inflammation and fibrosis of the conduction system,[18,20] and in other forms of myocarditis in the dog.[16] Alteration of potassium concentration can also block the AV conduction.[27] However, in 2/5 dogs plasma potassium concentration was measured and it was normal.

Low voltage R-amplitude was found in significant percentages in all study groups as well as survivors and non-survivors. Low voltage QRS is a common sign in pericardial effusion,[72] and in pleural effusion a correlation was found between the amount of fluid and the reduction of QRS-amplitude.[73] In this study, 4/11 of the dogs that had necropsy and valid ECG had small R-amplitude when recorded with filters. As 3/4 dogs with pericardial effusion had small R-amplitude and all 7 dogs that had no pericardial effusion had normal R-amplitude, the correlation between the presence of small R and pericardial effusion was statistically significant. Based on this correlation and the high prevalence of low R-amplitude in the dogs that did not have necropsy, the prevalence of pericardial effusion might have been much higher. Four survivors had survey thoracic radiographs taken and 2 of them showed mild pleural effusion. This further supports the assumption that effusion was also present in the survivors. Effusion into cavities have been described as rare in canine babesiosis,[5-7] but based on this study, it may have been overlooked. Electrical alternans is another sign of pericardial effusion which was seen in 5 dogs in

this study, however, there are conflicting results about its sensitivity.[72,74] In this study, none-of the dogs that had pericardial effusion on necropsy had electrical alternans, possibly because of the small volume. It was seen together with small R in 4/5 dogs. Two of these dogs were the dogs that showed evidence of mild pleural effusion on radiographs. Electrical alternans was also found to indicate cardiac electrical instability from various causes including ischaemia,[75] which is an important possible cause in this study.

Low-pass filters of the ECG can reduce the R-amplitude.[76-78] In this study, the filter effect was a facto, but low R-amplitude was also found when the filters were off. R-amplitude increased significantly 24 hours after initiation of treatment. The increase in R-amplitude indicated a possible influence of the disease process and indicated that the filter factor was not of major importance. For low-voltage R-amplitude to have electrophysiological importance other than axis deviation, it should be low in all leads,[54] as was found in this study. Small heart mass can cause low R-amplitude,[73] but there were no differences in body weight between the dogs with low or normal R-amplitude. The dogs with the low R was significantly younger, but this does not explain the increase of the R-amplitude after 24 hours.

Myocardial infarction can cause low voltage QRS.[54,79] It has been seen in clinical cases,[80] and is reported to be more common in left ventricular infarctions compared to right ventricle.[81] There are conflicting studies regarding R-wave amplitude alterations in experimental acute myocardial infarcts in dogs. In 1 study, R-amplitude following infarction decreased initially and later increased to a higher level.[82] Another study showed increased R-wave amplitude in the ischaemic area, but a decrease or no change in the border area.[83] All these studies investigated the alteration in R-amplitude during the first 5 minutes after induction of a myocardial infarction. Only 1 study investigated the changes in R-amplitude over a period of 5 hours of myocardial ischaemia in dogs, showed an immediate increase in amplitude followed by continuous loss of voltage.[84] This seems to be more applicable to canine babesiosis. Low voltage R-amplitude was also described in acute and chronic Chagas' disease together with decreased cardiac

function[18,20] and in other diffuse myocardial diseases.[85] In this study, all 4 dogs that showed low R-amplitude and died had macroscopic epicardial haemorrhages, however as 3/7 dogs with normal R-amplitude also had epicardial haemorrhages, this correlation was insignificant. Other histopathological findings namely necrosis in 2 dogs, inflammatory infiltrate in 2, microscopic haemorrhages in 4 and fibrin microthrombi in 3, were also not significantly correlated with the presence of low R-amplitude.

A possible mechanism for decreased R-amplitude in myocardial disease is a reduction in the ventricle activation process,[73] especially conductivity. An early study described the combination of prolonged, low voltage and notched QRS, all of which were seen in this study, as signs of myocardial disease and called them intraventricular block.[86] The R-amplitude is a sum of simultaneous fibre-to-fibre conduction through the ventricular myocardium from the subendocardial terminus of the Purkinje fibres to the epicardial surface.[87] Theoretically if either the simultaneity or the direction homogeneity of the conduction is disturbed, the R-amplitude should be lowered, a mechanism proposed to explain notching of the QRS.[88] Myocardial ischaemia or myocarditis are the main reason for low R due to decreased conductivity, and the alterations in R-amplitude in myocardial infarction were found are correlated to intramyocardial conduction time.[82,89] Another study suggested contractile failure as a cause for decreased R-wave amplitude.[90] The loss of R-amplitude was interpreted in 1 study as loss of electrical activity in the myocardium. The loss in R-amplitude was significantly correlated to creatine kinase depletion, indicating that myocardial damage is responsible for the loss of electrical activity.[84]

Low R-wave amplitude have been reported to in association with reduced left ventricular function.[91] Sepsis is reported to cause severe reduction in cardiac function,[31,39] and myocardial infarction can cause regional myocardial dysfunction resulting in dyssynergic contractions.[13,92] We postulated that the small R might have been a result of dyssynergic conduction. If so, dyssenergic contraction may follow. Electrocardiography is not the modality of choice to investigate mechanical cardiac function.[93] Heart failure has not been reported as complication of babesiosis, but cardiovascular functional studies are necessary to prove this.

Prolonged QRS was commonly seen in this study. This result, however, needs to be interpreted cautiously for the following reasons: 1) The QRS-duration was measured to the nearest 0.01 second, which compared with the measured range of QRS-duration (0.03-0.08 second) is not accurate enough for meaningful analysis. 2) The ECG filters can widen the QRS due to inadequate phase response.[94] 3) There was no change in the QRS-duration between the first and second ECG, implying that the initial prolongation could have been one of the above-mentioned technical causes rather than the disease process itself. Prolonged QRS represents delayed intraventricular conduction, and has been described in ventricular ischaemia and infarction,[69,82,83] myocarditis,[15,20] conduction blocks distal to the bundle of His, ventricular enlargement[54] and in hypo- or hyperkalaemia.[27] As discussed previously, other indications of ischaemia, necrosis, myocarditis and conduction alteration were seen in this study, and the prolongation of the QRS is consistent with these findings.

A notched R is a sign of myocardial intramural myocardial infarction, seen very often in canine experimental models of myocardial infarction.[54,95] The notch is derived from high frequency signals.[79] Vibrations of the wave of activation on the edge of infarction, competing generator sites within the ventricle myocardium, alteration in the radial spread of activation from endocardium to epicardium, and anisotrophy such as ventricular hypertrophy are all explanations for the appearance of notches.[88] It is possible that the electrocardiograph in our study (with or even without filters) was not sensitive enough to detect such notches and they were artifactual. However, the notches in this study were always seen at the end of the descending slope of the R-wave, a consistency, which would be unlikely for an artifact. Low-pass filters eliminate high frequency deflections such as notching and slurring, as described in the cat [78] and man. [76,77] On the other hand, filters can caused loss of the S-wave,[76] and slurring of the junction between the QRS and the ST,[77] which may cause a consistent artifactual notch. In this study, the R notches were evident with filtering, but even clearer without filters, supporting the likelihood that they had a real electrophysiologial meaning. Notched R was seen in significantly higher prevalence in the IMHA group. This may support the finding that IMHA is a common

cause of myocardial infarction in the dog.[26] Notching and slurring of the QRS complex has also been observed in dogs with Chagas' disease,[20] dogs with myocardial necrosis,[80] and other diffuse myocardial disease.[85] In this study, 2 dogs with notched R died. Both of them had inflammatory infiltrate, haemorrhages and fibrin microthrombi in various sites. One of the dogs also had necrosis in the apex.

There is no set standard for the dog's Q and S in canine ECG reference ranges.[54,96,97] Early studies reported a variety of ranges for Q-amplitude (0.05 – 1.2,[53] 0-0.51,[98] and 0-0.2mV.[99]) As none of the references gave details about filtering during the recording, this made it even more difficult to compare the Q-amplitudes observed in this study. Even in human medicine, where deep Q-wave is a diagnostic criterion for infarction, the definition of abnormality is vague, and includes the presence of Q, prolonged Q or deep Q.[100] In the absence of clear guidelines, this study evaluated the Q-wave relative to the R in lead II, and we noted the number of cases that had Q-wave bigger than ½ of the R. Forty dogs had a Q-wave bigger than ½ of the R, and 16 of them had a Q bigger than R.

High voltage Q-wave is rarely described in the canine literature. It was reported in dilation cardiomyopathy of English Cocker Spaniels in association with increased mass of the IV septum and the apical region of the left ventricle.[101] In these dogs, the high Q was accompanied by high R-amplitude, due to the increased mass that also caused right axis deviation. Pathologic Q was also reported in hypertrophic cardiomyopathy in both humans[102] and dogs,[54] but this is unlikely to be the cause of deep Q in this study. A big Q was also described in experimental studies in dogs heart that had complete heart block following ischaemia,[83] and in a case report on septic myocardial infarction with a deep QS.[103] In man, pathological Q, especially in the chest leads, is a diagnostic sign for myocardial infarction.[104] Transmural infarcts are more commonly associated with deep Q than subendocardial ones,[105] and left ventricular infarction is more commonly associated with deep Q compared to right ventricular infarction.[81] In man, abnormal Q was found to be indicative of more extensive myocardial damage and decreased cardiac wall motion,[106] and of general ischaemic conditions such as coronary artery disease and ischaemic cardiomyopathy.[107] One study reported a 100% sensitivity of pathological Q, ST

elevation or depression, or T abnormalities on the presenting ECG in people with acute cardiac ischaemia with syncope.[108] Since all these signs of myocardial ischaemia and infarction were found in this study, the finding of prominent Q, even without being completely standardised, supports the argument that the ECG pattern found in canine babesiosis is similar to the ECG pattern of myocardial ischaemia. The same set of signs was described in people suffering from viral myocarditis.[17] In myocarditis, Q-waves might indicate a more severe course in early illness.[109]

Five dogs showed S-amplitude ½ of the R-amplitude voltage or higher, and 4 of them had S-amplitude higher than the R (rS configuration). Deep S has been described in the dog with right heart enlargement and right bundle branch block, where it was reported together with prolonged QRS and right axis deviation.[54] In these 5 dogs the QRS-duration was normal and 4 dogs had left axis deviation, which also did not support right bundle branch block. Split R (R'), another sign suggesting right bundle branch block, was present in only 1 dog. It thus would appear that the deep Q and S in this study are associated with an axis deviation due to conduction abnormalities distal to the bundle branches or within the myocardium, similar to described for intraventricular block.[86] The wide range of axis deviations found in this study support this argument, rather than a block of a specific bundle branch or ventricular enlargement. Evidence for conduction abnormalities were seen with the other ECG findings in this study. Axis deviation has been reported in Chagas' disease,[18] and malaria,[51] both of which having similar cardiac lesions to canine babesiosis.

ST deviation, ST depression and ST coving are nonspecific signs of myocardial ischaemia in the dog,[54] ST changes have been described often in dogs that were used in experimental models of induced acute myocardial ischaemia.[27,69,83,110] Diagnosis of myocardial ischaemia, based on ST deviation in different leads, is routine in human cardiology.[100] ST depression has also been associated with reduction in left ventricular wall movement,[111] and has been reported in humans with malaria.[43] Despite that the use of ST in human medicine is based on canine experimental models, the sensitivity of ST alterations in canine medicine has been questioned.[65] However, it has been seen in dogs

with Chagas' disease,[20] myocardial infarctions[103] and atherosclerosis.[112] In this study, it is another sign supporting ischaemic changes within the myocardium in canine babesiosis. Another cause for ST deviation is ether hyper- or hypokalaemia,[27] however, no correlation between the 2 was found in this study.

T-amplitude was high (higher than R-amplitude/4) in a large percentage of dogs in this study, even when R-amplitude was recorded without filters. Abnormal and especially tall T-wave has been described for myocardial infarction and ischaemia in both humans and dogs,[54,103,104] however, T-wave abnormalities are considered of little diagnostic significance in canine clinical electrocardiography.[113] High T-wave is associated with hyperkalaemia.[27] In this study there was no correlation between the potassium concentration and the T-wave amplitude.

Attention has been given to drug effects on ECG in malaria.[28,114] The treatment in this study was not standarised and was individually tailored, but all dogs received antibabesial treatment (diminazine 59/62, imidocarb 2/62 and 1/62 was not recorded). There was no apparent ECG change that was specific for the second ECG (post treatment) and thus a drug effect is unlikely.

In summary, this study identified 2 ECG changes associated with poor prognosis, namely bradycardia and irregular rhythm. The other ECG changes represent a pattern that has previously been described in either myocarditis, especially Chagas' disease and myocardial ischaemia in both dogs and man. This pattern (small and notched R, deep Q, prolonged QRS and ST alterations) was evident in all the study groups, suggesting that it is associated with the pathophysiology of canine babesiosis in general and not with specific complication. These changes can be considered as normal variation in some dogs.[115] However, the fact that they were presented in consistent fashion and in relatively high prevalence in this study, indicates their pathophysiologic importance. The correlation between specific study groups and specific ECG changes was evident in very few parameters. This implies that there was no correlation between severity of the disease and the ECG changes. However, higher prevalence of notched R and ST alterations was

found in the IMHA group. This may indicate more ischaemia in this group, but there was no necropsy evidence to prove it. Based on this study the need for routine ECG monitoring for arrhythmia in canine babesiosis is questionable. For the other cardiac abnormalities, further studies of cardiac function are required to establish whether more specific treatment and monitoring are needed.

6.2 Cardiac pathology

Pericardial effusion was seen in 4 of the 16 dogs that had necropsy in this, and it was always clear and watery, consistent with the definition of hydropericardium.[116] In 1 study it was found to be the most common cardiac lesion in association with myocardial necrosis in dogs and cats.[80] In this study, only 1 dog with pericardial effusion had myocardial necrosis. A low albumin and increased capillary permeability, are 2 other possible mechanism of effusion, that have been described canine babesiosis.[3,6-8,117] The volume of fluid seen in the pericardium in this study was relatively small, and would not be expected to affect the heart function.[116]

Myocardial haemorrhages are seldom seen in dogs, and if seen are more common in the subepicardium.[9] However, in this study, they were not rare. Both macroscopic and microscopic haemorrhages did not show predilection for any specific site. Myocardial haemorrhages have been reported in hypoxia, acute infectious diseases,[116], and DIC,[11] all of which are part of the described pathogenesis of canine babesisis.[3] As coagulopathy was diagnosed, in this study, based on the presence of haemorrhages, it is not surprising that all dogs that had coagulopathy also had myocardial haemorrhages. Interesting is that 5/10 dogs with subepicardial haemorrhage, 6/9 with subendocardial haemorrhages and 6/11 with microscopic haemorrhages, did not have haemorrhages in any other organ. Haemorrhages limited to the epi- or the endocardial surface of the heart can be an agonal change, however this finding is relatively rare in the dog in comparison with horses and cattle,[116] and thus might instead indicate a specific babesiosis-related lesion.

Myocardial necrosis is a common, non-specific finding in systemic diseases, especially infectious and anaemic diseases. This necrosis is commonly diffuse,[116] while in this study the necrosis was commonly multifocal. Myocardial necrosis was seen in 8 dogs in this study and has previously been described in canine babesiosis.[7] Necrosis can only be apparent 12 hours after injury,[9,116] and could have been overlooked or underscored, because of the acute death seen in all cases in this study (all dogs died within 24 hours from hospitalisation). The nature of the necrotic lesion in our study, namely degeneration with inflammatory infiltrate but no fibroblasts, is consistent with acute necrosis of 12 hours to 4 days duration.[36] Myocardial necrosis can be a consequence of either coronary artery obstruction or inadequate oxygenation of the myocardial tissue.[80] Relatively common obstructive lesion in the dog and the cat are microthrombi as a result of hypercoagulable-state due to DIC or IMHA.[80] Endothelial injury is another cause of thrombosis,[116] and is assumed to occur in babesiosis.[11] Hypoxia associated with shock can also cause myocardial necrosis.[118]

There is an association between disseminated myocardial necrosis and central neurologic diseases,[116] possibly through neurologically induced catecholamine release causing coronary spasm.[118] Myocardial necrosis might also be induced by stress associated with a disease process.[118] Association between myocardial necrosis and brain lesions was described retrospectively in a large number of dogs. The brain lesion can be traumatic, infectious or space occupying.[119] Myocardial necrosis was also induced experimentally by creating intracranial haemorrhage in dogs.[120] This necrosis was accompanied by typical ECG changes and echocardiographic myocardial wall motion abnormalities.[120] The necrosis was multifocal and subepicardial, predominantly in the left ventricle.[119] In this study 3/8 dogs with myocardial necrosis had histologic lesions in the brain. Three other dogs, however, had brain lesions and no myocardial necrosis. No cause and effect relationship could thus be established. The acute death can also be a factor in the lack of association, since it there is a lag time of up to several days between the brain damage and the myocardial necrosis.[119]

All the conditions reported to be associated with myocardial necrosis, namely IMHA, DIC, brain involvement, hypoxia and shock are reported complications of babesiosis,[3] which were seen in this study. However, association between a specific complication and myocardial necrosis was not found. A cause and effect relationship between microthrombi and necrosis was not detected at any site, but of 10 sites with necrosis in this study, 7 also had fibrin microthrombi, indicating a possible association. Myocardial necrosis and infarctions in dogs are found predominantly in the subepicardium.[14] In necrosis due to hypoxia and arteriosclerosis, the site reported is predominantly the subendocardium of the papillary muscles of both ventricles.[118,121] This was not the case in this study and there was no predilection for any site of necrosis within the myocardium (e.g. endocardium, mid-myocardium or epicardium). The left ventricular papillary muscles were not overpresented, however combining the apex and the IV septum together with left papillary muscles as left ventricle sites revealed that 60% of necrosis was within the left ventricle. Thus, the distribution of necrosis is not classical to hypoxia and DIC and might represent a pattern of disseminated necrosis more typical to canine babesiosis.

Inflammatory changes in the myocardium are relatively frequent in the dog.[122] They are commonly secondary to myocardial necrosis or systemic infectious disease,[116] but are rarely considered as the primary disease.[122] The inflammatory infiltrate following necrosis is commonly neutrophilic.[80] In this study in 8 of the 10 sites with necrosis, inflammatory cells were present; however, there were 20 other sites where inflammatory infiltrate was seen without necrosis. The type of inflammatory cells included neutrophils and macrophages/lymphocytes in about the same numbers. Thus, besides the inflammatory reaction secondary to necrosis, there could have been an inflammatory reaction due to another process. Overwhelming inflammatory response was suggested as 1 of the mechanisms for tissue damage in canine babesiosis,[11] and it may be responsible for part of the myocardial inflammation seen in this study.

Fibrin microthrombi throughout the body are the hallmark of DIC.[123] In this study 12/16 dogs had myocardial fibrin microthrombi, but only 4 had coagulopathy and were

suspected to have DIC. However, the morphological diagnosis has serious limitations due to the fibrinolytic process that eliminates fibrin thrombi, and the similarities between fibrin thrombi and post-mortal clots.[123] Immune peroxidase stains are assumed to increase the sensitivity of the morphological diagnosis because they can react with fibrinogen and fibrin degradation products, but they are experimental at this stage.[124]

Immune mediated haemolytic anaemia is another condition associated with myocardial thrombosis[14,80] that has been described in canine babesiosis. Fibrin microthrombi containing aggregates of parasitised erythrocytes, is another possible cause for fibrin microthrombi.[6] Parasites were seen in capillaries within the myocardium in this study, but parasitic plaques similar to the description above were not identified.

In summary, myocardial lesions were seen in high prevalence in this study, but they were all non-specific lesions. It can, however, justifiably be said that the heart suffers from the same pathological processes, which have been described for other organs involvement in canine babesiosis, namely inflammatory reaction and ischaemia.[3]

The study showed no correlation between ECG changes and histopathological changes. This is consistent with the literature and other similar studies.[36,93] It reflects the non-specific properties of the ECG, which cannot differentiated cardiac and extracardiac causes of altered heart conduction, as well as the fact that the numbers of necropsies in clinical studies is always limited. Heart pathology is also often non-specific, since there are similar changes involved in many disease and agonal processes.[116,118] The changes seen in these studies are multifactorial, and canine babesiosis has a variety of metabolic and pathologic abnormalities that can contribute to both ECG and pathological changes.[3,118]

The long-term implications of the cardiac pathology detected in this study were not investigated. Chronic heart complications are exceptionally rare in malaria.[50] The prevalence of chronic myocardial diseases in our hospital is low (0.03%) comparing to the literature (0.5-1.1%),[125] which may also indicate a lack of chronic changes from

babesiosis, despite the high prevalence of the disease. A follow up study would be required to prove this argument.

CHAPTER 7

CONCLUSIONS

- Electrocardiographic changes were found in canine babesiosis.

- The following ECG changes were recorded: sinoatrial (7%) and atrioventricular blocks (4%), ventricular premature complexes (7%), low R-amplitude (23%), prominent Q (33%), axis deviations (40%), prolonged QRS (32%), ST depression and coving (28%), large T (42%), and notched R (28%). This pattern of changes has been described commonly in myocardial ischaemia, infarction and inflammation.

- The correlation between specific study groups and specific ECG changes was evident in very few parameters, namely higher prevalence of notched R and ST alterations in the IMHA group. This may indicate more myocardial ischaemia in this group.

- Changes over 24 hours were decrease in heart rate and increase in the R-amplitude.

- Irregular rhythm and sinus bradycardia were seen in significantly higher prevalence in the non-survivors, and appeared to be poor prognostic indicators. However, their prevalence in the whole study population was relatively low.

- No correlation was found between plasma potassium concentration and the ECG changes.

- Gross pathological changes were pericardial effusion (25%) and subepicardial (56%) and subendocardial haemorrhages (63%). Histological changes were haemorrhages (69%), necrosis (50%), inflammation (63%) and fibrin microthrombi (75%).

- The only correlation between pathology and ECG was low R-amplitude and pericardial effusion, but the number of dogs with valid ECG and necropsy was small (11), making correlation difficult.

- Based on this study, the clinical value of ECG monitoring in canine babesiosis is limited, and antiarrhytmic treatment is unlikely to be required. Further studies regarding cardiac function are thus needed, and the necessity for cardiovascular management should be based on those studies rather than ECG.

References

1. Taboada J, Merchant SR. Babesiosis of companion animals and man. Veterinary Clinics of North America: Small Animal Practice 1991; 21:103-123.

2. Lobetti RG. Canine babesiosis. Compendium on Continuing Education for the Practicing Veterinarian 1998; 20:418-430.

3. Jacobson LS, Clark IA. The pathophysiology of canine babesiosis: New approaches to an old puzzle. Journal of the South African Veterinary Association 1994; 65:134-145.

4. Bone RC, Balk RA, Cerra FB, Dellinger RP, Fein AM, Knasus WA, et al. Definitions for sepsis and organ failure and guidlines for the use of innovative therapies in sepsis. The ACCP/SCCM Consensus Conference Committee. American College of Chest Physicians/Society of Critical Care Medicine. Chest 1992; 101:1644-1655.

5. Moore DJ, Williams MC. Disseminated intravascular coagulation: A complication of *Babesia canis* infection in the dog. Journal of the South African Veterinary Association 1979; 50:265-275.

6. Gilles HM. Pathological processes in *Babesia canis*. 1951; Oxford University. BSc.

7. Breitschwerdt EB. Babesiosis. In: Greene CE, editor. Infectious diseases of the dog and cat. 1st ed. Philadelphia: W.B. Saunders Co., 1990:796-803.

8. Maegraith B, Gilles HM, Devakul K. Pathological processes in *Babesia canis* infections. Zeitschrift für Tropenmedizin and Parasitologie 1957; 8:485-514.

9. Hildebrandt PK. The organ and vascular pathology of babesiosis. In: Ristic M, Kreier JP, editors. Babesiosis. 1st ed. New York: Academic Press, 1981:459-473.

10. Wright IG, McKenna RV, Goodger BV. Acute *Babesia bovis* infections: plasma creatine kinase, lactate dehydrogenase and creatinine levels and associated muscle damage. Zeitschrift für Parasitenkunde 1981; 64:297-302.

11. Reyers F, Leisewitz AL, Lobetti RG, Milner RJ, Jacobson LS. Canine babesiosis in South Africa: more than one disease. Does this serve as a model for falciparum malaria? Annals of Tropical Medicine and Parasitology 1998; 92:503-511.

12. Boyden PA. Cellular electrophysiologic basis of cardiac arrhythmia. American Journal of Cardiology 1996; 78:4-11.

13. Rakita L, Vrobel T, Kaufman ES. Electrocardiography. In: Ayers ST, Grenvik A, Holbrook PR, Shoemaker WC, editors. Critical Care. 3rd ed. Philadelphia: WB Saunders Co, 1995:492-497.

14. Driehuys S, Van Winkle TJ, Sammarco CD, Drobatz KJ. Myocardial infarction in dogs and cats: 37 cases (1985-1994). Journal of the American Veterinary Medical Association 1998; 213:1444-1448.

15. Keim S. Conduction disturbances in the critically ill. In: Ayers ST, Grenvik A, Holbrook PR, Shoemaker WC, editors. Critical Care. 3 ed. Philadelphia: WB Saunders Co, 1995:497-502.

16. Patterson DF, Detweiler DK, Hubben K, Botts RP. Spontaneous abnormal cardiac arrhythmias and conduction disturbances in the dog a clinical and pathologic study of 3000 dogs. American Journal of Veterinary Research 1961; 22:355-369.

17. Dec GWJ, Waldman H, Southern J, Fallon JT, Hutter AMJ, Palacios I. Viral myocarditis mimicking acute myocardial infarction. Journal of the American College of Cardiology 1992; 20:85-89.

18. Barr SC, Holmes RA, Klei TR. Electrocardiographic and echocardiographic features of trypanosomiasis in dogs inoculated with North American *Trypanosoma cruzi* isolates. American Journal of Veterinary Research 1992; 53:521-527.

19. Laranja FS, Dias E, Nobrega G, Miranda A. Chagas' disease a clinical, epidemiologic, and pathologic study. Circulation 1956; 14:1035-1060.

20. Andrade ZA, Andrade SG, Maguire JM. Experimental Chagas' disease in dogs. Archives of Pathology and Laboratory Medicine 1981; 105:460-464.

21. Jacobson LS, Lobetti RG, Vaughan-Scott T. Blood pressure changes in dogs with babesiosis. Journal of the South African Veterinary Association 2000; 71:14-20.

22. Button C. Metabolic and electrolyte disturbances in acute canine babesiosis. Journal of the American Veterinary Medical Association 1979; 175:475-479.

23. Forrest DM, Walley KR, Russell JA. Impact of acid-base disorders on individual organ system. In: Ronco C, Bellomo R, editors. Critical Care Nephrology. 1st ed. Dordrecht: Kluwer Academic Publishers, 1998:316-326.

24. Lunney J, Ettinger SJ. Cardiac arrhythmias. In: Ettinger SJ, Feldman EC, editors. Textbook of Veterinary Internal Medicine. 4th ed. Philadelphia: W.B. Saunders, 1995:959-995.

25. Leisewitz AL, Guthrie AJ, Berry WL. Evaluation of the effect of whole-blood transfusion on the oxygen status and acid-bace balance of *Babesia canis* infected dogs using the oxygen status algorithm. Journal of the South African Veterinary Association 1996; 67:20-26.

26. Irwin PJ, Hutchinson GW. Clinical and pathological findings of *Babesia* infection in dogs. Australian Veterinary Journal 1991; 68:204-209.

27. Feldman EC, Ettinger SJ. Electrocardiographic changes associated with electrolyte disturbances. Veterinary Clinics of North America: Small Animal Practice 1977; 7:487-496.

28. Bertrand E, Clerc G, Renambot J, Odi Assamoi J, Chauvet J. 50 cases of acute malaria: symptomatic study, especially cardiac. Bulletin de la Sociêtê de Pathologie Exotique 1975; 68:456-466.

29. Kumar A, Brar R, Wang P, Dee L, Skorupa G, Khadour F, et al. Role of nitric oxide and cGMP in human septic serum-induced depression of cardiac monocyte contractility. American Journal of Physiology - Regulatory Integrative & Comparative Physiology 1999; 45:265-276.

30. White JN. Malaria. In: Cook GC, editor. Manson's Tropical Diseases. 20th ed. London: WB Saunders Co, 1996:1087-1164.

31. Parker MD. The heart in sepsis. In: Ayers ST, Grenvik A, Holbrook PR, Shoemaker WC, editors. Critical Care. 3rd ed. Philadelphia: WB Saunders Co, 1995:596-604.

32. Marretta SM, Matthiesen DT, Nichols R. Pyometra and its complications. Problems in Veterinary Medicine 1989; 1:50-62.

33. Davidson JR, Lantz GC, Salisbury SK, Kazacos EA, Bottoms GD. Effects of flumixin meglumine on dogs with experimental gastric dilatation-volvulus. Veterinary Surgery 1992; 21:113-120.

34. Muir WW. Gastric dilatation-volvulus in the dog, with emphasis on cardiac arrhythmias. Journal of the American Veterinary Medical Association 1982; 180:739-742.

35. Muir WW, Lipowitz AJ. Cardiac dysrhythmias associated with gastric dilatation-volvulus in the dog. Journal of the American Veterinary Medical Association 1978; 172:683-688.

36. Muir WW, Weisbrode SE. Myocardial ischemia in dogs with gastric dilatation-volvulus. Journal of the American Veterinary Medical Association 1982; 181:363-366.

37. McDonald RK, Roudebush P. ECG of the month. Journal of the American Veterinary Medical Association 1988; 192:1534-1535.

38. Singh K, Krishnamurthy D, Peshin PK. Endotoxic shock with and without dexamethasone pretreatment in anaesthetized buffalo calves-II: Haemodynamics, acid-base and blood gas changes. Indian Journal of Veterinary Surgery 1997; 18:8-11.

39. Parker MD, Shelhamer JR, Natanson C, Alling DW, Parrilo JE. Serial cardiovascular variables in survivors and nonsurvivors of human septic shock: Heart rate as an early predictor of prognosis. Critical Care Medicine 1987; 15:923-930.

40. Solomon MA, Correa R, Alexander HR, Koev LA, Cobb JP, Kim DK. Myocardial energy metabolism and morphology in a canine model of sepsis. American Journal of Physiology 1994; 266:H755-H768

41. Ristic M, Kreier JP. Malaria and babesiosis: similarities and differences. In: Ristic M, Ambroise-Thomas P, Kreier J, editors. Malaria and babesiosis research finding and control measures. 1st ed. Dordrecht: Martinus Nijhoff Publishers, 1984:3-33.

42. Merkel WC. *Plasmodium falciparum* malaria. Archives of Pathology 1946; 41:290-298.

43. Franzen D, Curtius JM, Heitz W, Hoof HW, Diehl V, Hilger HH. Cardiac involvement during and after malaria. Clinical Investigator 1992; 70:670-673.

44. Boonpucknaving V, Boonpucknaving S. The histopathology of malaria. In: Wernsdorfer WH, McGregor SI, editors. Malaria - Principles and practice of malariology. 1st ed. Edinburgh: Churchill Livingstone, 1988:673-708.

45. Sprague HB. The effect of malaria on the heart. American Heart Journal 1946; 31:426-430.

46. Bethell DB, Phuong PT, Phuong CXT, Nosten F, Waller D, Davis TME, et al. Electrocardiographic monitoring in severe falciparum malaria. Transactions of the Royal Society Tropical Medicine and Hygiene 1996; 90:266-269.

47. Rojas RA, Dezza D. Cardiac changes in malarial patients. American Heart Journal 1947; 33:702-703.

48. Charles D, Bertrand E. The heart and malaria. Medecine Tropicale 1982; 42:405-409.

49. Macpherson GG, Warrell MJ, White JN, Looareesuwan S, Warrell DA. Human cerebral malaria. American Journal of Pathology 1985; 119:385-401.

50. Sharma SN, Mohapatra AK, Machave YV. Chronic falciparum cardiomyopathy. Journal of the Association of Physician India 1987; 35:251-252.

51. Simonson E, Keys A. Experimental malaria in man. III. The changes in the electrocardiogram. Journal of clinical investigation 1950; 29:68-71.

52. Stout TT, van Bergeijk L, Bouma HG, Woittiez AJ. Electrocardiographic changes in acute malaria. Netherlands Journal of Medicine 1990; 37:124-128.

53. Hill JD. The electrocardiogram in dogs with standardized body and limb positions. Journal of Electrocardiology 1968; 1:175-182.

54. Tilley LP. Analysis of the canine P-QRS-T deflections. In: Tilley LP, editor. Essentials of Canine and Feline Electrocardiography Interpretation and Treatment. 3 ed. Philadelphia: Lea & Febiger, 1992:59-99.

55. Tilley LP. Pathophysiologic basis and hemodynamic effects of cardiac arrhythmias. In: Tilley LP, editor. Essentials of Canine and Feline Electrocardiography Interpretation and Treatment. 3 ed. Philadelphia: Lea & Febiger, 1992:255-273.

56. Calvert CA, Jacobs GJ, Pickus CW. Bradycardia-associated episodic weakness, syncope, and aborted sudden death in cardiomyopathyc Doberman pinchers. Journal of Veterinary Internal Medicine 1996; 10:88-93.

57. Rishniw M, Thomas WP. Bradyarrhythmias. In: Bonagura JD, editor. Kirk's Current Veterinary Therapy XIII Small Animal Practice. Philadelphia: W.B. Saunders, 2000:719-725.

58. Mangrum JP, DiMarco JP. The evaluation and management of bradycardia. The New England Journal of Medicine 2000; 342:703-709.

59. Moíse NS. Diagnosis and management of canine arrhythmias. In: Fox PR, Sisson D, Moíse NS, editors. Textbook of Canine and Feline Cardiology Principles and Clinical Practice. 2 ed. Philadelphia: W.B. Saunders, 1999:331-385.

60. Welzl C, Leisewitz AL, Jacobson LS, Vagughan-Scott T. Systemic inflammatory response syndrome in canine babesiosis. Proceedings, 9th Annual Congress of the European Society of Veterinary Internal Medicine; Peruga, Italy: 1999:49-50.

61. Jeraj K, Ogburn PN, Edwards WD, Edwards JE. Atrial standstill, myocarditis and destruction of cardiac conduction system: Clinicopathologic correlation in a dog. American Heart Journal 1980; 99:185-192.

62. Robinson WF, Thompson RR, Clarck WT. Sinoatrial arrest associated with primary atrial myocarditis in a dog. Journal of Small Animal Practice 1981; 22:99-107.

63. Calvert CA, Pickus CW, Jacobs GJ, Brown J. Signalment, survival and prognostic factors in Doberman pinchers with end-stage cardiomyopathy. Journal of Veterinary Internal Medicine 1997; 11:323-326.

64. Tilley LP. Analysis of the common canine cardiac arrhythmias. In: Tilley LP, editor. Essentials of Canine and Feline Electrocardiography Interpretation and Treatment. 3 ed. Philadelphia: Lea & Febiger, 1992:127-207.

65. Eckenfels A, Trieb G. The normal electrocardiogram of the conscious Beagle dog. Toxicology and Applied Pharmacology 1978; 47:567-584.

66. Kittleson MD, Schukei TW. Sinoatrial block in a dog. Journal of the American Veterinary Medical Association 1980; 177:332-334.

67. Simonsen E, Nielsen JS, Nielsen BL. Sinus node dysfunction in 128 patients a retrospective study with follow-up. Acta Medica Scandynavia 1980; 208:343-348.

68. Scarpa WJ. The sick sinus syndrome. American Heart Journal 1976; 92:648-660.

69. Sugi K, Karaguezian HS, Fishbein MC, McCullen A, Sato Y, Ganz W, et al. Spontaneous ventricular tachycardia associated with isolated right ventricular infarction, one day after right coronary artery occlusion in the dog: Studies on the site of origin and mechanism. American Heart Journal 1985; 109:232-244.

70. Branch CE, Robertson BT, Williams JC. Frequency of second-degree atrioventricular heart block in dogs. American Journal of Veterinary Research 1975; 36:925-929.

71. Gonzalez MD, Scherlag BJ, Mabo P, Lazzara R. Functional dissociation of cellular activation as a mechanism of Mobitz type II atrioventricular block. Circulation 1993; 87:1389-1398.

72. Berg RJ, Wingfield W. Pericardial effusion in the dog: A review of 42 cases. Journal of the American Animal Hospital Association 1984; 20:721-730.

73. Rush JE, Hamlin RL. Effect of graded pleural effusion on QRS in the dog. American Journal of Veterinary Research 1985; 46:1887-1891.

74. Bonagura JD. Electrical alternans associated with pericardial effusion in the dog. Journal of the American Veterinary Medical Association 1981; 178:574-579.

75. Smith JM, Clancy EA, Valeri CR, Ruskin JN, Cohen RJ. Electrical alternans and cardiac electrical instability. Circulation 1988; 77:110-121.

76. Berson AS, Pipberger HV. Electrocardiographic distortions caused by indequate high-frequency response of direct-writing electrocardiographs. American Heart Journal 1967; 74:208-218.

77. Dower GA, Moore DA, Ziegler WG, Osborne JA. On QRS amplitude and other errors produced by direct-writing electrocardiographs. American Heart Journal 1963; 65:307-321.

78. Schrope DP, Fox PR, Hahn AW, Bond B, Rosenthal S. Effects of electrocardiograph frequency filters on P-QRS-T amplitudes of the feline electrocardiogram. American Journal of Veterinary Research 1995; 56:1534-1540.

79. Goldberger AL, Bhargava V, Froelicher V, Covell J, Mortara D. Effect of myocardial infarction on the peak amplitude of high frequency QRS potentials. Journal of Electrocardiology 1980; 13:367-372.

80. Kidd L, Stepien RL, Amrheiw DP. Clinical findings and coronary artery disease in dogs and cats with acute and subacute myocardial necrosis: 28 cases. Journal of the American Animal Hospital Association 2000; 36:199-208.

81. Lewin RF, Arditti A, Strasberg B, Sclarovsky S, Mager A, Hellman C, et al. Predominant right ventricular infarction. Clinical and electrocardiographic features. European Heart Journal 1986; 7:425-430.

82. David D, Naito M, Michelson E, Watanabe E, Chen CC, Morganroth J, et al. Intramyocardial conduction: A major determinant of R-wave amplitude during acute myocardial ischaemia. Circulation 1982; 65:161-167.

83. Ino-Oka I, Maehara K, Kyono H, Yoshikata S, Shimizu Y, Tamotsu T. The effect of ventricular conduction velocity on ST-segment level in acute myocardial ischaemia. Japanese Heart Journal 1986; 27 Supplement:245-254.

84. Selwyn A, Welman E, Jonathan A. Electrocardiographic signs in experimental myocardial ischemia and infarction. European Journal of Cardiology 1978; 8:185-196.

85. Reynolds EW, Muller BF, Captain MC, Anderson GJ, Muller BT. High frequency component in the electrocardiogram a comparative study of normals and patients with myocardial disease. Circulation 1967; 35:195-206.

86. Oppenheimer BS, Rothschild MA. Electrocardiographic changes associated with myocardial involvement. Journal of the American Medical Association 1917; 69:429-431.

87. Hamlin RL, Smith CR. Anatomical and physiological basis for interpretation of the electrocardiogram. American Journal of Veterinary Research 1960; 21:701-708.

88. Flowers NC, Horan LG, Thomas JR, Tolleson WJ. The anatomic basis for high-frequency components in the electrocardiogram. Circulation 1969; 39:531-539.

89. David D, Naito M, Chen CC, Michelson E, Morganroth J, Schaffenburg M. R-wave amplitude variations during acute experimental myocardial ischemia: an inadequate index for changes in intracardiac volume. Circulation 1981; 63:1364-1371.

90. Nakagomi A, Takashi S, Kimura Y, Tamura Y, Miura M. Altered electrical and metabolic response of reperfused myocardium to ischaemia after recovery from preceeding ischaemia: evidence for ischaemia sensitised myocardium. Cardiovascular Research 1993; 27:-571

91. Panciera DL. An echocardiographic and electrocardiographic study of cardiovascular function in hypothyroid dogs. Journal of the American Veterinary Medical Association 1994; 205:996-1000.

92. Liu S, Fox PR. Myocardial ischemia and infarction. In: Bonagura JD, editor. Kirk's Current Veterinary Therapy XI Small Animal Practice. Philadelphia: W.B. Saunders, 1992:791-795.

93. Tilley LP. Principles of electrocardiographic recording. In: Tilley LP, editor. Essentials of Canine and Feline Electrocardiography Interpretation and Treatment. 3 ed. Philadelphia: Lea & Febiger, 1992:21-39.

94. Ditayler RV. Artefactual ST segment abnormalities due to electrocardiograph design. British Heart Journal 1985; 54:121-128.

95. Langer PH, DeMolt T, Hussey M. High-fidelity electrocardiography: Effects of induced localized myocardial injury in the dog. American Heart Journal 1966; 71:790-796.

96. Ettinger SJ, Bobinnec G, Côte E. Electrocardiography. In: Ettinger SJ, Feldman EC, editors. Textbook of Veterinary Internal Medicine. 5 ed. Philadelphia: W.B. Saunders, 2000:800-833.

97. Miller MS, Tilley LP, Smith FWK, Fox PR. Electrocardiography. In: Fox PR, Sisson D, Moíse NS, editors. Textbook of Canine and Feline Cardiology Principles and Clinical Practice. 2 ed. Philadelphia: W.B. Saunders, 1999:67-106.

98. Horwitz SA, Spanier MR, Wiggers HC. The electrocardiogram of the normal dog. Proceedings of the Society for Experimental Biology and Medicine 1953; 84:121-125.

99. Petersen ES, Ricketts HT, Brewer NR, Lints HA, Test CE, Tupikova NA. Electrocardiogram of the beagle dog. Proceedings of the Society for Experimental Biology and Medicine 1951; 77:330-332.

100. Finch C. Electrocardiography and vectorcardiography. In: Braunwald E, editor. Heart Diseases A Textbook of Cardiovascular Medicine. 3 ed. Philadelphia: W.B. Saunders, 1988:180-222.

101. Gooding JP, Robinson WF, Mews GC. Echocardiographic characterization of dilatation cardiomyopathy in the English cocker spaniel. American Journal of Veterinary Research 1986; 47:1978-1983.

102. Tendera M, Polonski L, Wodniecki J, Kardaszewicz P. Evolution of the electrocardiographic changes in patients with hypertrophic cardiomyopathy. Clinical Cardiology 1991; 14:891-896.

103. Fregin GF, Luginbuhl H, Guarda F. Myocardial infarction in a dog with bacterial endocarditis. Journal of the American Veterinary Medical Association 1972; 160:956-963.

104. The Joint European Society of Cardiology and the American College of Cardiology. Myocardial infarction redefined - a consensus document of the Joint European Society of Cardiology/American College of Cardiology Committee for the redefinition of myocardial infarction. Journal of the American College of Cardiology 2000; 36:959-969.

105. Antaloczy Z, Barcsak J, Magyar E. Correlation of electrocardiologic and pathologic findings in 100 cases of Q wave and non-Q wave myocardial infarction. Journal of Electrocardiology 1988; 21:331-335.

106. Terrosu P, Ibba GV, Franceschino V, Contini GM, Delpini A, Pes R. Angiographic correlate of post-reperfusion abnormal Q waves. Japanese Heart Journal 1988; 29:179-187.

107. Feld H, Priest S, Denson M. Importance of pathologic Q waves in patients with dilated cardiomyopathies. American Journal of Medicine 1993; 94:546-548.

108. Georgeson S, Linzer M, Griffith JL, Weld L, Selker HP. Acute cardiac ischemia in patients with syncope: importance of the initial electrocardiogram. Journal of General Internal Medicine 1992; 7:379-386.

109. Nakashima H, Katayama T, Ishizaki M, Takeno M, Honda Y, Yano K. Q wave and non-Q wave myocarditis with special reference to clinical significance. Japanese Heart Journal 1998; 39:763-774.

110. Mirvis DM, Ramanathan KB, Wilson JL. Regional blood flow correlates of ST segment depression in tachycardia-induced myocardial ischemia. Circulation 1986; 73:365-373.

111. Pichler M, Shah PK, Peter T, Singh B, Berman D, Shellock F, et al. Wall motion abnormalities and electrocardiographic changes in acute transmural myocardial infarction: implications of reciprocal ST segment depression. American Heart Journal 1983; 106:1003-1009.

112. Liu S, Tilley LP, Tappe JP, Fox PR. Clinical and pathological findings in dogs with atherosclerosis: 21 cases (1970-1983). Journal of the American Veterinary Medical Association 1986; 189:-227

113. Hahn AW, Hamlin RL, Patterson DF. Standards for canine electrocardiography. 1977; The Academy of Veterinary Cardiology Committee Reports.

114. Monlun E, Le Metayer P, Szwandt S, Neau D, Longy-Boursier M, Horton J, et al. Cardiac complications of halofantrine: a prospective study of 20 patients. Transactions of the Royal Society Tropical Medicine and Hygiene 1995; 89:430-433.

115. Detweiler DK. The dog electrocardiogram: A critical review. In: MaCfarlane PW, Lawrie TDV, editors. Comprehensive Electrocardiology Theory and Practice in Health and Disease. New York: Pergamon Press, 1988:1267-1329.

116. Robinson WF, Maxie MG. The cardiovascular system. In: Jubb KVF, Kennedy PC, Palmer N, editors. Pathology of Domestic Animals. 4 ed. San Diego: Academic Press, 1993:1-100.

117. Lobetti RG, Möhr AJ, Dippenaar T, Myburgh E. A preliminary study on the serum protein response in canine babesiosis. Journal of the South African Veterinary Association 2000; 71:38-42.

118. Van Fleet JF, Ferrans VJ. Myocardial diseases of animals. American Journal of Pathology 1986; 124:98-178.

119. King JM, Roth L, Haschek WM. Myocardial necrosis secondary to neural lesions in domestic animals. Journal of the American Veterinary Medical Association 1982; 180:144-148.

120. Elrifai A, Bailes JE, Shin S, Dianzumba S, Brillman J. Characterization of the cardiac effects of acute subarachnoid hemorrhage in dogs. Stroke 1996; 27:737-741.

121. Falk T, Jönsson L. Ischaemic heart disease in the dog: a review of 65 cases. Journal of Small Animal Practice 2000; 41:97-103.

122. Luginbühl H, Detweiler DK. Cardiovascular lesions in dogs. Annals New York Academy of Sciences 1965; 127:517-540.

123. Schiefer B, Seracy G. Disseminated intravascular coagulation and consumption coagulopathy. The Canadian Veterinary Journal 1975; 16:151-159.

124. Shimamura K, Kozima M. Removal of fibrin thrombi in disseminated intravascular coagulation (DIC). Archives of Pathology and Laboratory Medicine 1981; 105:659-663.

125. Sisson D, O'Grady MR, Calvert CA. Myocardial diseases of dogs. In: Fox PR, Sisson D, Moíse NS, editors. Textbook of Canine and Feline Cardiology Principles and Clinical Practice. 2 ed. Philadelphia: Saunders, 1999:581-619.