THE SCINTIGRAPHIC EVALUATION OF THE PULMONARY PERFUSION PATTERN OF DOGS HOSPITALISED WITH BABESIOSIS

by

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Submitted in partial fulfilment of the requirements for the degree of MMedVet (Diagnostic Imaging) in the Faculty of Veterinary Science, University of Pretoria

Pretoria

October 2007

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Dedication

To Gert, Lyné and little Annika

Acknowledgements

I am indebted to the following people for their knowledge, advice, hard work, dedication, patience and valuable inputs:

Ms Elmaré Kilian for her never-ending enthusiasm, hard work, loyal support, dedication and friendship

The radiographers, Bev Olivier, Liani Kitshoff, Melanie McLean, and radiography assistants, Jerry Khoza, Hermans Mampsa and John Masola of the Onderstepoort Veterinary Academic Hospital for their hard work and willingness to always help, even at awkward times

The numerous final year students and nurses who assisted in the handling of patients

The UPBRC staff, and especially Mr Mario Smuts, for the supply of, and excellent care and management of the control dogs

The Section of Clinical Pathology, Department of Companion Animal Clinical Studies, with special thanks to Mrs Elsbé Myburg

The Section Pathology, Department of Production Animal Clinical Studies, with special thanks to Dr Sarah Clift

The numerous clinicians of the Section Small Animal Medicine, Department of Companion Animal Clinical Studies for their assistance and excellent patient care

Ms René Ehlers and Mrs Rina Owen for performing the statistical analysis

Dr Frans Naudé for his expertise and assistance in the classification of the scintigraphic images

Prof Andrew Leisewitz and Prof Irene Dormehl for their valuable inputs and patience

My supervisor, Prof Robert Kirberger, for his continued support, advice and mentorship and for sharing with me his incredible knowledge of diagnostic imaging

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Glossary

Abbreviations used in the text:

^{81m}Kr	Krypton-81m
^{99}Mo	Molybdenum-99
^{99m}Tc	Technetium-99m
ARDS	Acute respiratory distress syndrome
B	*Babesia*
bpm	Beats per minute
CACS	Companion Animal Clinical Studies
CB	Canine babesiosis
COPD	Chronic obstructive pulmonary disease
CT	Computed tomography
DIC	Disseminated intravascular coagulation
E	*Ehrlichia*
ECG	Electrocardiographic
EIPH	Exercise-induced pulmonary haemorrhage
ICU	Intensive care unit
IMHA	Immune-mediated haemolytic anaemia
L	Left
LEAP	Low-energy all purpose
MAA	Macroaggregated albumin
MODS	Multiple organ dysfunction syndrome
MRI	Magnetic resonance imaging
OVAH	Onderstepoort Veterinary Academic Hospital
PIOPED	Prospective Investigation of Pulmonary Embolism Diagnosis
PISA-PED	Prospective Investigative Study of Acute Pulmonary Embolism Diagnosis
PTE	Pulmonary thromboembolism
Q	Perfusion
R	Right
RES	Reticulo-endothelial system
SD	Standard deviation
SIRS	Systemic inflammatory response syndrome

SPECT	Single photon emission computed tomography
UPBRC	University of Pretoria's Biomedical Research Centre
V	Ventilation

Summary

THE SCINTIGRAPHIC EVALUATION OF THE PULMONARY PERFUSION PATTERN OF DOGS HOSPITALISED WITH BABESIOSIS

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A hypercoagulable state has been demonstrated in human falciparum malaria in mild and complicated forms of the disease. Disseminated intravascular coagulation (DIC) was implicated by some authors, but deemed a rare occurrence by others. The possibility of coagulopathy in *Babesia canis rossi* infections in the canine patient has also been suggested in the literature, but minimal work has been done to evaluate the clinicopathological nature of it in further detail. In the canine babesiosis (CB) pathogenesis thought-process, DIC has been implicated. A DIC-like syndrome, as evidenced by intravascular fibrin deposition and haemorrhage into muscles and tissues was found at *post mortem* in one study. On the basis of these findings, it was postulated that DIC might be a serious complication of severe *Babesia* infection in the dog. Clinical DIC (haemorrhagic diathesis) is however seldom seen. It was also hypothesised in the literature that the multiple organ dysfunction syndrome (MODS) demonstrated in the complicated form of *Babesia* was caused, in addition to tissue damage due to local hypoxia, by microthrombi as a result of a coagulopathy. This needs to be further investigated.

Pulmonary thromboembolism (PTE) has not been implicated in CB, however thromboemboli in the lungs were found in dogs with immune-mediated haemolytic anaemia (IMHA) for which a similar mechanism of venous stasis, hypercoagulability and endothelial

damage (as found in CB) is proposed. In humans, PTE is believed to be a major underdiagnosed contributor to mortality in 5 to 15% of hospitalised adults. If early diagnosis of PTE can be achieved, the mortality rate can certainly be decreased. A similar situation with resultant serious implications in complicated CB cases may exist. Clinically, PTE is suspected if a patient with a known prothrombotic condition develops sudden dyspnoea and tachypnoea. These clinical symptoms are frequently seen in complicated CB patients and may, in addition to being a compensatory mechanism for the metabolic acidosis and anaemia, be attributed to thrombus-induced mechanical changes in lung function.

Pulmonary scintigraphy provides a sensitive means of diagnosing PTE. It was (and some authors still do) believed that a ventilation scintigraphic scan should be done in association with a perfusion scan to increase the specificity and accuracy of diagnoses. However, authors of the recent PISA-PED study in humans proposed that the sensitivity and specificity of a perfusion scan, without a ventilation scan, in patients with suspected PTE was sufficient. The incidence of PTE or the use of pulmonary perfusion scintigraphy in CB dogs has never been studied.

The objective of this study was to prospectively evaluate the scintigraphic pulmonary perfusion pattern in hospitalised Babesia dogs in an attempt to ascertain whether a scintigraphic pattern consistent with PTE does indeed occur in these patients. The study consisted of a normal control group of nine mature healthy Beagle dogs aged 36 – 43 months and weighing 9.9 – 15kg and a Babesia group with 14 dogs of a variety of breeds that were naturally infected with *Babesia*, aged 6 – 103 months and weighing 6.3 – 25.5kg. Pulmonary perfusion scintigraphy was performed after making thoracic radiographs and performing a blood gas analysis in both groups.

The scintigraphic images were visually inspected for changes suggestive of PTE. Surprisingly, not a single dog in the Babesia group had segmental or wedge-shaped perfusion defects which would have resulted in a high probability for PTE. The scintigraphic pulmonary perfusion pattern demonstrated was not significantly different between the two groups ($p = 1.00$). Many dogs in both groups had a mottled appearance on the right and left dorsal oblique images, which was not believed to be consistent with clinically relevant PTE. This study provides baseline data that may be used to further investigate the pulmonary perfusion pattern in *Babesia* dogs.

1. Objective and benefits

1.1 Objective

The objective of this study was to scintigraphically evaluate the pulmonary perfusion pattern of hospitalised dogs naturally infected with *Babesia canis rossi*; to investigate the possible occurrence of scintigraphic evidence of pulmonary thromboembolism (PTE) in these dogs and to obtain baseline and additional data for use in further studies.

1.2 Benefits

This study helps in providing increased insight into the pathogenesis of canine babesiosis (CB), especially as regards the much-debated possibility of coagulopathy in the complicated form of the disease.

The additional data obtained will serve as baseline information for further studies and similar cases. It falls under the Faculty research theme of "Important conditions of companion animals in Sub-Saharan Africa".

The collected data may be used for additional publications investigating any correlation between the occurrence of PTE and the measured clinical parameters or outcome of disease as well as the thoracic radiographic findings in CB.

This project forms part of the research requirements for Dr Sweers's MMedVet(Diagnostic Imaging) degree.

2. Research question and hypothesis

2.1 Research question

This study was designed to answer the question: "Does a scintigraphic pattern consistent with PTE occur in dogs hospitalised with naturally occurring infection with *B canis rossi*?"

2.2 Hypothesis

Scintigraphic evidence of PTE does occur in hospitalised patients with naturally occurring infection with *B canis rossi*.

3. Literature review

3.1 Nuclear medicine

3.1.1 Introduction

Nuclear medicine deals with the *in vivo* use of radiopharmaceuticals for imaging of the different regions or organs of the body, including the lungs, for diagnosis of disease. The detected distribution of emitted radiation within the lung allows for the evaluation of the functional and morphological status of the organ[1]. The optimal dosage of radioactivity to acquire the required diagnostic information with the least amount of radiation dosage to the patient is required and is regularly achieved with a radiopharmaceutical with short physical half-life which emits only low energy gamma radiation[1]. Technetium-99m (^{99m}Tc), with its six-hour half-life and 140keV gamma ray emission is an excellent example of such a radionuclide. It is currently the most widely used radionuclide in nuclear medicine and is generated in a ^{99}Molybdenum/^{99m}Technetium (^{99}Mo/^{99m}Tc) generator system that is readily available and replaced weekly or two-weekly[1].

3.1.2 Pulmonary scintigraphy in veterinary medicine

Scintigraphic imaging of pulmonary perfusion and ventilation can be performed in the veterinary patient; however ventilation (V) studies require specialised equipment and are quite difficult to perform due to the patient cooperation needed to obtain interpretable results[2]. Perfusion (Q) studies are easier to perform in animals and have been used in small animals mostly for the detection of PTE and in equines with chronic obstructive pulmonary disease (COPD), exercise-induced pulmonary haemorrhage (EIPH) or to lateralise pathology[2].

Pulmonary scintigraphy has been used for the detection of several diseases or conditions in veterinary patients including heartworm[3,4], hypertrophic cardiomyopathy and pulmonary artery thrombosis in a cat[5], embolism during total hip replacement[6] as well as PTE caused by disseminated intravascular coagulation (DIC) of unknown aetiology[7]. Other pulmonary pathology like abscesses or neoplasia can also be detected by these studies[2]. Pulmonary scintigraphy has never been attempted in patients infected with *Babesia canis*.

3.1.3 Pulmonary perfusion scintigraphy

3.1.3.1 Radiopharmaceutical

^{99m}Tc-human macroaggregated albumin (^{99m}Tc-MAA) is used after preparation from a lyophilised radiopharmaceutical kit ("Pulmotek", Atomic Energy Corporation of South Africa, Pretoria, South Africa), which contains a suspension of approximately four million albumin particles[8] to which sodium^{99m}Tc-pertechnetate is added and allowed to incubate for a period of time[2]. It should preferably be used directly after preparation and definitely within six hours of preparation[1]. Hood *et al*[9] used ^{99m}Tc tagged macroaggregated *dog* serum albumin to minimise the possibility of allergic reactions.

The dosage in humans weighing 70kg is 200 000 to 350 000 particles in 37 to 148MBq (1 – 4mCi) ^{99m}Tc-pertechnetate[8]. According to Berry *et al*[2], the dosage in dogs is 17.5 to 74MBq (0.5 – 2mCi), however, a slightly higher dosage of 111MBq (3mCi) was used in a study evaluating PTE associated with canine total hip replacement[6]. The number of particles to be administered to a patient should be calculated according to body weight to avoid obstruction of too many pulmonary capillaries[2]. The minimum and maximum particles per kilogram bodyweight to inject in humans are 2 857 and 5 000 respectively[8]. This corresponds well with a similar value published for use in dogs[9].

3.1.3.2 Mechanism of localisation and acquisitions

Because of the size of the ^{99m}Tc-MAA particles, with more than 90% having a diameter between 10 and 60µm (maximum size of 150µm), they physically lodge by a purely mechanical process in the first capillary bed encountered after intravenous injection. This in effect implies blockage in the pulmonary capillaries[8], as these small vessels have a diameter of approximately 7 to 10µm[10]. Aggregates smaller than the capillary diameter are taken up by the reticulo-endothelial system (RES), mostly within the first five minutes after injection. If even mixing of particles in the venous blood has taken place, their distribution within the lung will be proportional to blood flow within the lungs, thus permitting the evaluation of the perfusion pattern in the lungs by the image generated from the emitted radiation[2,11]. It has been found that only 1 to 5% of the pulmonary capillaries will be blocked if the correct dose of particles is

injected[2]. This small temporary mechanical impediment to pulmonary arterial blood flow is normally physiologically insignificant[11].

The biological half-life of ^{99m}Tc-MAA is four to six hours[2]. Image acquisition can thus begin immediately after intravenous injection, but may be delayed for a few hours if necessary. Although blockage by the particles is immediate, it will last for several hours before being degraded to smaller particles[2,11]. Three processes play a role in the clearance of the particles[12]. Bloodstream enzymes immediately begin to dissolve the particles from its outer surfaces, thus shrinking them in size. Additionally, respiratory motion causes the lung tissue to expand and contract continuously, thus elongating and compressing the particles. Thirdly, the constant rhythmic blood pressure being delivered eventually pushes the particles out into the systemic circulation. These removed particles are now small enough to be removed by the RES, especially in the liver and spleen. The fate of the released technetium residue was previously unknown because of its short physical half-life[12], however it is believed that the kidneys excrete the majority of the oxidized (and very small amount of free) ^{99m}Tc-pertechnetate that is released with dissociation of the radiopharmaceutical *in vivo*[2]. Some renal activity may be noted at the end of a scintigraphic perfusion study as a result of this process[10]. Although the radioactivity ends up in the urine, because of the low dosage of ^{99m}Tc administered per patient, the amount and contamination risk is negligible especially when considering the benefit of the procedure against risk. It is, however, good practice to still take the necessary precautions regarding distance and handling of patients.

Acquisition of images is recommended to be at least for 300K to 500K counts per image over several minutes. Right and left lateral, dorsal right and left oblique as well as dorsal and ventral images should be obtained as a minimum in small animals[2].

3.1.3.3 Risks and contraindications for pulmonary scintigraphy

The radiation dose resulting from the administration of a pharmaceutical should always be considered in relation to its diagnostic or therapeutic value[8]. The approximate radiation dose to the tissue would be 0.0066Gray (0.66rads) per 111MBq (3mCi) given[1].

Administration of the agent should be carefully considered and rather be avoided in pregnant or lactating bitches. The number of particles to inject should be decreased in cases of

pulmonary hypertension[1]. It has been reported that in several dogs with severe PTE death occurred after injection[2]. It is also contraindicated in patients with a known history of hypersensitivity reactions to materials containing human serum albumin[8]. The minimum toxic dose of albumin exceeds the usual imaging dose by a factor of 1000[11]. Anaphylactic reactions after injection should always be considered, but have not been reported in small animals.

If a right to left shunt is present, uptake by the brain, liver and kidneys will be seen due to unwanted activity ending up in the systemic circulation. Embolism due to the lodging of the particles in these organs could be of major consequence and the procedure is thus contraindicated in these patients[2,13]. However, a very small percentage of particles are smaller than 8µm and will thus pass through the pulmonary capillary bed and some renal, splenic and hepatic activity may be present. This will not exceed 5% of the injected dose by five minutes post injection in a non-shunting patient[2].

3.1.3.4 Interpretation of a perfusion scintigram

A normal perfusion scintigram is recognised by a homogenous distribution pattern throughout the entire lung field, with normal photopaenic areas corresponding to the cardiac notch on right lateral and ventral views as well as the mediastinum[2,9,14]. A normal (thus negative) perfusion scintigram virtually excludes the presence of clinically significant PTE[2,14-16]. Studies in experimental animal models have shown that a pulmonary scintigram may detect 97% of occlusive emboli larger than 2mm in diameter[17], but may fail to detect smaller or incompletely occlusive emboli[17,18].

Activity in the thyroid and salivary glands or gastric mucosa on the initial images is due to the presence of free pertechnetate and is indicative of non-satisfactory radiopharmaceutical binding[2]. A minimal degree of the above activity may be expected due to 95% tagging of the MAA, leaving a small quantity of free technetium to follow its normal path of bio-routing[12].

An abnormal pulmonary scintigram is recognised by photopaenic defects as a result of abnormal pulmonary perfusion. With PTE, a wedge-shaped and pleural-based photopaenic defect is usually seen with a lobar or segmental distribution[2,14,15]. False positive results may be obtained in the presence of pleural or diaphragmatic abnormalities such as pleural fluid, masses, diaphragmatic herniation or paralysis, displacing the lung edge medially and

cranially[2]. Lung mass lesions like abscesses, neoplasia or granulomas may be seen as high contrast photopaenic areas within the lung parenchyma. Alveolar infiltrates are seen as similar, but low contrast, photopaenic defects within the parenchyma, corresponding to similar sized areas of soft tissue opacification of the lung on thoracic radiographs[2]. Thus, prior to the scintigraphic procedure, survey radiographs of the thorax need to be made to rule these conditions out[15].

If blood is allowed to clot within the syringe during injection, the lungs will disproportionately absorb the radioactive aggregates causing an apparent uneven distribution of activity ("hot clots") even in normal patients[11]. For the same reason, all air in the syringe must be removed prior to injection.

3.1.3.5 Research findings for pulmonary perfusion scintigraphy in the veterinary patient

The regional distribution of pulmonary ventilation and perfusion was studied in three healthy conscious standing Greyhounds by the infusion of 5% dextrose with a continuous eluate containing ^{81m}Krypton (^{81m}Kr)[19]. It was found that ventilation and perfusion were equally distributed throughout the lungs. These findings may however not be representative due to the small number of animals studied and the use of Greyhounds, which cannot be classified as representative of most breeds. The use of ^{81m}Kr with a half-life of 13 seconds as perfusion agent is also less optimal than ^{99m}Tc as most of the former will have evolved into alveolar gas on its first pass through the pulmonary capillaries[19].

De Vries, Clercx and van den Brom[20-24] did extensive quantitative pulmonary scintigraphic studies on canine patients to investigate several aspects. They developed a method for the analysis of ^{99m}Tc-phytate colloid aerosol inhalation and ^{99m}Tc-MAA perfusion distribution patterns and compared the scintigrams based on the activity of corresponding pixels[23]. They mostly expressed mismatching (regional and intraregional) of ventilation and perfusion. In a summary of their first results of inhalation and perfusion studies in healthy dogs[20], they reported that the V/Q ratio in the anaesthetised sternal recumbent dog decreased from cranial to caudal lung regions. They hypothesised that gravity is therefore not the only determinant of V/Q ratio distribution in the canine patient as it is assumed to be in man, but believed that other factors such as regional differences in lung compliance may play a role.

They found that the left lung was significantly less ventilated (47.6%) and perfused (46.5%) than the right lung. However, the distribution of V/Q values did not significantly differ between left and right.

A comparison of inhalation to perfusion ratio between sternal recumbent anaesthetised dogs with a barrel-shaped thorax (Beagles) and a deep thorax (Greyhound-type) has also been made[24]. The mean V/Q ratio was also found to decrease from cranial to caudal in both types, with the decrease more sustained in the right lung. The total decrease, however, was less in Greyhound-type dogs as compared to the Beagles. In Beagles, a dorsal to ventral increase in the mean V/Q ratio by approximately 50% of its initial dorsal zone value was seen. In Greyhound-types it decreased slightly from dorsal to ventral (as seen in humans), with the exception of the most ventral zone. It was hypothesised that the height of the thorax in Greyhound-type dogs could permit the gravitational force to exert more influence than in Beagles[24].

Anaesthesia in dogs induces sites with very low V/Q ratios and a shift of perfusion to the more dorsal and caudal regions of the lung[20,25]. It also induces significant changes in the shape of both dorsal and lateral lung images, believed to be due to enhanced muscle relaxation permitting a further lateral enlargement of ventral zones and vertical flattening of the caudal zones[25].

Comparisons of lung shape, configuration and perfusion in the standing and sternal recumbent patient indicated that in sternal recumbency the lungs are flattened vertically with enlargement of the ventral zones and the lung parenchyma being pushed more caudally and dorsally[20,25]. Perfusion decreased to the caudal third of the lung, thought to be mainly due to the pressure exerted by the abdominal content through the diaphragm and to the shift of blood towards the better ventilated regions[25]. No significant age-related changes were found[20].

In another study[21], lobar and sublobar airway obstruction was induced in dogs. With sublobar obstruction, the relative ventilation to the lung containing the obstruction was increased and blood was shifted from the obstructed segment to the rest of the same lung. The mean V/Q ratio was increased to both the obstructed segment and the lung containing the obstruction. Collateral ventilation at sublobar level is thus very efficient in the canine patient. With lobar obstruction, air and blood (to a lesser extent) was diverted away from the obstructed

segment to the contralateral lung. The intraregional V to Q mismatching was increased in both the obstructed segment and the rest of the same lung.

The diagnostic value of quantitative analysis of ^{99m}Tc aerosol inhalation and perfusion scintigrams was studied in an experimentally induced canine PTE model, in which a 1% solution of inert agar was injected intravenously[22]. The more caudal zones, cranial extremities and peripheral lung regions were found to be the most heavily embolized on the scintigrams, with perfusion redistributed from these regions to the more central zones. The pattern of embolisation is believed to be a result of the higher density of pulmonary units (and thus a thinner vascular system) in the more peripheral layer of the lung parenchyma and thicker caudal lung zones[22]. The perfusion density factor was calculated and found decreased in the heavily embolized regions, believed to be a reflection of vasoconstriction in the embolized regions as a result of the local release of mediators[22]. These mediators are also responsible for increased permeability to plasma proteins resulting in pulmonary oedema[22]. The pulmonary hemodynamic effects are already present after five minutes of the embolic incident and remains for at least an hour[22,26]. Another study[4] demonstrated large perfusion defects as well as subsequent V/Q abnormalities in six dogs that underwent pulmonary artery occlusion using a Swan-Ganz catheter.

In humans, gravity-dependant perfusion and ventilation gradients exist, thus resulting in the most dependant part of the lung receiving a greater amount of blood flow and air when compared to the non-dependant parts of the lung[2]. In the standing conscious dog, similar gravity-dependant ventilation gradients are seen[2].

In both humans and animals, an autoregulatory mechanism occurs where blood flow is shunted away from less- or non-ventilated areas towards better-ventilated areas of the lung[2]. Pulmonary abnormalities result in ventilation-perfusion mismatches[2].

3.2 Pulmonary thromboembolism

3.2.1 Possible causes and importance of diagnosis

No typical signalment or breed predisposition exists for the obstruction of a pulmonary vessel by a thrombus[27]. Any condition causing hypercoagulability of the blood, stasis of blood

flow or vascular endothelial injury will predispose the patient to thrombus formation[27,28]. Sepsis and DIC are prothrombotic conditions that resort under these criteria[27-29]. Several changes in circulating blood, including increased platelet reactivity, coagulation factors, fibrinolytic inhibitors and decreased coagulation inhibitors or fibrinolytic activity as well as increased or abnormal lipids are all implicated in hypercoagulability and thrombosis[30]. Intravascular haemolysis of any aetiology (including *Babesia*[31]) is a common trigger for DIC[32].

In humans, PTE is thought to be a major contributor to mortality in 5 to 15% of adults in hospitals[29]. It is highly underdiagnosed, with only 30% of human patients with anatomically important pulmonary emboli being diagnosed correctly[28,33]. Small thrombi or emboli may go unrecognised as more than 50% of the arterial bed must be occluded before a significant increase in pulmonary pressure is noted[34]. It stands to reason that by increasing the number of diagnoses and the percentage of patients diagnosed early, the mortality rate due to PTE can be decreased[33].

3.2.2 Respiratory and cardiovascular sequelae to pulmonary thromboembolism

Thrombi release humoral factors that stimulate constriction of the small peripheral airways and shift ventilation to better-perfused regions. The increased airway resistance reduces lung volume and compliance, which will eventually result in airway closure and alveolar collapse with clinically recognisable dyspnoea[29]. Pulmonary oedema in non-embolized regions results from increased hydrostatic pressure due to additional blood flow diverted away from the occluded vessels. The released humoral factors cause increased permeability of the pulmonary vasculature. Another feature of PTE is decreased surfactant production with alveolar collapse and excessive leakage of transudate into the alveoli[29]. Infarction is uncommon but can occur with very distal pulmonary vasculature occlusion[29]. Pleural effusion may occur due to increased capillary permeability in the visceral pleura adjacent to the infarction and an increased intrapleural negative pressure secondary to decreased lung volume[29]. The cross-sectional area of the pulmonary vascular bed is decreased with PTE, but the large reserve capacity and dilation of individual capillary segments can overcome the change in blood flow. In normal dogs more than 60% of the pulmonary vasculature must be occluded before significant increased vascular resistance will lead to decreased pulmonary arterial blood flow[29]. With the increased vascular resistance, the right ventricle must work harder and may fail if not able to compensate[29].

3.2.3 Ventilation-perfusion ratios in pulmonary thromboembolism

The entire lung's V/Q regions contribute to the oxygenation of blood, being insufficient with moderate to severe PTE. Different V/Q ratios are found in different parts of the lungs, with normal ventilation and absent perfusion in embolized regions[14,29,35].

3.2.4 Clinical signs of pulmonary thromboembolism

The most common clinical signs reported are acute onset dyspnoea, tachypnoea and depression[28]. It can also be subclinical and mild signs may be missed easily[27,29]. In canine patients with experimentally induced PTE, the respiratory and heart rates were significantly increased immediately and within the first five minutes after the injection of agar, but gradually returned to the initial values[22]. A suspicion of PTE is raised after the sudden onset of dyspnoea in a patient with a known prothrombotic condition[27,36].

3.2.5 Ante mortem diagnosis of pulmonary thromboembolism

Diagnosis of PTE remains difficult, with haematology and biochemistry of limited value. Two radiographic patterns, namely oligemia and single or multiple alveolar pulmonary infiltrates have been described[27,28,34]. Pleural-based, wedge-shaped alveolar opacification is rarely seen[27,34]. Visualisation of the lobar pulmonary artery does not exclude PTE as the clot within the vessel may be of similar soft tissue opacity thus still creating the impression of an arterial silhouette[34]. Main pulmonary artery segment enlargement, right-sided cardiomegaly and pleural effusion have been reported[27,28].

Electrocardiographic (ECG) findings may indicate a sinus tachycardia (most commonly) and other changes indicative of an acute right ventricular compromise. Elevations of pulmonary arterial, right ventricular, right atrial and central venous pressure are all non-specific for PTE[27].

Arterial blood gas values may be useful in diagnosis of PTE, however normal values do not exclude it[27,28]. Canine patients with experimentally induced PTE revealed no significant differences in blood gas values within five minutes or the next half hour after injection[22]. Arterial blood gas values of three dogs with PTE, breathing room air, were compared with

normal values and tabulated by Dennis[27]. An increased alveolar-arterial oxygen concentration difference may be the most sensitive single blood parameter for diagnosis of PTE[27]. Arterial hypoxaemia and hypocapnoea may also be found[27,28]. Blood pH is variable due to the influences of respiratory alkalosis and metabolic acidosis[27].

Identifying evidence of pulmonary arterial hypertension with echocardiography may help to support a diagnosis of PTE[37]. Poor right ventricular contraction, paradoxical septal motion, pulmonary artery dilatation and high-velocity tricuspid and pulmonary regurgitant jets may be found. In the absence of pulmonic stenosis, pulmonary hypertension may be indicated by increased right heart pressures (calculated by utilising the regurgitant jets' velocity) [37].

All of the above tests may prove non-specific for PTE, but may serve as an excluding mechanism of other disease processes[27,28,36].

D-dimer is a laboratory marker of coagulation, which can be used in the detection of early embolism in humans and small animals[37]. This test is very sensitive (false negatives are uncommon) but lacks specificity, as its concentration may be high in dogs with hepatic disease as well as in dogs with haemabdomen or even neoplastic disease. If a D-dimer assay is negative however, it essentially rules *out* thromboembolism[37].

Spiral (high resolution) computed tomography (CT) is currently widely used in human medicine for the diagnosis of PTE[38]. This method is still not widely employed in veterinary medicine due to higher cost, lack of availability of equipment and experience as well as other logistical difficulties.

Diagnosis of PTE using magnetic resonance imaging (MRI) seems possible[39,40], however is not yet a reliable diagnostic technique in patients with relatively high false-positive rates obtained[15] and due to the higher cost and unavailability of equipment in the veterinary setting.

When pulmonary scintigraphic findings are inconclusive or the necessary scanning equipment is not available, pulmonary angiography is performed for definitive diagnosis. It is the most accurate technique currently available, however is very invasive, more difficult to perform and interpret, and requires general anaesthesia with an increased risk in critically ill

patients[27,28,41]. In dogs, it was reported that PTE greater than 2mm diameter might be detected with digital subtraction angiography[42].

Pulmonary scintigraphy is a useful screening test for PTE in humans[28] and has been found to be sensitive and specific for experimental PTE in the canine[27,28]. A normal pulmonary perfusion scintigram excludes PTE[28], but an abnormal one does not necessarily establish a diagnosis of PTE[27,28]. Some authors believe that the addition of a pulmonary ventilation scintigram increases the specificity[27,28].

In humans, a Prospective Investigation of Pulmonary Embolism Diagnosis (PIOPED) study was carried out by performing perfusion and ventilation scintigrams and then interpreted, together with thoracic radiographs, based on probabilistic criteria[43-45]. Scans were then classified as high, low, intermediate or undeterminate probability[38,43-45]. This study gained tremendous popularity in the nuclear medicine fraternity, probably due to it being widely published and marketed[38]. A study in human patients found that major short-term morbidity or death in patients with low probability scans was infrequent[46].

Since the PIOPED study, several other study groups have presented better results, but the advances of these studies are still not recognized sufficiently by nuclear medicine practitioners[38]. The Prospective Investigative Study of Acute Pulmonary Embolism Diagnosis (PISA-PED) was developed to determine the sensitivity and specificity of the pulmonary perfusion scintigram in human patients with suspected PTE, *without* a pulmonary ventilation scintigram[33]. Scans were classified as normal, near normal, abnormal and suggestive of PTE or abnormal and not suggestive of PTE. Pulmonary angiography, as the diagnostic gold standard, was performed in all cases with abnormal scintigrams. The sensitivity and specificity of the abnormal scintigrams suggestive of PTE was 86% and 93% respectively[36]. It has therefore been suggested that a sole perfusion scan can be considered the nuclear medicine examination of choice in the diagnostic work-up of PTE[33].

Low cost, safety, rapid and easy execution, low absorbed dose and high negative predictive value are all advantages of pulmonary scintigraphy[38]. Pulmonary scintigraphic performance using the PIOPED interpretive criteria may be less than the results currently obtained using spiral CT[38]. However, by using the PISA-PED study criteria, results are comparable or even superior to spiral CT[38]. Additionally, the use of single photon emission

computed tomography (SPECT) may reveal increased sensitivity and specificity when comparing it to standard planar techniques[38]. However, one study revealed no improvements with SPECT[15]. If available, scintigraphy may the best available method for screening of dogs with suspected PTE[41].

3.2.6 Pulmonary thromboembolism and the lungs at *post mortem*

Distribution of embolic material is influenced by its specific gravity[22]. Fresh blood clots and agar clots have comparable consistency and strength[22]. Macroscopic examination of lungs of canine patients injected with a 1% solution of inert agar coloured with carbon particles to induce PTE, revealed diffuse punctiform deposition of the coloured agar[22]. Other macroscopic abnormalities were not seen, however the patients were euthanased after the experiment and the (unknown) time between the embolic insult and death may have been too short to induce visible macroscopic abnormalities. Microscopically, diffuse emboli in all lung lobes were seen although the pulmonary scintigrams indicated the cranial extremities, caudal zones and peripheral lung regions of both lungs to be the most heavily embolized[22]. The diameter of embolized vessels varied between 0.04mm and 1.25mm[22].

3.3 Canine babesiosis

3.3.1 Introduction

In South Africa, CB is one of the most important infectious diseases of dogs[47]. Between 1988 and 1993, 11.69% of sick dogs presented to the Onderstepoort Veterinary Academic Hospital (OVAH) were diagnosed with CB, of which 31.4% were classified as serious enough to be admitted to the small animal medicine clinic for more intensive treatment[47]. The highly virulent *Babesia canis rossi*, which is transmitted by *Haemophysalis leachi*, is the strain found in South Africa[48-51].

Typically, an uncomplicated form of the disease, characterized by anaemia, pyrexia, anorexia, listlessness, splenomegaly, tachypnoea and a waterhammer pulse can be found[52,53]. A complicated form with clinical findings depending on the organ involved also exists. This includes lung involvement with respiratory symptoms[51,52]. This atypical form is believed to be due to the systemic inflammatory response syndrome (SIRS), which could then develop into

multiple organ dysfunction syndrome (MODS)[53]. Most, if not all of the patients admitted to the OVAH with CB suffer from the complicated form of the disease.

3.3.2 Canine babesia and human malaria – similar conditions?

Canine babesiosis and human malaria, caused by *Plasmodium falciparum*, are both vector-borne parasites that primarily infect the red blood cells of their hosts[51,54,55]. There are many similarities between the two diseases, both varying from uncomplicated to complicated and more severe disease[55-58]. Several authors have looked comparatively at both diseases and the possibility of using *Babesia* (either bovine or canine) as a model for falciparum malaria, and especially cerebral malaria, in humans[52,54,55,57]. It was found that these diseases could be used as models, even if only partially, for each other[53,54]. To search for clues to the understanding of CB, Jacobson and Clark[52] stressed the need for the clarification of the pathophysiological mechanisms of CB via further research and stated that babesiosis researchers should base their hypotheses on recent discoveries in human malaria, due to the striking similarities that exist between these two diseases.

3.3.2.1 Coagulopathy in human malaria

Activation of the coagulation cascade has been reported to occur in human malaria, even in mild disease[59,60]. The infected erythrocytes are believed to have procoagulant activity, activating the coagulation cascade directly[59], cytokine release are procoagulant[60] and endothelial damage also play a role[59]. As early as 1966, some researchers incriminated DIC in the pathogenesis of severe, complicated malaria with cerebral manifestations[56,60,61]. Disseminated intravascular coagulation, as termed and defined by McKay[62], is the pathological activation of the blood coagulation mechanism leading to widespread intravascular clotting involving particularly the arterioles and capillaries. A study published in 1989 concluded that coagulation abnormalities occurring in severe malaria is rarely pathologically significant[59].

3.3.2.2 Coagulopathy in canine babesiosis

The different complications of the disease are believed to be as a result of excessive inflammatory host response rather than the parasite itself[51,52]. The SIRS is caused by the

release of inflammatory mediators as a result of the severe tissue hypoxia with widespread tissue damage that occur in CB[51]. It precedes the development of MODS[51].

Babesiosis is mentioned as one of the diseases and conditions in small animals that is associated with DIC, which may result in thrombosis[31]. Haemolysis, vascular endothelial damage, acidosis, hypoxia, vascular stasis, shock and possibly an endotoxaemic-like state all predispose patients to DIC[31,51]. Disseminated intravascular coagulation has been reported in splenectomised calves infected with *Babesia argentina*[63] and *Babesia bovis*[64], in sheep experimentally infected with *Babesia ovis*[65,66], in 24% out of 63 cases of canine babesiosis in Hungary[67] and in several dogs with the severe (complicated) form of CB[56]. It has been suggested that the atypical symptoms of CB may be a manifestation of DIC localised primarily to a particular organ system[56]. The mechanisms by which *B canis* induce DIC are still unknown, but several were suggested by Moore and Williams[56].

Some researchers believe that the hypercoagulable state in CB is because of profound disturbance in fibrinogen metabolism rather than DIC[58,68,69]. Procoagulant activity has been shown in virulent strains of *Babesia bovis*, in which the organisms produce proteases, which may induce increased plasma kallikrein activity, which in turn can activate the intrinsic coagulation cascade at factor XII[68]. In bovine babesiosis, with activation of the coagulation system, large amounts of thrombin and thrombin-like enzymes as well as other enzymes are released which act as chemo-attractants for polymorphonuclear leucocytes and appear to act specifically in the capillary beds of the lungs. This causes the sequestration of erythrocytes and neutrophils[54]. The procoagulant activity and cytoadherence of infected red blood cells play a role in local vascular stasis[54].

With these indications of activation of the coagulation system in naturally infected as well as experimentally infected cases[50], it is however still not known if this results in increased stickiness and retention of infected red blood cells in the microvasculature, thus forming thrombi. Another explanation could be the obstruction of blood flow at capillary level due to the deposition of fibrin clots as a result of DIC[50,56]. The injection of heparin in a *B canis canis* infected dog resulted in an increased peripheral parasitaemia and packed cell volume, which could also further substantiate that the coagulation system may be involved in erythrocyte-retention in deep tissues[50].

3.3.2.3 Human malaria and the lungs

Sequestration of infected red blood cells in important organs, including the lungs, has been associated with biochemical, physiological and morphological abnormalities[58-60]. Due to a sudden increase in pulmonary capillary permeability pulmonary oedema may result[60]. Intravascular thrombi are rarely seen at autopsy in fatal cases[60].

3.3.2.4 Canine babesiosis and the lungs

Pulmonary damage may occur due to microthrombi formed in the hypercoagulable phase of DIC or due to tissue hypoxia induced by mediators in a SIRS and MODS state[31,51,52,56]. With MODS in humans, secondary pulmonary injury is a frequent occurrence and usually early, occurring 24 to 72 hours after the primary injury[52]. Lung injury is often unrecognised in complicated CB and may reflect the diagnostic methods employed and a species difference between humans and dogs[52,70].

Pulmonary oedema is a frequent serious complication of CB[51,52]. Little is known about the pathophysiology of lung oedema in CB, but several mechanisms have been proposed. Wright *et al*[54] suggested that both bovine Babesiosis (caused by *B bovis*) and human falciparum malaria induce a syndrome similar to acute respiratory distress syndrome (ARDS) in which there is massive sequestration of erythrocytes and neutrophils in pulmonary capillaries. This results in increased pulmonary vascular permeability and subsequent oedema[54]. Increased permeability can also result from DIC, PTE and hepatic disease[52]. Disseminated intravascular coagulation of pulmonary tissue was demonstrated microscopically, with congestion and oedema in a fatal case of CB[52,56]. Pulmonary thromboembolism has not been implicated as a cause for pulmonary oedema in CB[52]. It is believed that ARDS is a major cause of lung oedema in CB[52].

Pulmonary thromboembolism was found on *post mortem* examination in dogs with immune-mediated haemolytic anaemia (IMHA)[71]. The mechanism for IMHA-associated PTE is unknown, however like with CB, is also believed to be due to venous stasis, hypercoagulability and endothelial damage[71]. If the same mechanisms involved in IMHA-associated PTE are implicated in CB, the potential for PTE in cases of CB must exist.

3.3.3 Blood gas in canine babesiosis

Many critically ill dogs are respiratory-compromised at admission or during hospitalisation[72]. A similar observation is made in patients naturally infected with *Babesia canis* presented to, and admitted at the OVAH[51,52]. Severe anaemia, as found in complicated CB, can cause hypoxaemia[72,73]. The latter can cause hyperventilation which in turn can lead to hypocapnoea and respiratory alkalosis[35,72,73]. Pulmonary disease (including PTE and ARDS) is a common respiratory cause of acidosis[72]. Blood gas analysis can be vital in assessment and monitoring of these patients[72,73].

Mixed acid-base disturbances are extremely common in critically ill veterinary patients[35]. Mixed acid-base disturbances in severe CB were evaluated and described by Leisewitz *et al*[74] in 2001. The most common combination of abnormalities was hyperchloraemic acidosis, organic metabolic acidosis partially due to hyperlactataemia, hyperphosphataemic acidosis, dilutional acidosis and respiratory alkalosis.

A study of *Babesia*-infected dogs in a small animal intensive care setting[75] found that at admission, the mean blood oxygen partial pressure was elevated and the partial pressure of carbon dioxide was very depressed due to respiratory compensation for the existing metabolic acidosis. The patients were found to be hypoxaemic due to the reduced oxygen-carrying capacity of the blood as a result of severe anaemia. The patients demonstrated severe oxygen deficiency at tissue level, believed to be the cause for MODS in complicated cases. These variables were improved with blood transfusion.

4. Materials and Methods

4.1 Overview

This study was a prospective, minimally invasive experiment with a normal control group (n = 9) and *Babesia*-infected group (n = 14). Thoracic radiographs, blood gas analysis and pulmonary perfusion scintigraphy were performed in both groups. The scintigraphic pulmonary perfusion pattern was visually evaluated and compared between the two groups in an attempt to identify a pattern suggestive of PTE.

4.2 Study design

4.2.1 Sample groups

Beagle dogs, on loan from the University of Pretoria's Biomedical Research Centre (UPBRC), were used as normal control dogs.

Naturally *Babesia*-infected, client-owned dogs admitted to the hospital's small animal medicine section for further intensive treatment were used as the Babesia dogs. The dogs were from a variety of breeds, with one Spaniel, one Labrador retriever, one Husky, one Rottweiler, one Japanese akita, one Staffordshire bull terrier, one Dalmatian, one Bassett, one Sharpei and five crossbreeds.

4.2.1.1 Selection of control dogs

In order to ascertain their general health status, a complete physical examination (documenting habitus, temperature, pulse, respiration and mucous membrane colour), peripheral blood smear examination and full haematology was performed prior to being included in the study. Only dogs that were clinically healthy, in good physical condition, free of blood parasites (specifically *Babesia* and *Ehrlichia canis*) and with all haematology values within normal limits were included.

4.2.1.2 Selection of Babesia dogs

Dogs were selected from patients admitted to the OVAH for further treatment (i.e. blood transfusion therapy or treatment for other complications). An attempt was made to select patients with a uniform body weight for ease of evaluation of the scintigraphic images. Only dogs with respiratory symptoms (i.e. dyspnoea, tachypnoea, cough or blood-tinged frothy nasal discharge) and a peripheral blood smear positive for *B canis*, but negative for *E canis* and with no clinical suspicion of ehrlichiosis were considered. Haematocrit and in-saline agglutination status did not affect inclusion. Dogs were excluded if they had any illness or accident in the preceding weeks, any history of a lung condition, any known history of hypersensitivity to materials containing human serum albumin, pulmonary hypertension, right-to-left shunting, demonstrated any significant thoracic radiographic changes not attributable to *Babesia*, pregnant or lactating bitches, narrow-chested or severely obese dogs.

4.2.1.3 Pre-imaging management of control dogs

Control dogs were housed at the UPBRC kennels and fed and managed according to UPBRC standard protocols. Dogs were transferred to the OVAH scintigraphy section on the morning of the scintigraphic procedure.

4.2.1.4 Pre-imaging management of Babesia dogs

Babesia dogs were admitted via the OVAH Outpatient section to the section of small animal medicine and were caged and managed in the intensive care unit (ICU). Dogs were treated in accordance with standard treatment protocols for *B canis* currently in use at the OVAH, with specific treatment at the discretion of the attending clinician. In most cases it also included a blood transfusion. Regular (at least hourly) turning of a patient was done if it was required; however it was only necessary in one dog.

4.2.1.5 Baseline data collection of control dogs

Each dog's age, sex, sterilisation status, body weight, habitus, temperature, pulse, respiration and mucous membrane colour was recorded. Habitus was scored from 1+ to 5+, with 1+ being extremely lethargic and 5+ being normal bright and alert. The temperature,

pulse and respiration were again recorded prior to the scintigraphic procedure. A panting dog's respiratory rate, where it was impossible to accurately count the breaths per minute, was taken at a fixed value of 100 breaths per minute.

A peripheral blood smear was made and evaluated for the presence of blood parasites (specifically *B* and *E canis*) and a sample for a full haematology obtained. The latter recorded values for haematocrit, red cell count, haemoglobin concentration, mean corpuscular volume, mean corpuscular haemoglobin concentration, red cell distribution width, white cell count, absolute mature neutrophil, immature neutrophil, lymphocyte, monocyte, eosinophil, basophil and thrombocyte count and the presence of anisocytes, normoblasts, reticulocytes, spherocytes, lymphoblasts, monoblasts, active monocytes and toxic granulocytes.

Thoracic radiographs, consisting of a right and left lateral and dorsoventral view, were made (not more than four hours prior to the scintigraphic procedure) in all dogs except one, where a ventrodorsal view was made.

A femoral arterial blood sample was obtained for blood gas analysis, immediately prior to the scintigraphic examination and the following parameters were recorded: partial pressure of oxygen and carbon dioxide, pH, sodium, potassium, calcium, haematocrit, standard and actual bicarbonate, blood and extra-cellular fluid base excess, total carbon dioxide content, calcium ion concentration adjusted to pH 7.4, estimated oxygen saturation, estimated oxygen content, arterial oxygen tension-inspired oxygen fraction ratio, arterial-alveolar oxygen tension difference and arterial-alveolar oxygen tension ratio.

4.2.1.6 Baseline data collection of Babesia dogs

Baseline data collection was part of the minimum data base information routinely obtained in CB patients and was under the supervision of the attending clinician. Each dog's age, sex, sterilisation status, body weight, habitus, temperature, pulse, respiration and mucous membrane colour was recorded. The temperature, pulse and respiration were again recorded prior to the scintigraphic procedure.

A peripheral blood smear was made and evaluated for the presence of blood parasites (specifically *B* and *E canis*). An in-saline agglutination test was performed and a sample for

haematology obtained with recorded values as for the control dogs. Additional values in some dogs were obtained at the discretion of the attending clinician. These included total serum protein, albumin, globulins, albumin:globulin ratio, urea, creatinine, sodium, potassium, total calcium, ionised calcium, phosphate and blood glucose. Thoracic radiography and a femoral arterial blood gas analysis were done as for the control dogs. Again, the set of radiographs consisted of a right and left lateral and dorsoventral view in all dogs except one, where a ventrodorsal view was made.

4.2.1.7 Post-imaging management of control dogs

After the scintigraphic examination, dogs were transferred to the nuclear medicine isolation facility where they were caged for 24 hours and monitored for any change in habitus or respiration. Dogs were fed according to the UPBRC standard protocol and allowed to urinate and defecate in a designated isolated area. After the 24-hour period, dogs were transferred back to the UPBRC unit.

4.2.1.8 Post-imaging management of Babesia dogs

After the scintigraphic examination, dogs were transferred to an ICU cage in a specifically designated isolation area to minimise radiation risk. The ICU personnel were trained in the safety precautions to limit radiation exposure to personnel and other dogs. Routine monitoring, treatment and feeding proceeded at the discretion of the attending clinician. The special isolation cage markings were removed the following day when the radiation emitted from the dog measured less than 20µSv per hour. Dogs were discharged from the ICU upon recovery. The cadaver of a dog that died later in the course of the study was transported to the Pathology section where a complete *post mortem* examination was performed.

4.2.2 Radiograph evaluation

Radiographs were evaluated prior to the scintigraphic procedure for the possible presence of pathology not attributable to *Babesia*. All radiographs were evaluated again at a later stage for any abnormalities and classified from 1 to 4, as follows:

1. Normal
2. Near normal, but not suggestive of PTE and pulmonary arteries defined
3. Near normal, but not suggestive of PTE and some pulmonary arteries not defined
4. Abnormal and suggestive of PTE

4.2.3 Arterial blood gas

4.2.3.1 Sample collection

A blood sample was drawn from the femoral artery into a lithium-heparinised 1ml syringe, using a 26G needle. After filling and removal of the syringe, all air bubbles were promptly removed and the needle sealed to room air by inserting its open end into a rubber stopper. Prolonged firm pressure was applied over the arterial puncture site immediately after the sample collection to prevent haematoma formation.

4.2.3.2 Analysis

The arterial blood was analysed using a Rapidlab 348 pH/blood gas analyser (Chiron Diagnostics, Halstead, England). The parameters measured were discussed above under section 4.2.1.5. In clinical cases, the results of the blood gas analysis were made available to the attending clinician to be used at their discretion for additions or alterations in treatment.

4.2.4 Scintigraphy

4.2.4.1 Procedure

Quality control was performed on the equipment in the morning or early afternoon on the day a scintigraphic study was to be performed. The dogs were transported from the

UPBRC (control dogs) or the ICU (Babesia dogs) to the nuclear medicine facility, where either an Elscint Apex 410 (Elgens, Haifa, Israel) or Siemens Orbiter gamma camera (Siemens medical systems, Iselin, NJ, USA) was used with a parallel-hole, low-energy all purpose (LEAP) collimator to perform the scintigrams. Two gamma cameras were used as the Siemens Orbiter gamma camera broke halfway through the study and could not be repaired. The scintigrams in the Babesia dogs were performed as soon as logistically possible, in all dogs after completion of the blood transfusion if administered.

The radiopharmaceutical, ^{99m}Tc-MAA, was ordered from a commercial company (Syncor) to contain 40 000 particles and 37MBq (1mCi) radioactivity in 2ml saline. It was delivered in a lead container and kept in the lead protected compartment of the hot lab. The dosage within the needle and syringe was measured in a radionuclide dose calibrator (Capintec counter, model CRC-15R) immediately before and after administration. Dogs were positioned in sternal recumbency on a perspex-covered table. A 22G Jelco catheter was placed in the cephalic vein and flushed with sterile saline immediately before and after the radiopharmaceutical injection. Immediately prior to injection, the syringe was gently inverted a few times to ensure an even suspension of particles. The agent was slowly injected over five seconds to ensure even distribution of particles in the blood. The time of injection was recorded.

Five minutes after the ^{99m}Tc-MAA injection, a combination of diazepam (at 0.2mg/kg) and morphine (at 0.2mg/kg) was given intravenously via the same cephalic catheter for sedation of all control dogs and four Babesia dogs whose temperament precluded them from lying still for the duration of the scans.

After five minutes, the dog's position was checked to ensure that it was optimal. The gamma camera was moved around the sternally recumbent dog to obtain dorsal, dorsal right oblique, dorsal left oblique and ventral images. Thereafter, the dog was placed in lateral recumbency to obtain left and right lateral images from ventrally. All images were acquired for 120 seconds and recorded in both colour and black-and-white. Images obtained with the Elscint and Siemens gamma cameras were stored in 128x128 and 256x256 matrices respectively. Immediately after the procedure, the control dogs were transferred to the nuclear medicine isolation cages and Babesia dogs to the ICU.

4.2.4.2 Evaluation

The colour and black-and-white scintigrams for each dog were *visually* evaluated by both the author and a human nuclear medicine specialist and classified into different groups based on a consensus opinion. The following classification system adapted from the PISA-PED study[33] was used:

1. Normal (with no perfusion defects)
2. Near normal (with no perfusion defects, but photopaenic defects caused by an enlarged heart, hilus or mediastinum only)
3. Abnormal and suggestive of PTE (single or multiple wedge-shaped perfusion defects)
4. Abnormal and not suggestive of PTE (single or multiple perfusion defects, other than wedge-shaped)

4.3 Statistical analysis

Descriptive statistics, the Fischer's exact test and Mann-Whitney rank sum test were used using SAS (SAS Institute Inc, Cary, NC, USA) and BMDP (BMDP Statistical software Inc, Los Angeles, CA, USA) statistical programmes. Statistical significance was set at $p < 0.05$.

4.4 Ethical considerations

The Animal Use and Care Committee of the Faculty of Veterinary Science, University of Pretoria, approved this study (reference 36-5-627).

5. Results

5.1 General

5.1.1 Control group

There were one intact male, two neutered males and six intact female Beagle dogs. The age mean was 41 months, 2.87 standard deviation (SD) with a range from 36 – 43 months. The body weight mean was 12.28kg, 1.78 SD with a range of 9.9 – 15kg. All dogs maintained normal 5+ habitus throughout, had pink mucous membranes and no blood parasites were found. No complications were observed after the scintigraphic procedure. The temperature means at admission and prior to the scintigraphic procedure were 38.97°C (0.24 SD and range of 38.6 – 39.3°C) and 38.23°C (0.33 SD and range of 37.5 – 38.6°C) respectively. The pulse mean at admission and prior to the scintigraphic procedure were 82bpm (7.94 SD and range of 72 – 96bpm) and 76bpm (6.71 SD and range of 66 – 84bpm) respectively. The respiration rate mean at admission and prior to the scintigraphic procedure were 85.33 breaths per minute (29.12 SD and range of 32 – 100 breaths per minute) and 45.89 breaths per minute (31.79 SD and range of 20 – 100 breaths per minute) respectively. The high respiration rate at admission was due to the fact that seven out of the nine dogs were panting, and thus assigned a fixed respiration rate of 100. Prior to the procedure, dogs were more settled in the new environment and only two of the dogs were panting at that stage. Haematology results were normal (Table 1).

5.1.2 Babesia group

There were nine intact males, four intact females and one sterilised female dog. Four out of the fourteen dogs (dogs 12, 13, 17 and 21) were classified as severe uncomplicated babesiosis due to a haematocrit of <15% (0.15l/l)[52]. Seven dogs (dogs 11, 14, 18, 19, 20, 22 and 23) were classified as complicated babesiosis as they presented with icterus[52]. One dog (dog 16) with IMHA (with a positive in-saline agglutination test on a few consecutive days) and one dog (dog 10) with haemoconcentrated babesia ("red biliary") were also classified as cases of complicated babesiosis[52]. The last dog (dog 15) with a haematocrit of 15.7% (0.157l/l), negative in-saline agglutination test and no icterus was admitted due to a severe

thrombocytopaenia. A severe thrombocytopaenia is a routine finding in both complicated and uncomplicated babesiosis[52], and this dog could thus not be accurately classified.

The age mean was 23.36 months, 26.01 SD with a range from 6 – 103 months. The body weight mean was 15.13kg, 4.92 SD with a range of 6.3 – 25.5kg. The difference in the body weight mean was not found to be statistically significant between the two groups ($p = 0.10$). Only one dog (dog 10) did not receive a blood transfusion since it had a haematocrit of 41% (0.41 l/l). This same dog required turning at regular intervals and was the only non-survivor that died a few days later due to complications not related to the scintigraphic procedure. Half of the dogs had a habitus of 1+ and the other half a habitus of 2+ at presentation. Mucous membrane colour was red in one dog, pale and yellow in two dogs, yellow in five dogs and pale in the remaining six dogs. An in-saline agglutination test was not performed in one dog, was positive in one dog and negative in the remaining 12 dogs. The temperature means at admission and prior to the scintigraphic procedure were 39.3°C (1.41 SD and range of 36.4 – 40.9°C) and 38.14°C (0.62 SD and range of 36.7 – 39.2°C) respectively. The pulse mean at admission and prior to the scintigraphic procedure were 142.57bpm (29.24 SD and range of 80 – 200bpm) and 121.86bpm (21.94 SD and range of 84 – 154bpm) respectively. The respiration rate mean at admission and prior to the scintigraphic procedure were 44.14 breaths per minute (13.23 SD and range of 20 – 70 breaths per minute) and 61.93 breaths per minute (31.99 SD and range of 28 – 120 breaths per minute) respectively. Haematology and serum chemistry results are given in table 1.

On *post mortem* examination, the single non-survivor revealed multiple proteinaceous coagulums (versus classic, coarse fibrillar thrombi as expected for PTE) in numerous small and medium-sized arteries of the lungs. A few thrombi were visualised in several histological sections of the myocardium and there were thromboemboli in serosal blood vessels of the intestines.

Table 1: Haematology and blood chemistry results for group 1 (normal dogs) and group 2 (Babesia dogs)

Parameter	Unit	Normal values	Group 1				Group 2			
			n	Mean	Standard deviation	Range	*n*	Mean	Standard deviation	Range
Haemoglobin concentration	g/l	120-180	9	162.33	9.18	148.0-178.0	14	56.94	30.12	32.0-152.0
Red cell count	$x10^{12}$/l	5.5-8.5	9	6.88	0.33	6.31-7.53	14	2.45	1.21	1.44-6.23
Haematocrit	l/l	0.37-0.55	9	0.46	0.02	0.43-0.51	14	0.16	0.08	0.09-0.41
Mean corpuscular volume	fl	60-77	9	66.91	1.67	64.2-69.3	14	65.41	3.95	59.4-72.7
Mean corpuscular haemoglobin concentration	g/dl of cells	32-36	9	35.29	0.39	34.7-35.8	14	35.29	1.49	32.6-37.1
Red cell distribution width	%		9	15.92	1.08	14.0-17.1	14	17.71	3.12	13.6-24.6
White cell count	$x10^9$/l	6.0-15.0	9	10.52	1.84	7.8-12.5	14	15.82	11.24	5.1-41.3
Absolute mature neutrophil count	$x10^9$/l	3.0-11.5	9	6.06	1.77	3.43-8.25	12	9.11	8.50	2.6-33.45
Absolute immature neutrophil count	$x10^9$/l	0.0-0.5	9	0.13	0.23	0.0-0.69	12	2.13	2.74	0.05-9.77
Absolute lymphocyte count	$x10^9$/l	1.0-4.8	9	2.94	0.67	1.72-3.83	14	2.10	1.13	0.53-4.81
Absolute monocyte count	$x10^9$/l	0.15-1.35	9	0.66	0.32	0.17-1.09	14	1.56	1.43	0.11-5.37
Absolute eosinophil count	$x10^9$/l	0.10-1.25	9	0.73	0.34	0.34-1.4	14	0.04	0.06	0.0-0.21
Absolute basophil count	$x10^9$/l	0.0-0.1	9	0.05	0.09	0.0-0.25	14	0.01	0.02	0.0-0.08
Thrombocyte count	$x10^9$/l	200-500	9	349.56	66.74	263.0-497.0	14	30.37	39.15	0.0-96.0
Reticulocyte percentage	%	0.5-1.5	0	-	-	-	11	8.91	7.04	0.75-22.2
Total serum protein	g/l	53-75	0	-	-	-	14	52.92	12.98	36.0-78.0
Albumin	g/l	27-35	0	-	-	-	9	17.58	3.18	13.4-23.2
Globulin	g/l	20-37	0	-	-	-	9	35.16	11.26	17.5-55.9
Albumin:globulin ratio		0.6-1.2	0	-	-	-	9	0.55	0.22	0.3-1.06
Urea	mmol/l	3.6-8.9	0	-	-	-	1	22.30	-	-
Creatinine	µmol/l	40-133	0	-	-	-	6	86.00	88.63	29.0-265.0
Sodium	mmol/l	140-155	0	-	-	-	9	137.01	4.66	128.0-142.0
Potassium	mmol/l	3.6-5.1	0	-	-	-	11	3.36	0.82	2.46-4.86
Total calcium	mmol/l	2.2-2.9	0	-	-	-	1	1.93	-	-
Ionised calcium	mmol/l		0	-	-	-	9	1.23	0.09	1.12-1.38
Phosphate	mmol/l	0.9-1.6	0	-	-	-	1	2.71	-	-
Blood glucose	mmol/l	3.3-5.5	0	-	-	-	9	4.32	0.89	2.3-5.3

5.2 Arterial blood gas

Eight (88.89%) control and 11 (78.57%) Babesia dogs' blood gas analysis was performed within five minutes of obtaining the sample. In one (11.11%) control and two (14.29%) Babesia dogs the analysis was performed between six to ten minutes after obtaining the sample. In one (7.14%) Babesia dog the analysis was performed at 20 minutes after obtaining the sample; however the sample was refrigerated during this time. The results for the arterial blood gas analysis are given in table 2.

The Mann-Whitney rank sum test was performed to determine whether there were significant differences between the means of the two groups for ten variables. There was no statistical significant difference between the partial pressure of carbon dioxide ($p = 0.05$), partial pressure of oxygen ($p = 0.90$), pH ($p = 0.75$), actual bicarbonate ($p = 0.05$), estimated oxygen saturation ($p = 0.90$), arterial oxygen tension:inspired oxygen fraction ratio ($p = 0.66$), arterial-alveolar oxygen tension difference ($p = 0.19$) and arterial-alveolar oxygen tension ratio ($p = 0.19$). There was a statistically significant difference for the standard bicarbonate ($p = 0.03$) and estimated oxygen content ($p = 0.0001$).

Table 2: Blood gas analysis results for group 1 (normal dogs) and group 2 (Babesia dogs)

Parameter	Unit	Normal values	Group 1				Group 2			
			n	Mean	Standard deviation	Range	*n*	Mean	Standard deviation	Range
Partial pressure of carbon dioxide	mmHg	30-40	9	34.1	3.27	26.6-37.3	14	30.41	4.75	19.3-38.5
Partial pressure of oxygen	mmHg	>90	9	80.47	9.31	63.3-89.7	14	78.38	11.69	49.7-92.8
pH		7.35-7.45	9	7.34	0.17	6.89-7.43	14	7.40	0.03	7.36-7.48
Sodium	mmol/l	140-155	9	140.67	3.84	131.0-144.0	14	136.79	4.41	127.0-140.0
Potassium	mmol/l	3.6-5.1	9	4.24	0.27	3.95-4.87	14	3.55	0.57	2.69-4.69
Calcium	mmol/l	1.15-1.32	9	1.35	0.12	1.03-1.47	14	1.13	0.16	0.83-1.38
Haematocrit	l/l		9	0.44	0.05	0.39-0.54	2	0.18	0.08	0.12-0.23
Actual bicarbonate	mmol/l		9	19.16	5.53	4.8-23.8	14	18.07	2.83	13.8-23.8
Standard bicarbonate	mmol/l	18-24	9	20.53	5.37	6.5-24.7	14	19.55	2.10	17.5-24.4
Extra-cellular fluid base excess	mmol/l		9	-6.31	8.26	-28.0-(-0.3)	14	-6.51	2.88	-9.5-0.0
Blood base excess	mmol/l		9	-5.33	8.34	-27.2-0.3	14	-5.87	2.50	-8.4-(-0.2)
Total carbon dioxide content	mmol/l		9	20.13	5.60	5.6-24.8	14	18.96	2.94	14.4-24.8
Calcium ion concentration adjusted to pH 7.4	mmol/l		9	1.40	0.04	1.37-1.5	14	1.14	0.16	0.87-1.4
Estimated oxygen saturation	%		9	93.19	5.60	79.1-96.5	14	94.24	3.52	84.6-97.5
Estimated oxygen content	ml/dl		9	21.28	1.73	17.4-23.0	14	8.77	4.45	4.5-18.0
Arterial oxygen tension : inspired oxygen fraction ratio		>3	9	3.53	0.42	2.77-3.93	14	3.46	0.51	2.3-4.14
Arterial-alveolar oxygen tension difference	mmHg		5	15.94	6.03	8.0-23.8	11	13.44	13.57	0.4-46.2
Arterial-alveolar oxygen tension ratio			5	0.82	0.07	0.73-0.91	11	0.86	0.14	0.52-0.99

5.3 Thoracic radiographs

Three (33.33%) control and seven (50%) Babesia dogs demonstrated a class 1-type pattern. Three (33.33%) control and four (28.57%) Babesia dogs demonstrated a class 2-type pattern. Three (33.33%) control and three (21.43%) Babesia dogs demonstrated a class 3-type pattern. The classification for each dog is given in table 3. The radiographic classification patterns observed for the two groups did not differ significantly ($p = 0.76$, Fischer's exact test).

5.4 Pulmonary perfusion scintigrams

5.4.1 Control group

All control dogs' scintigrams were performed using the Siemens Orbiter gamma camera. The injected radiopharmaceutical dosage mean was 24.05MBq (0.65mCi), SD was 12.21MBq (0.33mCi) and a range of 6.66 – 42.92MBq (0.18 – 1.16mCi). Using the PISA-PED classification, five (55.56%) dogs were classified as class 1 and four (44.44%) dogs as class 4. The classification for each dog is given in table 3. The colour and black-and-white scintigraphic images for the control dogs are shown in figure 1(A – C) and 2(A – C) respectively.

5.4.2 Babesia group

The Siemens Orbiter gamma camera was used in five dogs (dogs 10 – 14) and the Elscint Apex 410 gamma camera in nine dogs (dogs 15 – 23). The scintigrams were performed 16 to <20 hours after admission in six dogs, 20 to <25 hours after admission in six dogs and >25 hours after admission in two dogs. The latter two dogs' studies (dog 14 and dog 23) were done at 46 hours; 50 minutes and 36 hours; 5minutes respectively. The injected radiopharmaceutical dosage mean was 37.37MBq (1.01mCi), SD was 6.66MBq (0.18mCi) and a range of 28.86 – 52.17MBq (0.78 – 1.41mCi). Using the PISA-PED classification, six (42.86%) dogs were classified as class 1 and seven (50%) dogs as class 4. Only one (7%) dog (dog 10) demonstrated a class 2 pattern, due to an enlarged cardiac silhouette. The classification for each dog is given in table 3. The colour and black-and-white scintigraphic images for the Babesia dogs are shown in figures 1(D – H) and 2(D – H) respectively.

Although both colour and black-and-white images were used to classify each dog's perfusion pattern, the evaluators found that the black-and-white images were the most useful in this regard.

The Fischer's exact test revealed that the PISA-PED classification patterns observed for the two groups did not differ significantly ($p = 1.00$) and no statistic correlation was found between the radiographic and scintigraphic classification patterns observed for each dog ($p = 1.00$).

Table 3: Radiographic and scintigraphic classification for the two study groups

Dog number	Group	Radiographic classification	PISA-PED classification
1	Control	2	4
2	Control	1	4
3	Control	1	1
4	Control	2	1
5	Control	2	1
6	Control	3	4
7	Control	1	1
8	Control	3	1
9	Control	3	4
10	Babesia	3	2
11	Babesia	2	4
12	Babesia	1	1
13	Babesia	3	4
14	Babesia	3	1
15	Babesia	1	1
16	Babesia	2	4
17	Babesia	1	1
18	Babesia	2	4
19	Babesia	1	4
20	Babesia	1	4
21	Babesia	1	1
22	Babesia	1	1
23	Babesia	2	4

6. Discussion

This study presents original scintigraphic evidence of the pulmonary perfusion pattern in dogs hospitalised with babesiosis in an attempt to demonstrate the presence of PTE. There are no typical signalment or breed predispositions for PTE[27], but any condition causing hypercoagulability of the blood, stasis of blood flow or vascular endothelial injury predispose the patient to thrombus formation[27,28]. Sepsis and DIC are prothrombotic conditions that resort under these criteria[27-29]. Several changes in circulating blood, including increased platelet reactivity, coagulation factors, fibrinolytic inhibitors and decreased coagulation inhibitors or fibrinolytic activity as well as increased or abnormal lipids are all implicated in hypercoagulability and thrombosis[30]. Intravascular haemolysis of any aetiology (including *Babesia* spp. infection) is a common trigger for DIC[32]. Although clinical DIC is seldom seen, it was postulated that it might be a serious complication of severe *Babesia* infection in the dog, after one study revealed a DIC-like syndrome on *post mortem* examination[56]. Also hypothesised, was that the MODS demonstrated in the complicated form of *Babesia* was caused (in addition to tissue damage due to local hypoxia) by microthrombi as a result of a coagulopathy[51]. With indications of activation of the coagulation system in naturally infected as well as experimentally infected cases[50], it is however still not known if this results in increased stickiness and retention of infected red blood cells in the microvasculature, thus forming thrombi. Another explanation could be the obstruction of blood flow at capillary level due to the deposition of fibrin clots as a result of DIC[50,56].

Although PTE has never been published to be present at *post mortem* in CB, thromboemboli were demonstrated in the lungs at *post mortem* in dogs with IMHA (for which a similar mechanism of venous stasis, hypercoagulability and endothelial damage as found in CB is proposed)[71]. In the one Babesia dog that died, a *post mortem* examination revealed some proteinaceous coagulums in numerous small and medium-sized arteries. This was not the classic, coarse fibrillar thrombi that would be expected with PTE.

High-resolution spiral CT, digital subtraction angiography and pulmonary angiography (and even MRI) may have been more “gold standard methods” to diagnose PTE, however were not available at our institution and would have required general anaesthesia carrying increased risk in our critically ill patients. D-dimers may also be used to diagnose thromboembolism[37] however at the time of the study this diagnostic method was not available for the use in

animals in South Africa. It is also not specific to the lung, and should be interpreted in association with the patient's clinical signs. Acute onset dyspnoea, tachypnoea and depression are common clinical signs in PTE[28], but may also be found in complicated CB as a result of the severe anaemia, which causes hypoxaemia and compensatory hyperventilation[72,73]. Finding these clinical signs may thus not always raise one's suspicion for PTE, and would thus not be as helpful when using D-dimers as an absolute diagnostic test.

Arterial blood gas values may be useful in the diagnosis of PTE, however normal values do not exclude it[27,28]. In one study, canine patients with experimentally induced PTE revealed no significant differences in blood gas values within five minutes or the next half hour after injection[22]. In another study an increased arterial-alveolar oxygen concentration (tension) difference was thought to be the most sensitive single blood parameter to diagnose PTE in three dogs[27]. Arterial hypoxaemia and hypocapnoea may also be found[27,28]. The blood pH varies due to the influences of respiratory alkalosis and metabolic acidosis[27]. The arterial blood gas analysis results demonstrated no statistically significant difference between the two groups for partial pressure of carbon dioxide (which indicate that respiratory alkalosis was also not present in the Babesia group) or oxygen, pH, actual bicarbonate, estimated oxygen saturation, arterial oxygen tension:inspired oxygen fraction ratio, arterial-alveolar oxygen tension difference or arterial-alveolar oxygen tension ratio. The oxygen parameters as a measure of lung function were all good, indicating that there was no blood gas indication of V/Q mismatching, which would be expected in clinically significant PTE. The standard bicarbonate and estimated oxygen content differed significantly between the two groups, the latter could be explained by the presence of anaemia in the Babesia group. Acid-base disturbances were not specifically evaluated in this study. Mixed acid-base disturbances in severe canine babesiosis has been described by Leisewitz *et al*[74] with the most common combination of abnormalities found being hyperchloraemic acidosis, organic metabolic acidosis partially due to hyperlactataemia, hyperphosphataemic acidosis, dilutional acidosis and respiratory alkalosis.

The aim of this study was to answer the question: "Does a scintigraphic pattern consistent with PTE occur in dogs hospitalised with naturally occurring infection with *B canis rossi*?" It was hypothesised that scintigraphic evidence of PTE would indeed occur in these hospitalised patients; this was however found not to be the case in this limited study. Babesia dogs demonstrated a similar distribution pattern to a group of normal control dogs. It is

possible that a more subtle difference in the spatial distribution of perfusion may have existed between the two groups if a more quantitative evaluation of the pulmonary perfusion scintigrams was done, but this was beyond the scope of this study.

A mottled appearance was seen especially on the dorsal oblique images of many normal and Babesia dogs. The reason for this is unknown and has not been specifically reported previously in normal dogs. It is possible that this pattern may be consistent with multiple small emboli or chronic obstructive pulmonary disease[2], however since many normal dogs also appeared similar, is not believed to be true in this case. Another possibility is that the appearance is due to pulmonary infiltrates; however this was not seen on the radiographs and would again not explain the appearance in the normal dogs. A third, but less likely, possibility is that the appearance is due to uneven distribution because of injection error. The occurrence of this was limited by multiple precautionary measures, i.e. all dogs were kept in sternal recumbency during injection and for five minutes thereafter, the syringe was gently inverted a few times before injection to ensure good mixing of the particles, the injection was made slowly and evenly and care was taken not to have air in the syringe or to inject air during the injection. Due to uncooperative patients (especially the normal dogs), it was decided to rather use a catheter to avoid the possibility of having the dog move and thus possibly injecting the radiopharmaceutical subcutaneously. It is possible that the use of a catheter and not direct injection into the vein may have had an effect on the above (i.e. having some gas or blood clots in the catheter hub). If blood is allowed to clot within the syringe or in the catheter during injection (in abnormal and normal patients), disproportionate absorption of the radioactive aggregates in the lung will cause an apparent uneven distribution of activity. For similar reasons, all air should be removed from the syringe or the catheter prior to injection. Both will rather result in “hot clots” and not photopaenic defects[11].

The general aim in this study was to obtain an injected dose of 37MBq (1mCi) radiopharmaceutical, although the reference dosage in dogs is 17.5 to 74MBq (0.5 – 2mCi)[2]. Three of the nine control dogs (dogs 5, 6 and 9) and none of the Babesia dogs received a dosage below 17.5MBq. The reason for the difference in the injected dose between the two groups is unknown as the scintigraphic method employed and the method of injection was identical for both groups and the radiopharmaceutical did not leak subcutaneously. The visual evaluation of scintigrams was not affected negatively, as the perfusion pattern in the three control dogs that received less than 17.5MBq radiopharmaceutical did not differ significantly

from the other dogs. It is believed that this may only have resulted in a difference if a quantitative analysis of the perfusion patterns would have been done, which was beyond the scope of this study. Pulmonary thromboembolism would have resulted in wedge-shaped perfusion defects, which would have been seen during the visual inspection done and tested in this study. In fact, one of the initial ten control group dogs was excluded from the study due to an extremely low injected dose as some radiopharmaceutical leaked subcutaneously at the injection site, which resulted in a very pixelated appearance of the images. This pixilation made visual inspection more difficult, but still resulted in a recognisable perfusion pattern that would have demonstrated a wedge-shaped perfusion defect if it was indeed present.

Some authors still believe that a ventilation scintigraphic scan should be done in association with a perfusion scan to increase the specificity and accuracy of diagnoses[27,28], thus the absence of a ventilation study may be thought to be a possible limitation of this study. However, authors of the recent PISA-PED study in humans proposed that the sensitivity and specificity of a perfusion scan, without a ventilation scan, in patients with suspected PTE was sufficient[33].

Another possible limitation of this study is the large variation in the time to performing the scintigrams in the Babesia dogs. This time variation occurred due to a variety of logistical reasons, varying from awaiting owner consent prior to performing a study to ordering of radiopharmaceutical and awaiting delivery. The effect this may have had on the study is difficult to determine, however it is believed that if any dog would have had clinically significant PTE, it would still be present and thus diagnosed at the times the scintigrams were performed in the dogs in this study.

Another limitation of this study is the small number of cases as well as the selection of cases. In order to investigate the perfusion pattern in dogs of similar size and conformation, Babesia dogs in the same weight range as the normal control Beagles were selected. This became a logistical problem as most Babesia cases seen at the OVAH fall in the weight ranges above and below the selected weight range, thus resulting in a small study population. The selection of cases did not result in specifically selecting those individuals clinically suspected of PTE, however as mentioned before, PTE and CB share similar clinical signs, which would make selection difficult. Also, in dogs with experimentally induced PTE, the respiratory and heart rates were significantly increased immediately and within the first five minutes after the

injection of agar, but gradually returned to the initial values[22]. Again this may mimic dogs with babesiosis. The Babesia dogs in this study, except one dog that could not be accurately classified, were all suffering from either the severe uncomplicated or complicated form of the disease. Future studies could attempt to look more at critically respiratory ill patients or dogs showing blood gas abnormalities suggestive of V/Q mismatching in an attempt to select cases suspected of PTE. Studies incorporating more detailed *post mortem* evaluation of the lungs and possibly other organs, as well as D-dimer testing for the presence of thrombi should be embarked upon in future.

The objective of this study was to prospectively evaluate the scintigraphic pulmonary perfusion pattern in hospitalised Babesia dogs, in an attempt to ascertain whether a scintigraphic pattern consistent with PTE does indeed occur in these patients. Surprisingly, not a single dog in the Babesia group had wedge-shaped perfusion defects which would have resulted in a high probability for PTE. The scintigraphic pulmonary perfusion pattern demonstrated was not significantly different between a group of normal control and Babesia dogs ($p = 1.00$), thus indicating that in this limited study PTE was not a complicating factor in the Babesia dogs.

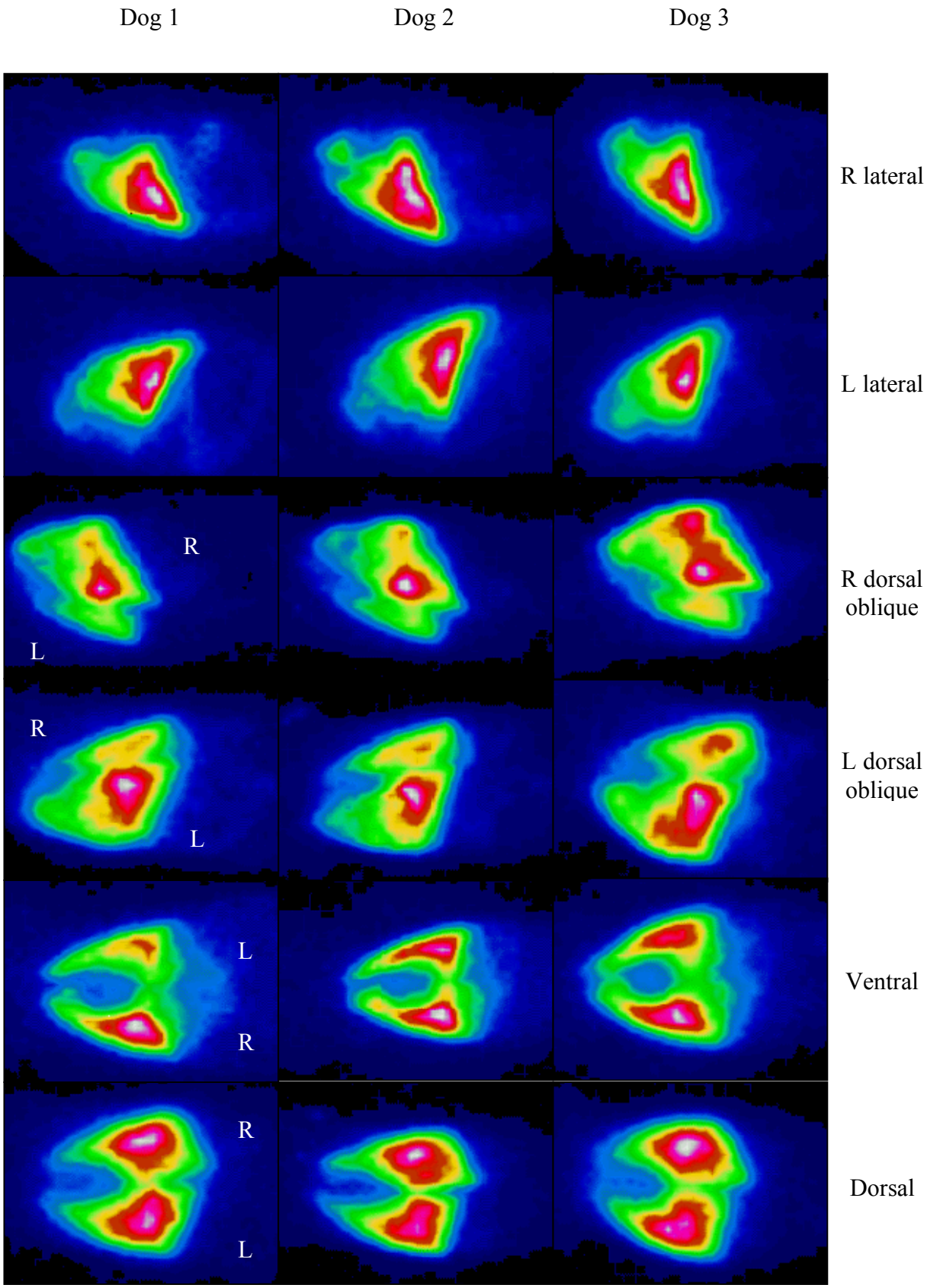

Figure 1A: Colour scintigraphic images for Dogs 1 – 3 (Control group) Cranial is to the left of the images

Dog 4 Dog 5 Dog 6

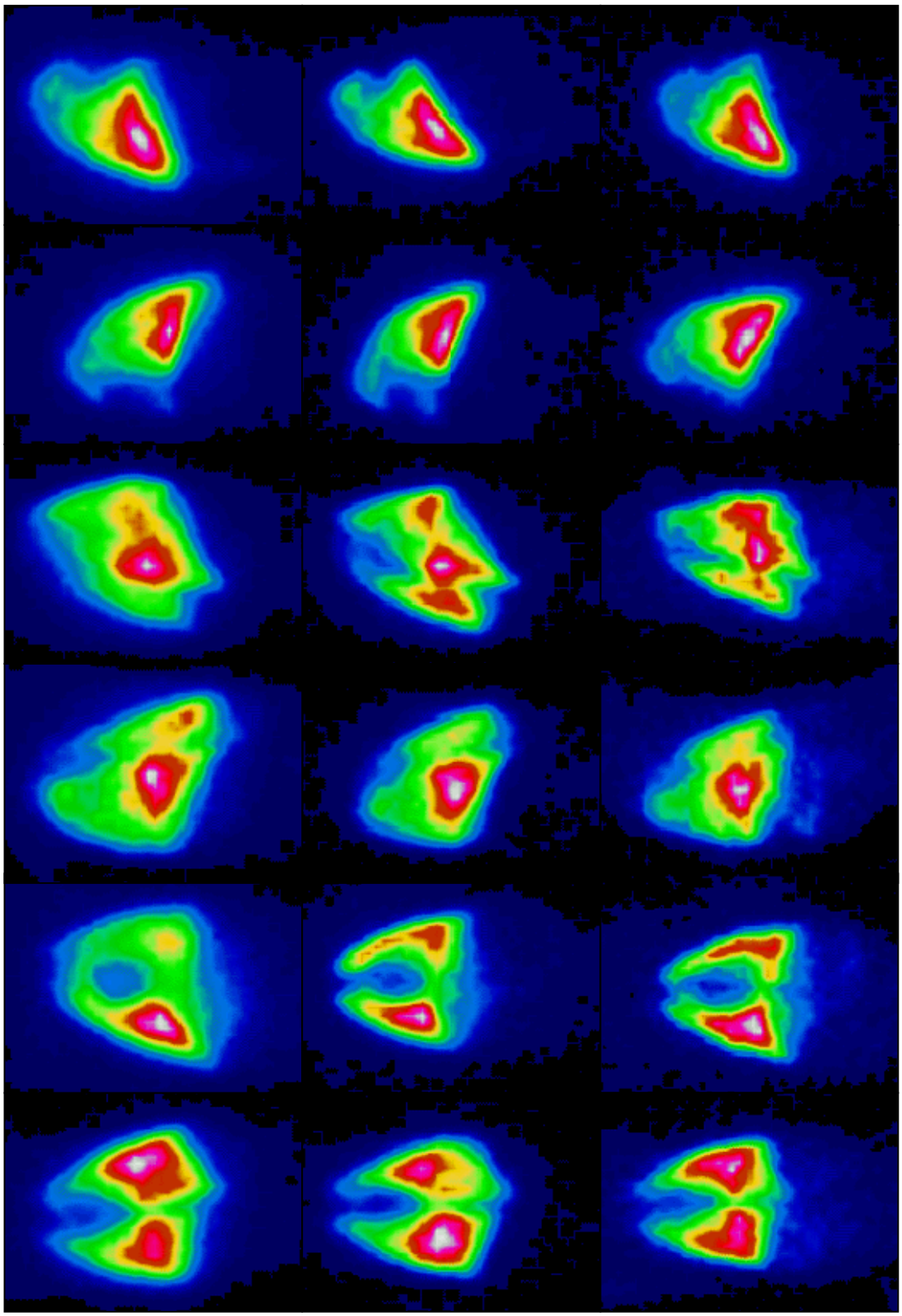

Figure 1B: Colour scintigraphic images for Dogs 4 – 6 (Control group)
Cranial is to the left of the images. See Fig 1A for right (R) and left (L) markers

Dog 7 Dog 8 Dog 9

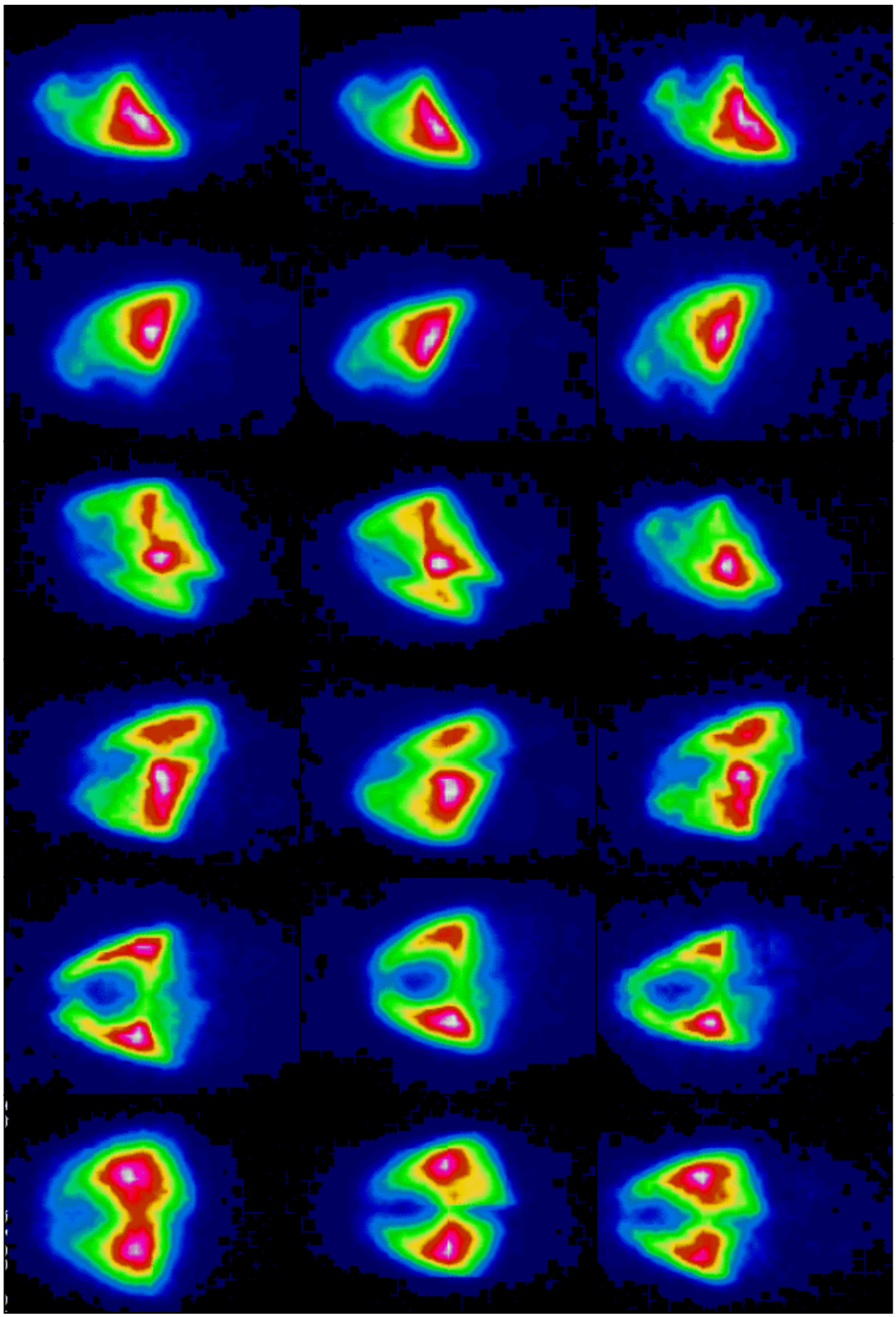

Figure 1C: Colour scintigraphic images for Dogs 7 –9 (Control group)
Cranial is to the left of the images. See Fig 1A for right (R) and left (L) markers

Dog 10 Dog 11 Dog 12

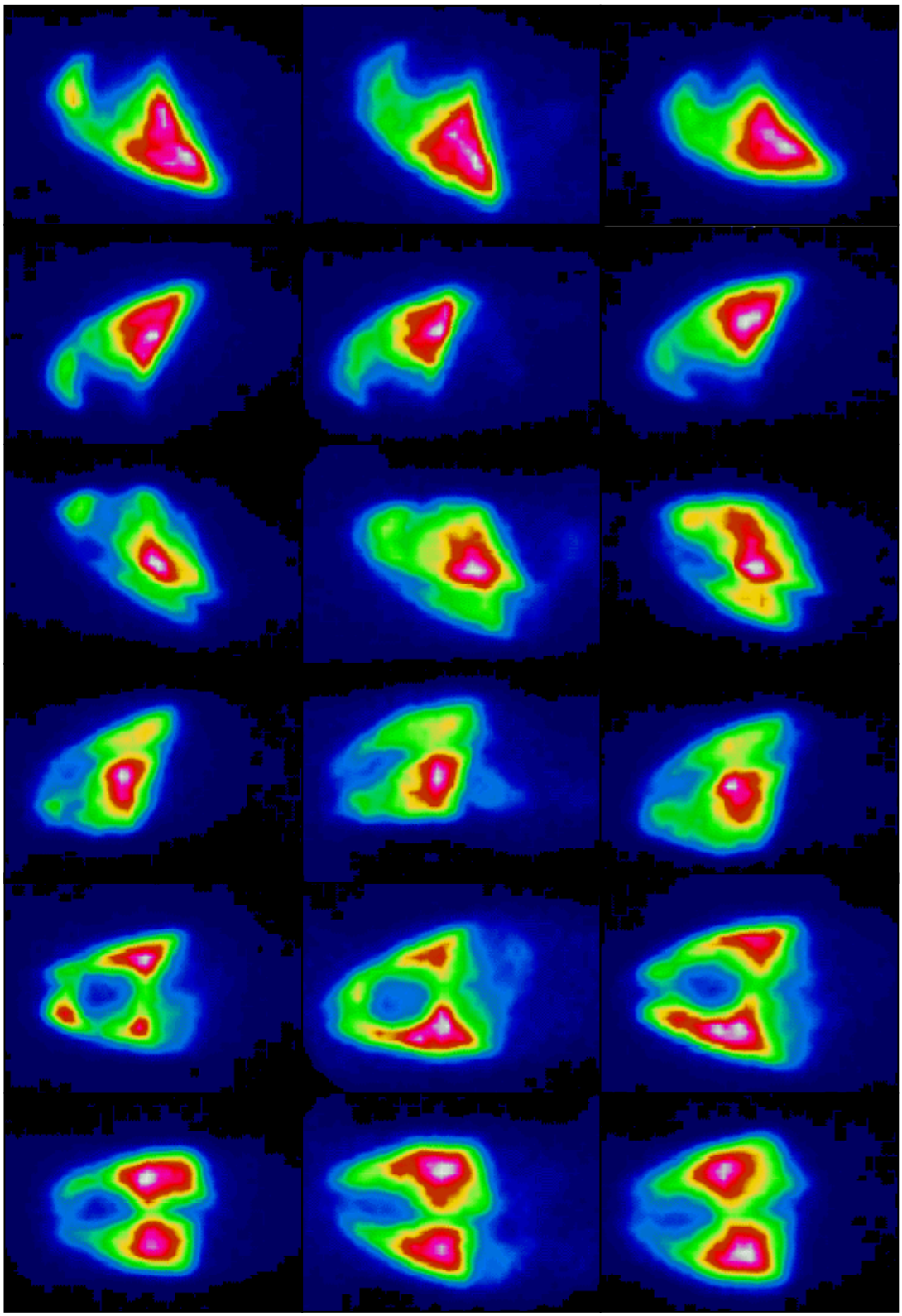

Figure 1D: Colour scintigraphic images for Dogs 10 – 12 (Control group) Cranial is to the left of the images. See Fig 1A for right (R) and left (L) markers

Dog 13 Dog 14 Dog 15

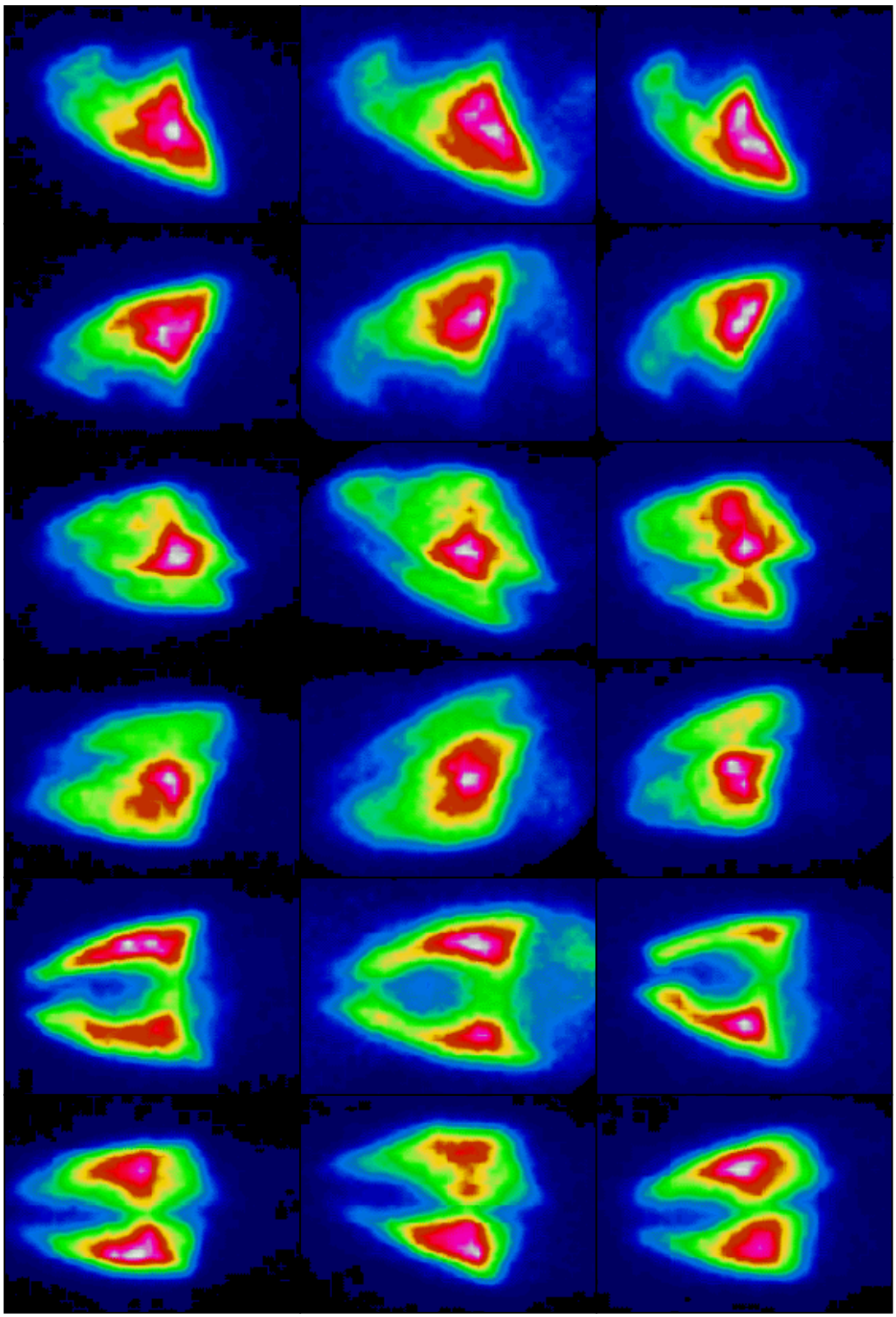

Figure 1E: Colour scintigraphic images for Dogs 13 – 15 (Control group) Cranial is to the left of the images. See Fig 1A for right (R) and left (L) markers

Dog 16 Dog 17 Dog 18

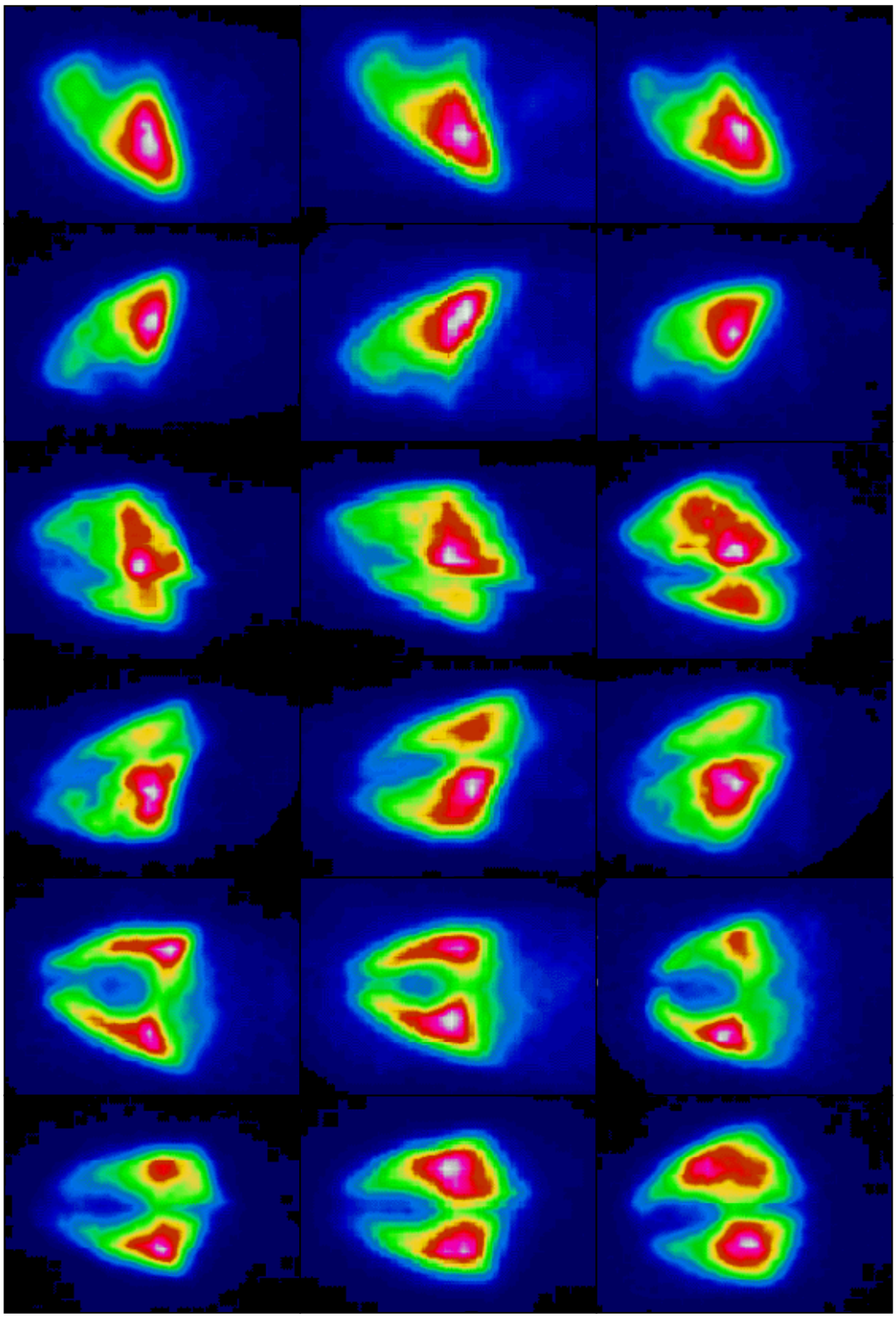

Figure 1F: Colour scintigraphic images for Dogs 16 – 18 (Control group) Cranial is to the left of the images. See Fig 1A for right (R) and left (L) markers

Dog 19 Dog 20 Dog 21

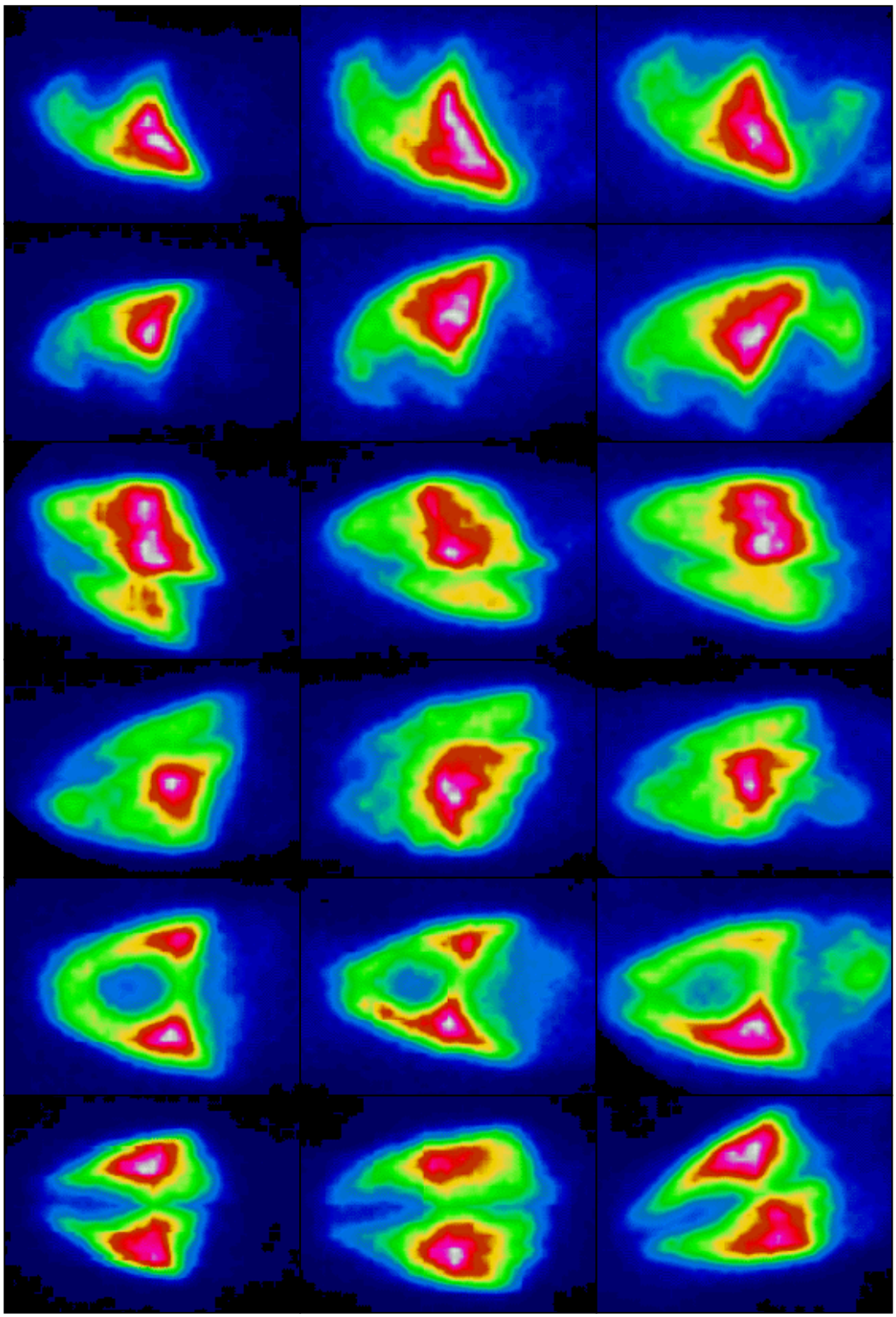

Figure 1G: Colour scintigraphic images for Dogs 19 – 21 (Control group) Cranial is to the left of the images. See Fig 1A for right (R) and left (L) markers

Dog 22 Dog 23

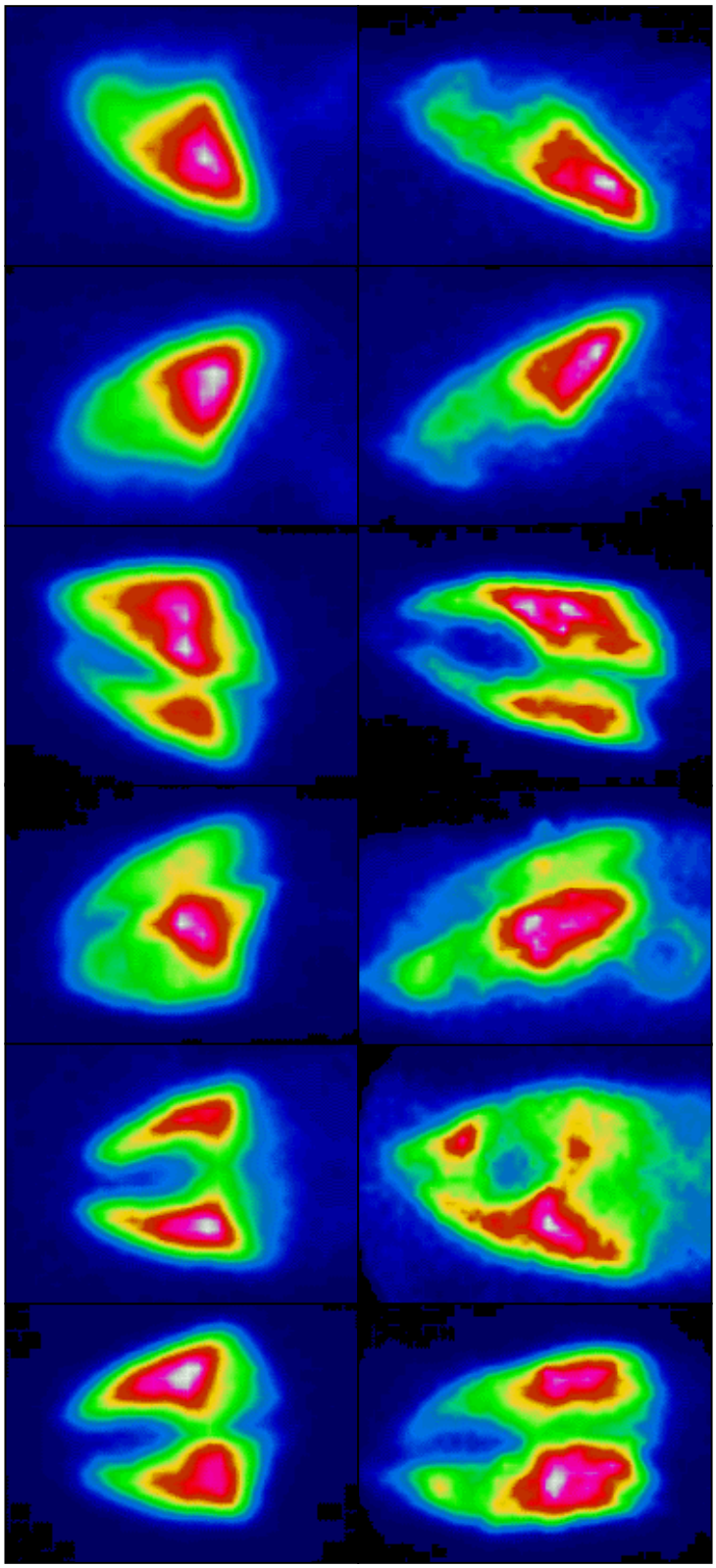

Figure 1H: Colour scintigraphic images for Dogs 22 – 23 (Control group) Cranial is to the left of the images. See Fig 1A for right (R) and left (L) markers

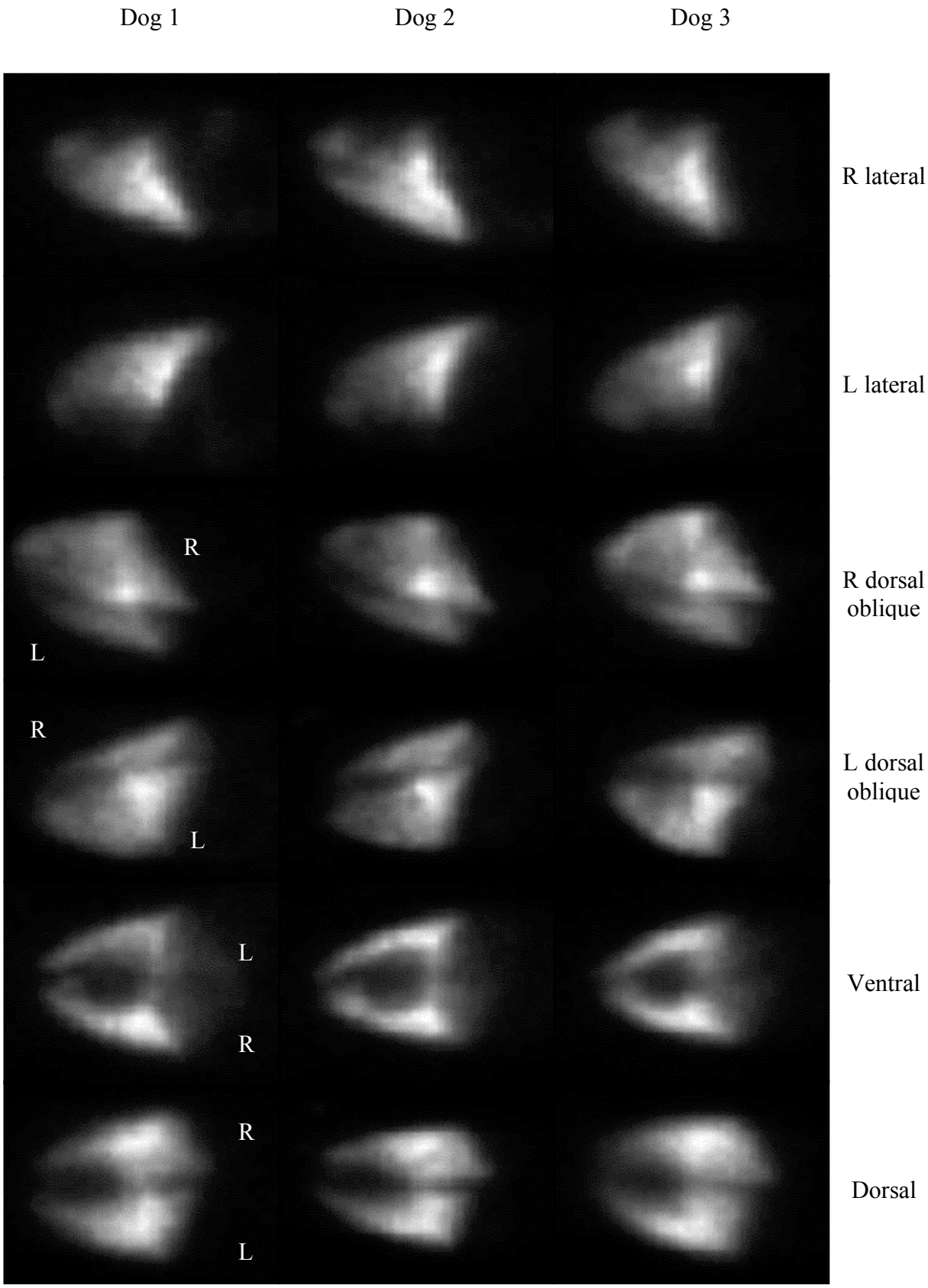

Figure 2A: Black-and-white scintigraphic images for Dogs 1 – 3 (Control group) Cranial is to the left of the images

Dog 4 Dog 5 Dog 6

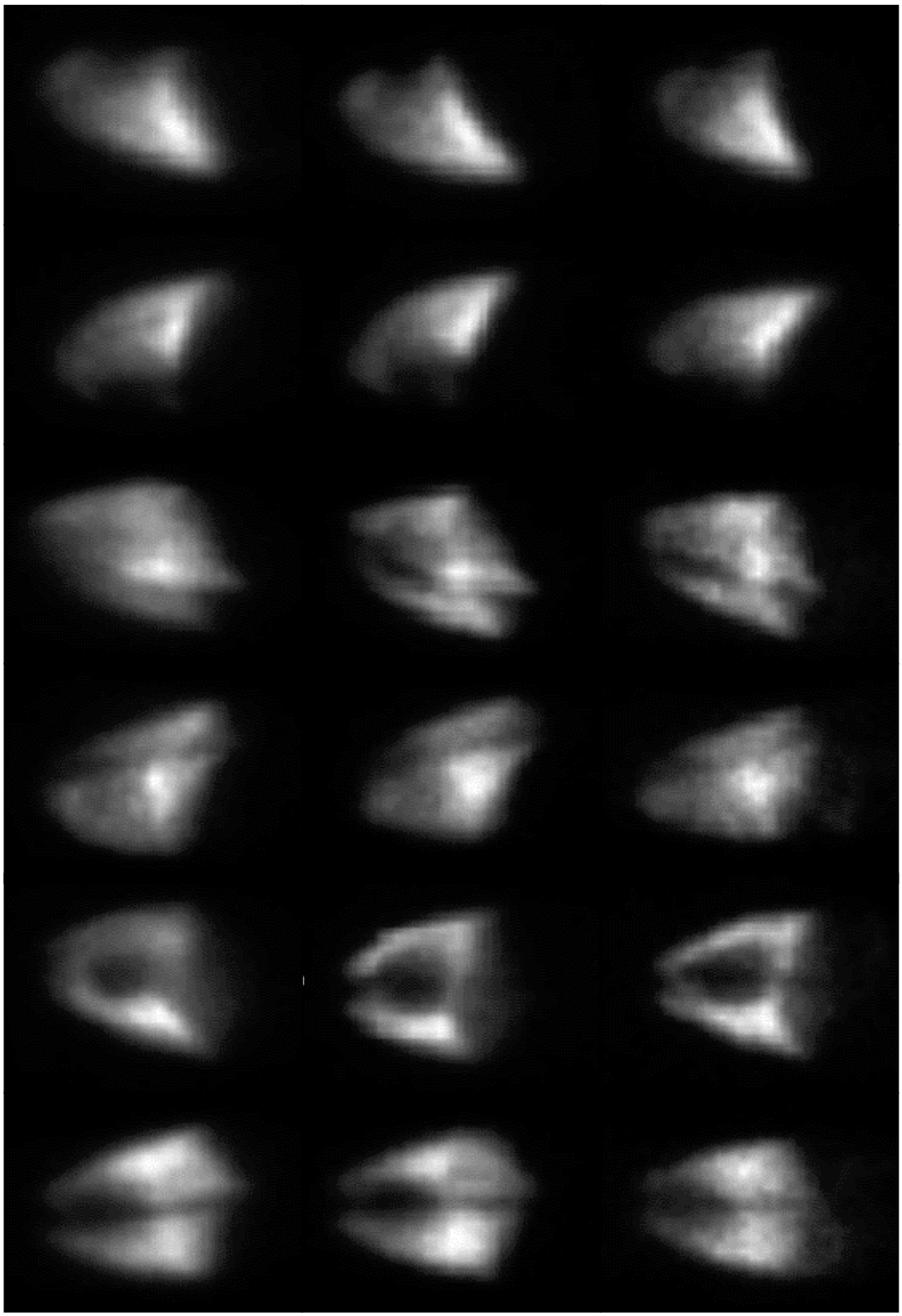

Figure 2B: Black-and-white scintigraphic images for Dogs 4 – 6 (Control group) Cranial is to the left of the images. See Fig 2A for right (R) and left (L) markers

Dog 7 Dog 8 Dog 9

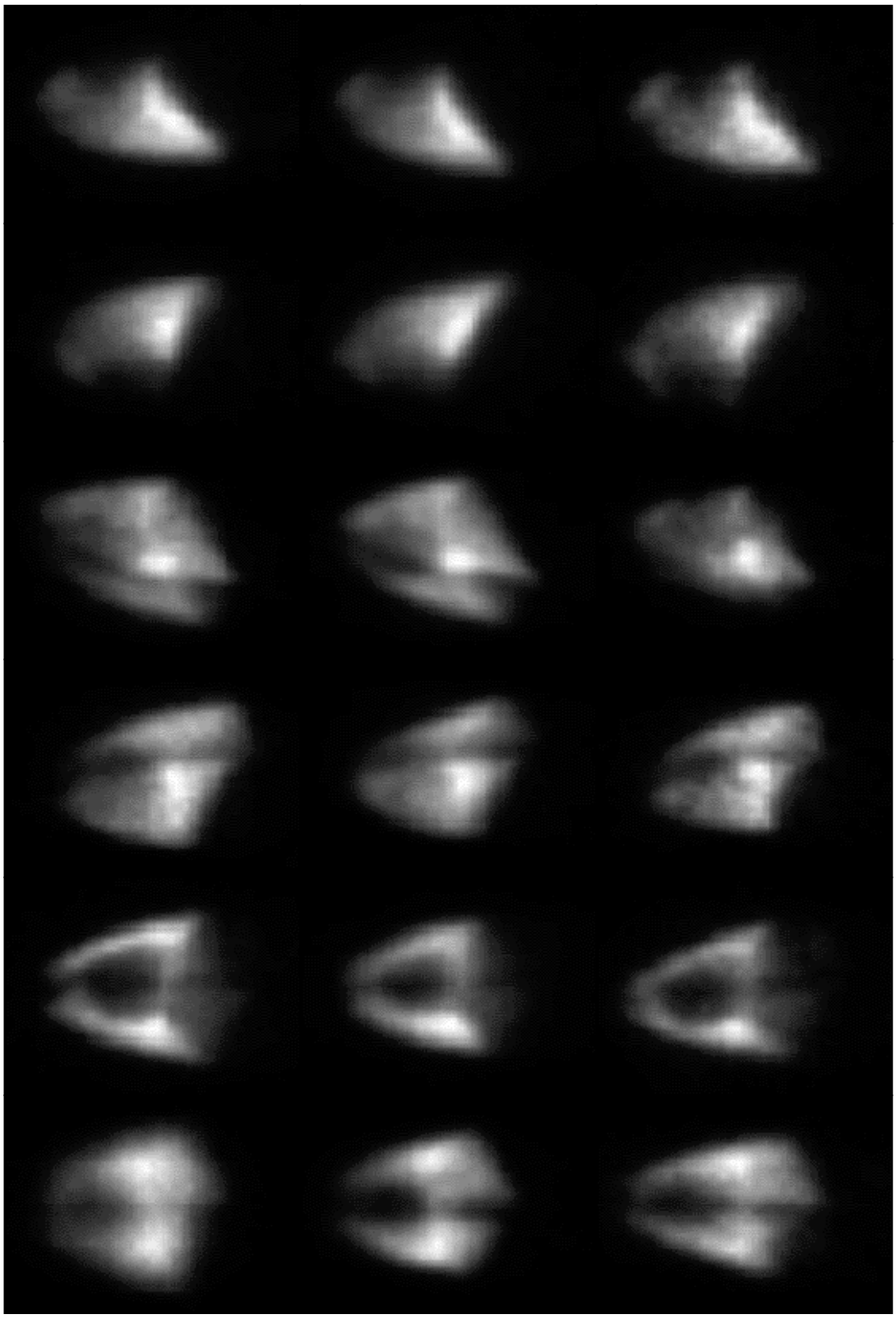

Figure 2C: Black-and-white scintigraphic images for Dogs 7 –9 (Control group) Cranial is to the left of the images. See Fig 2A for right (R) and left (L) markers

Dog 10 Dog 11 Dog 12

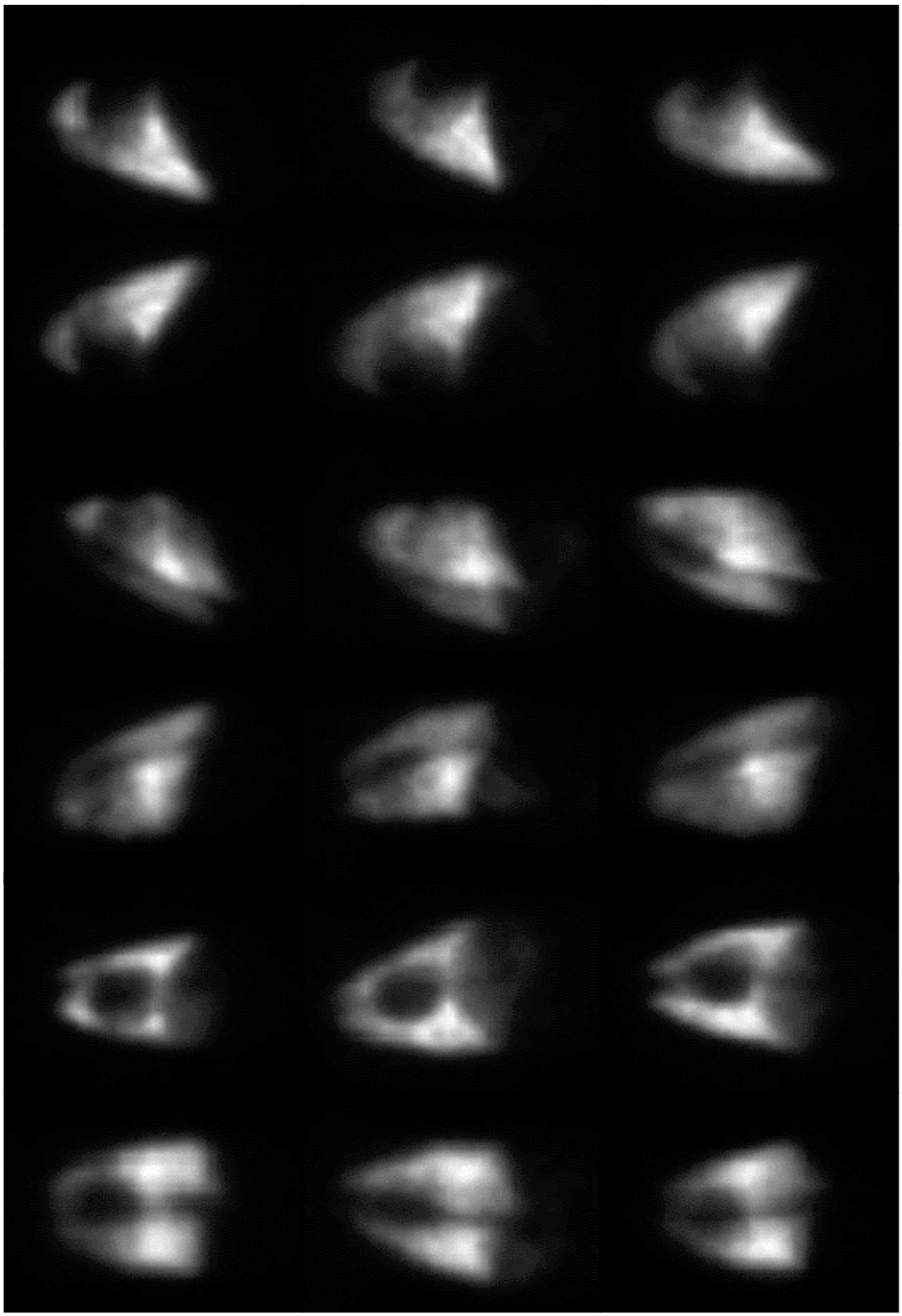

Figure 2D: Black-and-white scintigraphic images for Dogs 10 – 12 (Control group) Cranial is to the left of the images. See Fig 2A for right (R) and left (L) markers

Dog 13 Dog 14 Dog 15

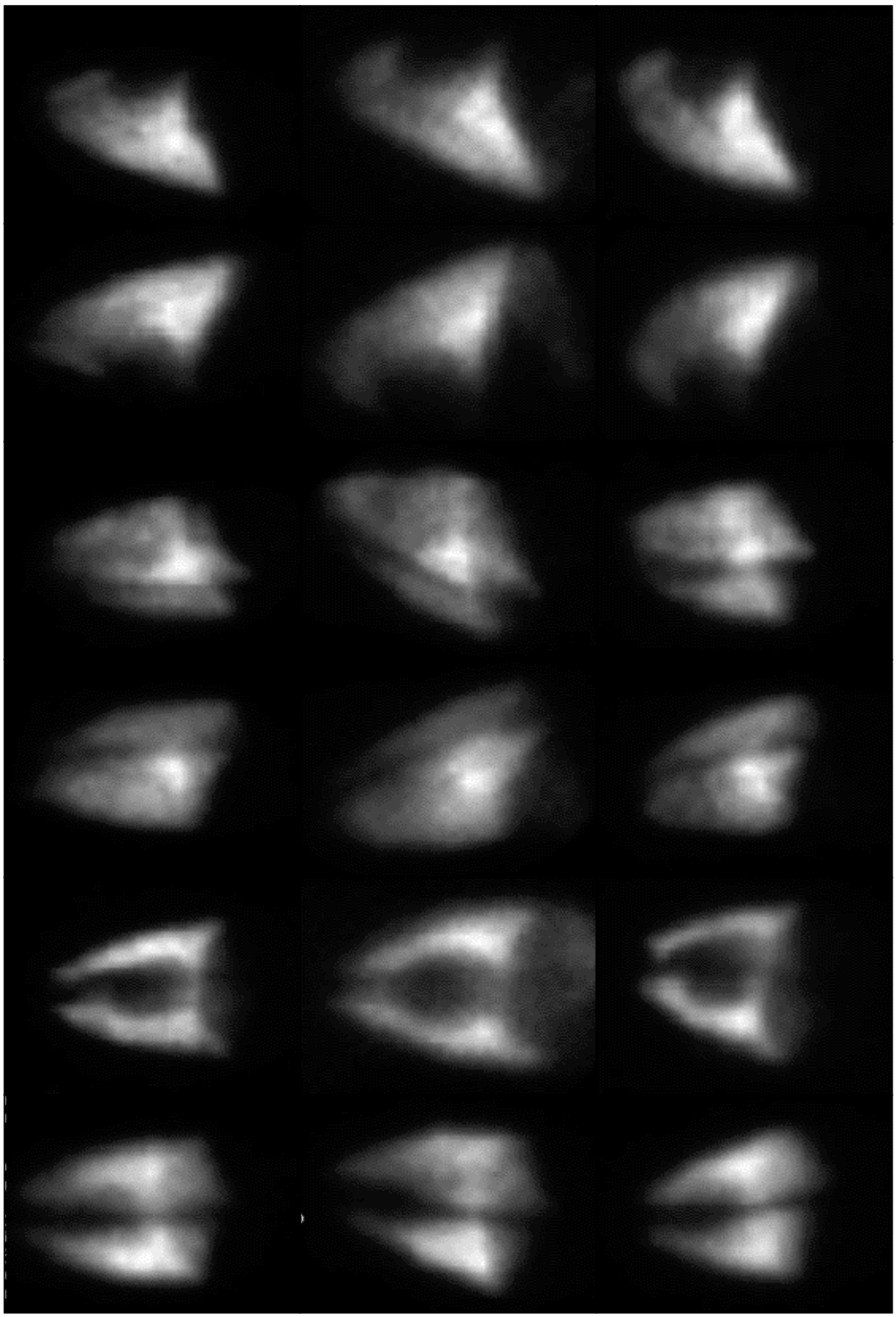

Figure 2E: Black-and-white scintigraphic images for Dogs 13 – 15 (Control group) Cranial is to the left of the images. See Fig 2A for right (R) and left (L) markers

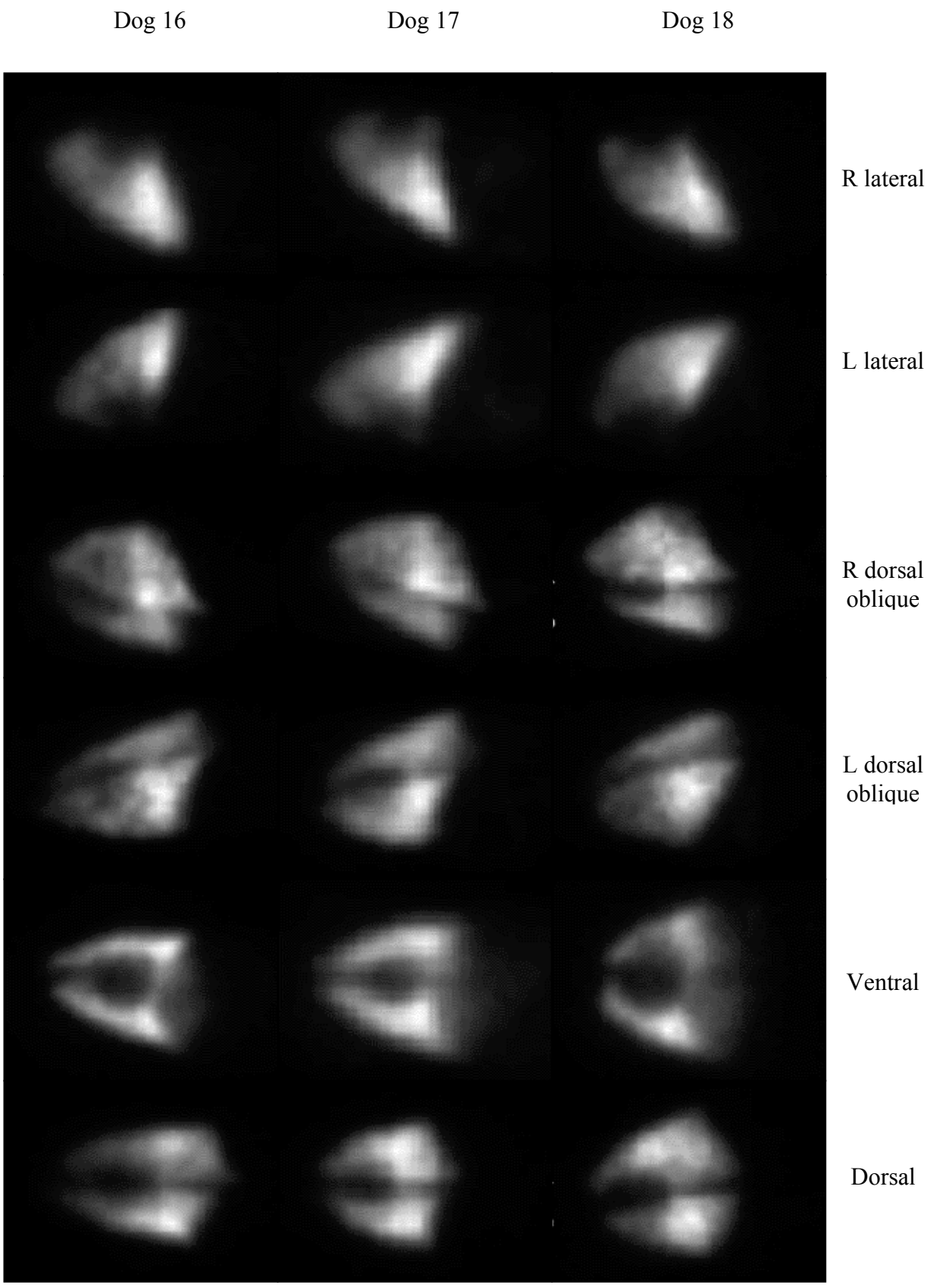

Figure 2F: Black-and-white scintigraphic images for Dogs 16 – 18 (Control group) Cranial is to the left of the images. See Fig 2A for right (R) and left (L) markers

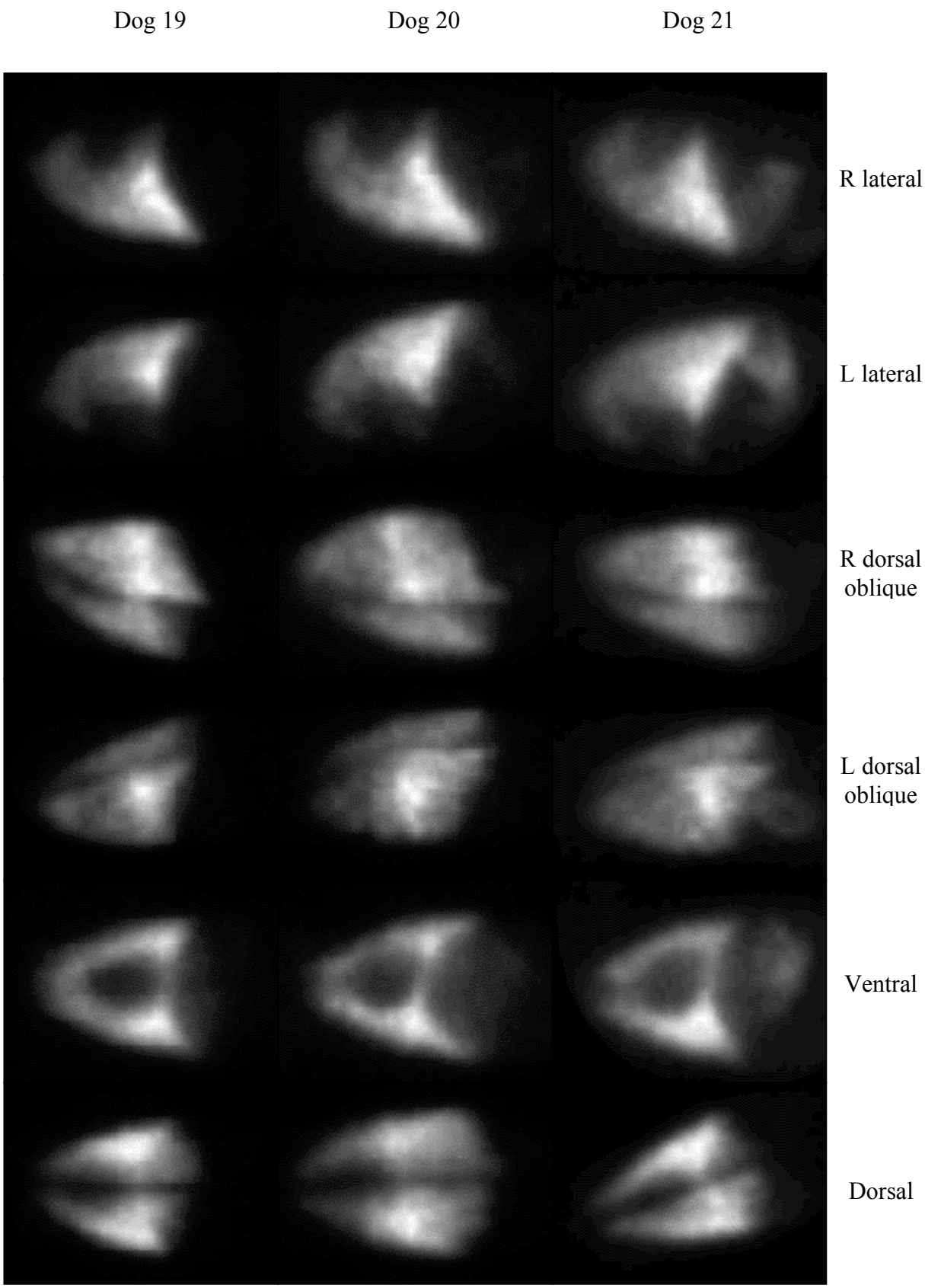

Figure 2G: Black-and-white scintigraphic images for Dogs 19 – 21 (Control group) Cranial is to the left of the images. See Fig 2A for right (R) and left (L) markers

Dog 22 Dog 23

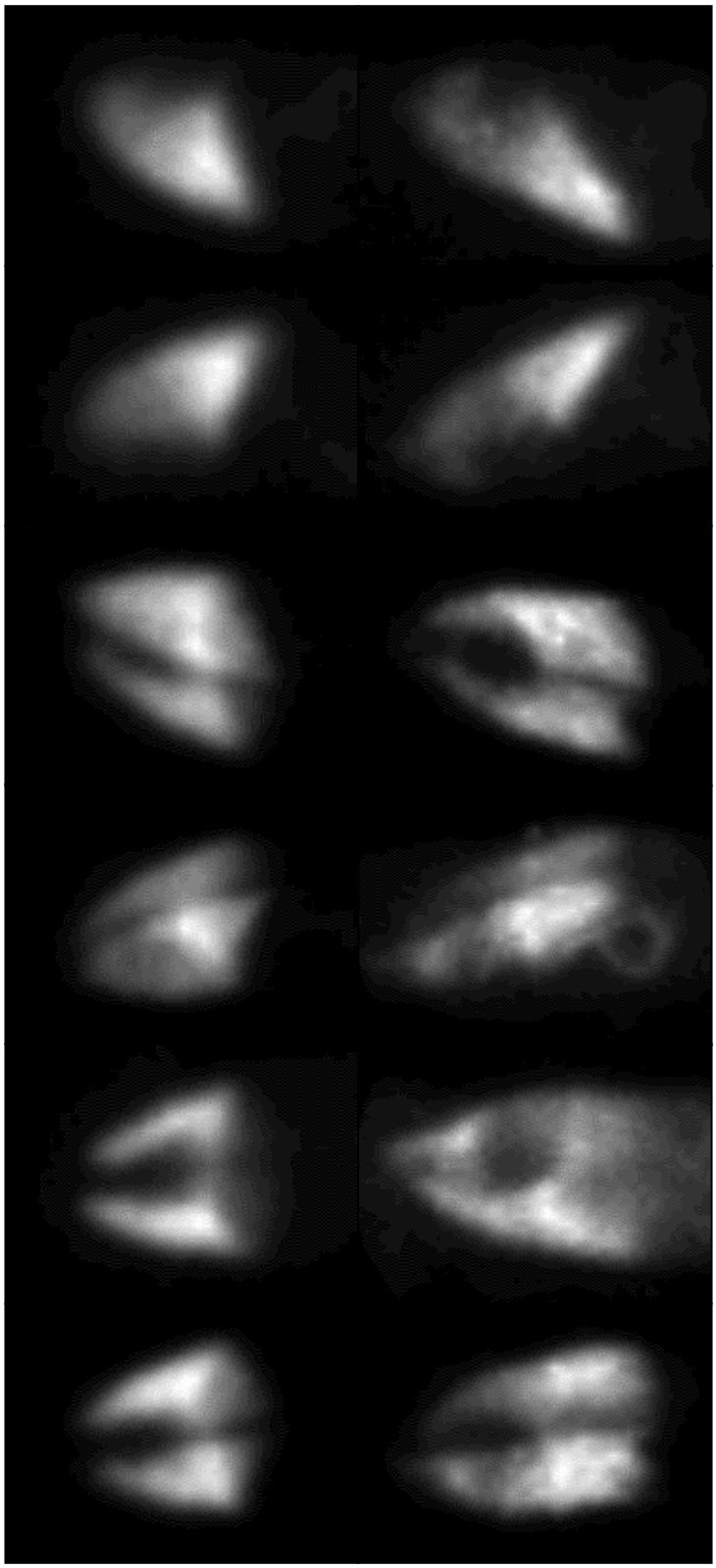

Figure 2H: Black-and-white scintigraphic images for Dogs 22 – 23 (Control group) Cranial is to the left of the images. See Fig 2A for right (R) and left (L) markers

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