The scintigraphic evaluation of the pulmonary perfusion pattern of dogs hospitalised with babesiosis

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ABSTRACT

The possibility of coagulopathy in *Babesia canis rossi* infections in the canine patient has been suggested in the literature, but minimal work has been done to evaluate the clinicopathological nature of it in further detail. Pulmonary thromboembolism (PTE) has not yet been implicated in canine babesiosis (CB), but may also be one of the causes of the sudden dyspnoea and tachypnoea that are frequently seen in complicated CB patients. The objective of this study was to prospectively evaluate the scintigraphic pulmonary perfusion pattern in hospitalised dogs with babesiosis in an attempt to ascertain whether a scintigraphic pattern consistent with clinically relevant PTE does indeed occur in these patients. The study consisted of a normal control group of 9 mature healthy Beagle dogs (group 1) and a Babesia group with 14 dogs of a variety of breeds that were naturally infected with *Babesia* (group 2). 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, but not a single dog in group 2 had pleural-based, wedge-shaped perfusion defects which would have resulted in a high probability for clinically relevant PTE. The scintigraphic pulmonary perfusion pattern demonstrated was not significantly different between the 2 groups ($P = 1.00$).

Key words: canine babesiosis, pulmonary perfusion, pulmonary thromboembolism, scintigraphy.

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INTRODUCTION

In South Africa, canine babesiosis (CB) is one of the most important infectious diseases of dogs[36]. Typically, an uncomplicated form of the disease, characterised by anaemia, pyrexia, anorexia, listlessness, splenomegaly, tachypnoea and a waterhammer pulse can be found[18,40]. A complicated form with clinical findings depending on the organ involved also exists. This includes lung involvement with respiratory symptoms[18,23].

No typical signalment or breed predisposition exists for the obstruction of a pulmonary vessel by a thrombus[12]. Any condition causing hypercoagulability of the blood, stasis of blood flow or vascular endothelial injury will predispose the patient to thrombus formation[12,21]. Sepsis and disseminated intravascular coagulation (DIC) are prothrombotic conditions that resort under these criteria[11,12,21]. 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[7]. Intravascular haemolysis of any aetiology (including *Babesia*[37]) is a common trigger for DIC[6]. Disseminated intravascular coagulation has been reported in splenectomised calves infected with *Babesia argentina*[9] and *Babesia bovis*[10], in sheep experimentally infected with *Babesia ovis*[42,43], in 24 % of 63 cases of canine babesiosis in Hungary[24] and in several dogs with the severe (complicated) form of CB[27]. It has been suggested that the atypical symptoms of CB may be a manifestation of DIC localised primarily to a particular organ system[27]. The mechanisms by which *Babesia canis* induce DIC are still unknown, but several were suggested by Moore and Williams[27]. Some researchers believe that the hypercoagulable state in CB is because of profound disturbance in fibrinogen metabolism rather than DIC[32,39,41].

Pulmonary thromboembolism (PTE) was found on *post mortem* examination in dogs with immune-mediated haemolytic anaemia (IMHA)[19]. The mechanism for IMHA-associated PTE is unknown; however, as with CB, is also believed to be due to venous stasis, hypercoagulability and endothelial damage[19]. If the same mechanisms involved in IMHA-associated PTE are implicated in CB, the potential for PTE in cases of CB must exist. In humans, PTE is thought to be a major contributor to mortality in 5 –15 % of adults in hospitals[11]. It is highly under-diagnosed, with only 30 % of human patients with clinically relevant pulmonary emboli being diagnosed correctly[21,31]. 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[31]. It has been reported in dogs that 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[13].

Diagnosis of PTE remains difficult, with haematology and biochemistry of limited value. Two radiographic patterns, namely oligaemia and single or multiple regions of alveolar pulmonary infiltrates have been described in dogs[12,13,21]. Pleural-based, wedge-shaped alveolar opacification is rarely seen[12,13]. 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[13]. Main pulmonary artery segment enlargement, right-sided cardiomegaly and pleural effusion have been reported[12,21]. Electrocardiographic (ECG) findings may

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indicate changes that are non-specific for PTE[12]. Arterial blood gas values may be useful in diagnosis of PTE; however, normal values do not exclude it[12,21]. Identifying evidence of pulmonary arterial hypertension with echocardiography may help to support a diagnosis of PTE[29]. All of the above tests may prove non-specific for PTE, but may serve as an excluding mechanism of other disease processes[12,21,26].

D-dimer is a laboratory marker of coagulation, which can be used in the detection of early embolism in humans and small animals[29]. 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[29]. Spiral (high resolution) computed tomography (CT) is currently widely used in human medicine for the diagnosis of PTE[14]. 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[3,38]. This is not yet a reliable diagnostic technique in either human or veterinary patients, with relatively high false-positive rates obtained in human studies[2] and also due to the higher cost and unavailability of equipment in the veterinary setting. Pulmonary angiography is the most accurate technique currently available for dogs – it is, however, invasive, more difficult to perform and interpret, and requires general anaesthesia with an increased risk in critically ill patients[12,20,21].

Pulmonary scintigraphy is a useful screening test for PTE in humans[21] and has been found to be sensitive and specific for experimental PTE in the canine[12,21]. Neither the incidence of PTE nor the use of pulmonary perfusion scintigraphy in CB dogs have ever been studied. With this method, ^{99m}Tc-human macroaggregated albumin (^{99m}Tc-MAA) with a biological half-life of 4–6 hours is used[5]. Because of the size of the ^{99m}Tc-MAA albumin particles (more than 90 % having a diameter between 10 and 60 μm), they physically lodge by a purely mechanical process in the 1st capillary bed encountered after intravenous injection. This in effect implies blockage in the pulmonary capillaries[4], as these small vessels have a diameter of approximately 7 to 10 μm[25]. 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[5,30]. A normal perfusion scintigram is recognised by a homogeneous 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[5,16,33]. A normal (thus negative) perfusion scintigram virtually excludes the presence of clinically significant PTE[2,5,17,33]. Studies in experimental animal models have shown that a pulmonary scintigram may detect 97 % of occlusive emboli larger than 2 mm in diameter[1], but may fail to detect smaller or incompletely occluding emboli[1,15,28]. In addition to the latter 2 reasons, when there is a substantial delay between the occurrence of the PTE and the scintigram, recanalisation or dissolution of the thrombus may occur, thus resulting in a false negative scan[15]. Small emboli located in the peripheral basal zones of the lungs may be missed, as would small areas of decreased perfusion with little change in radioactivity as compared with surrounding normal tissues due to a shine-through effect[15]. An abnormal pulmonary scintigram is recognised by photopaenic defects as a result of abnormal pulmonary perfusion. With clinically relevant PTE, a wedge-shaped pleural-based photopaenic defect is usually seen with a lobar or segmental distribution[2,5,33]. 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[5] and survey radiographs are thus essential. Some authors believe that the addition of a pulmonary ventilation scintigram increases the diagnostic specificity of PTE[12,21]. 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[31]. Scans were classified as normal (class 1), near-normal (class 2), abnormal and suggestive of PTE (class 3) or abnormal and not suggestive of PTE (class 4). 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[26]. 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[31].

The objective of this study was to prospectively evaluate the scintigraphic pulmonary perfusion pattern in hospitalised dogs with babesiosis and compare these with a group of normal control dogs, in an attempt to ascertain whether a scintigraphic pattern consistent with clinically relevant PTE does indeed occur in CB patients.

The study was approved by the University of Pretoria's faculty ethics committee (reference 36-5-627).

MATERIALS AND METHODS

This study was a prospective, minimally invasive experiment with a normal control group (n = 9) (group 1) and naturally *Babesia*-infected group (n = 14) (group 2).

Group 1 consisted of 1 intact and 2 neutered males and 6 intact female healthy Beagle dogs on loan from the University of Pretoria's Biomedical Research Centre (UPBRC). In order to ascertain their general health status, a complete physical examination (habitus, temperature, pulse, respiration and mucous membrane colour), peripheral blood smear examination and full haematology was performed prior to being included in the study. The blood smear was obtained and evaluated the day before the scintigraphic study. Only dogs that were clinically healthy, in good physical condition, free of blood parasites (specifically *Babesia* and *Ehrlichia canis*) and with normal haematology values were included. Dogs were housed at the UPBRC kennels and fed and managed according to UPBRC standard protocols. Dogs were transferred to the Onderstepoort Veterinary Academic Hospital (OVAH) scintigraphy section on the morning of the scintigraphic procedure.

Group 2 consisted of naturally *Babesia*-infected, client-owned dogs which were selected from patients admitted to the OVAH for further treatment (*i.e.* blood transfusion therapy or treatment for other complications). Dogs were caged and managed in the intensive care unit (ICU). The dogs were from a variety of breeds, including a Spaniel, Labrador retriever, Husky, Rottweiler, Japanese akita, Staffordshire bull terrier, Dalmatian, Bassett, Sharpei and 5 crossbreeds. An attempt was made to select patients with a uniform body weight for ease of evaluation of the scintigraphic images. Dogs with 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 classified according to a previously published classification system for CB[18]. 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* (and which may result in false positives or masking of PTE), pregnant or lactating bitches, narrow-chested or severely obese dogs. 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, which in most cases also included a blood transfusion. Regular (at least hourly) turning of a patient was done if it was required.

Baseline data collection (at admission) for both groups included each dog's age, sex, sterilisation status, body weight, habitus, temperature, pulse, respiration and mucous membrane colour. Habitus was scored from 1+ (extremely lethargic) to 5+ (normal bright and alert). The temperature, pulse and respiration were again recorded immediately 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 at admission and evaluated for the presence of blood parasites (specifically *B.* and *E. canis*) and a sample for complete haematology obtained. An in-saline agglutination test and other additional tests were performed in some group 2 dogs at the discretion of the attending clinician.

Thoracic radiographs, consisting of a right and left lateral and dorsoventral view, were made, not more than 4 hours prior to the scintigraphic procedure, in all dogs except 1 in each group, where a ventrodorsal view was made. All radiographs were visually evaluated by 2 veterinary diagnostic imaging specialists (LS and RK) for any abnormalities and classified from 1 to 4, as follows:

Class 1. Normal.

Class 2. Near normal, but not suggestive of PTE and pulmonary arteries visually defined.

Class 3. Near normal, but not suggestive of PTE and some pulmonary arteries not visually defined.

Class 4. Abnormal and suggestive of PTE.

Immediately before the scintigraphic examination, a femoral arterial blood sample was obtained for blood gas analysis using a Rapidlab 348 pH/blood gas analyser (Chiron Diagnostics, Halstead, England). 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.

The dogs were transported from the UPBRC (group 1) or the ICU (group 2) 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. The scintigrams in group 2 dogs were performed as soon as logistically possible, but after completion of the blood transfusion if administered. The ^{99m}Tc-MAA was ordered from a commercial company (Syncor) to contain 40 000 particles and 37 MBq (1 mCi) radioactivity in 2 mℓ saline. Immediately before injection, the syringe was gently inverted a few times to ensure an even suspension of particles. The radiopharmaceutical was slowly administered (*via* a cephalic catheter with the dog in sternal recumbency) over 5 seconds to ensure even distribution of particles in the blood. Five minutes after the ^{99m}Tc-MAA injection, a combination of diazepam (at 0.2 mg/kg) and morphine (at 0.2 mg/kg) was given intravenously via the same cephalic catheter for sedation of all group 1 and 4 group 2 dogs whose temperament precluded them from lying still for the duration of the scans. The catheter was flushed with sterile saline immediately before and after the radiopharmaceutical and sedative injections. The gamma camera was moved around the sternally recumbent dog to obtain dorsal, dorsal right oblique, dorsal left oblique and ventral images. This scan procedure was initiated 5 minutes after injection of the sedative or 10 minutes after injection of the ^{99m}Tc-MAA in dogs that received no sedation. Thereafter, the dog was placed in lateral recumbency to obtain left and right lateral images from ventrally (with the camera below the table). All static images were recorded in both colour and black-and-white and acquired for 120 seconds using a 128 × 128 (Elscint gamma camera) or 256 × 256 (Siemens gamma camera) matrix. Immediately after the procedure, group 1 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. Group 2 dogs were transferred to an ICU cage in a specifically designated isolation area to minimise radiation risk and the special isolation cage markings were removed when the radiation emitted from the dog measured less than 20 μSv per hour. Dogs were discharged from the ICU upon recovery. The colour and black-and-white scintigrams for each dog were visually evaluated by a veterinary diagnostic imaging specialist (LS) and a human nuclear medicine specialist (FN) and classified into different groups based on a consensus opinion. The following classification system adapted from the PISA-PED study[31] was used:

Class 1. Normal (with no perfusion defects) (Fig. 1).

Class 2. Near normal (with no perfusion defects, but photopaenic defects caused by an enlarged heart, hilus or mediastinum only).

Class 3. Abnormal and suggestive of PTE (single or multiple wedge-shaped perfusion defects) (Fig. 2).

Class 4. Abnormal and not suggestive of PTE (single or multiple perfusion defects, other than wedge-shaped) (Fig. 3).

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 $P < 0.05$.

RESULTS

General

Group 1

The age, body weight, temperature, pulse and respiration rate results are given in Table 1. The high respiration rate at admission was due to the fact that 7 out of the 9 dogs were panting, and thus assigned a fixed respiration rate of 100. Before the procedure, dogs were more settled in the new environment and only 2 of the dogs were panting at that stage. 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.

Group 2

Four dogs (dogs 12, 13, 17 and 21) were classified as severe uncomplicated babesiosis due to a haematocrit of <15 % (0.15l/l)[18]. Seven dogs (dogs 11, 14, 18, 19, 20, 22 and 23) were classified as complicated babesiosis, as they presented with

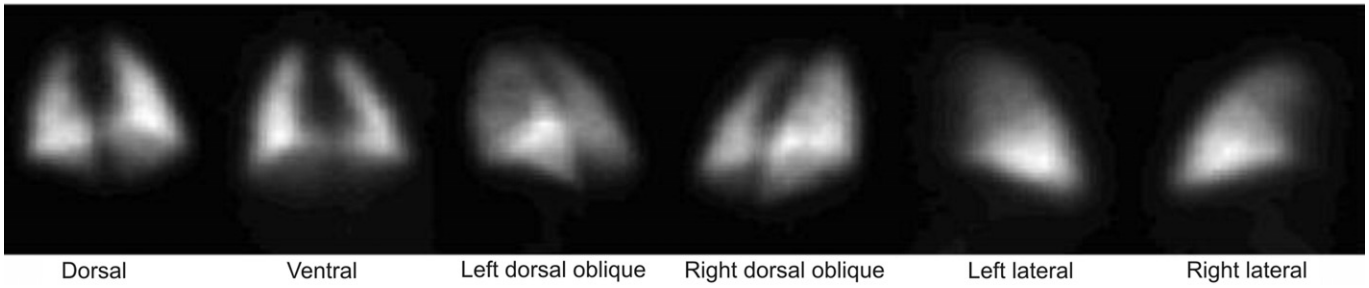

Fig. 1: **Black-and-white pulmonary perfusion scintigrams of dog 22 with a normal class 1 pattern. Note the homogeneous distribution of radiopharmaceutical throughout the entire lung field. For all images, cranial is to the top and caudal to the bottom**.

icterus[18]. One dog (dog 16) with IMHA (with a positive in-saline agglutination test on a few consecutive days) and 1 dog (dog 10) with haemoconcentrated babesia ('red biliary') were also classified as complicated[18]. The last dog (dog 15) with a haematocrit of 15.7 % (0.157 ℓ/ℓ), negative in-saline agglutination test and no icterus was admitted due to severe thrombocytopaenia. Severe thrombocytopaenia is a routine finding in both complicated and uncomplicated babesiosis[18], and this dog could thus not be accurately classified. The age, body weight, temperature, pulse and respiration rate results are given in Table 1. The difference in the body weight mean was not found to be statistically significant between the 2 groups ($P = 0.10$). Only 1 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 1 dog, pale and yellow in 2 dogs, yellow in 5 dogs and pale in the remaining 6 dogs. An in-saline agglutination test was not performed in 1 dog, was positive in 1 dog and negative in the remaining 12 dogs. Haematology and serum chemistry results were consistent with dogs suffering from CB, but are not presented here as it is beyond the scope of this study.On *post mortem* examination, the single non-survivor (dog 10) revealed multiple proteinaceous coagula

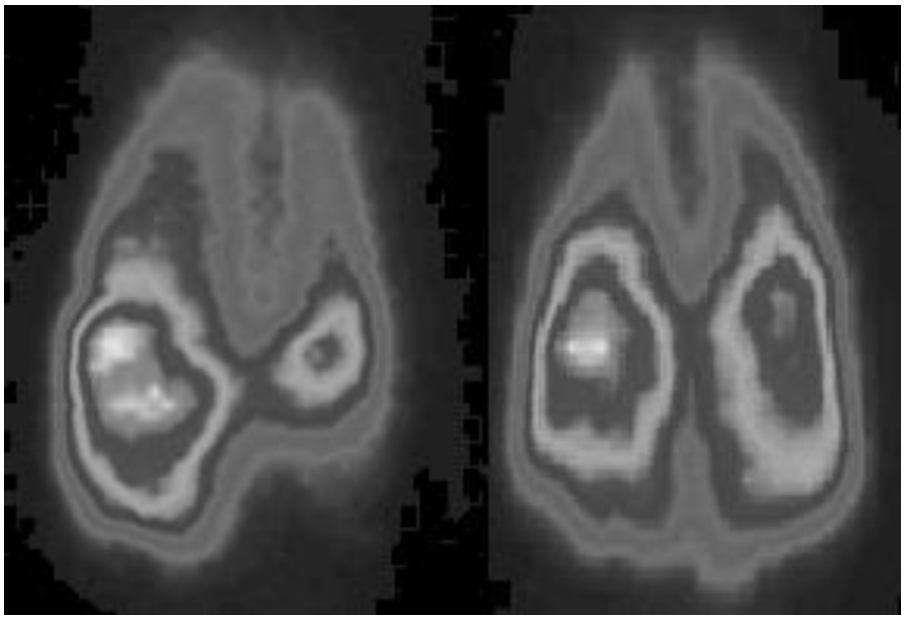

Fig. 2: **Colour** (converted to black-and-white) **dorsal pulmonary perfusion scintigrams of 2 different dogs. The right lung lobe is to the right of each image. The image on the left is of a dog that was not part of this study. Note the typical large basal photopaenic area in the right lung lobe consistent with a class 3 pattern which would be highly suggestive of pulmonary thromboembolism. This dog had confirmed foreign material emboli. The image on the right is of a dog (part of this study) demonstrating a normal class 1 pattern**.

(*versus* classic, coarse fibrillar thrombi as expected for PTE) in numerous small and medium-sized arteries of the lungs. A few thrombi were observed in several histological sections of the myocardium and there were thromboemboli in serosal blood vessels of the intestines.

Arterial blood gas

The results of 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 2 groups for 10 variables. There was no statistically 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

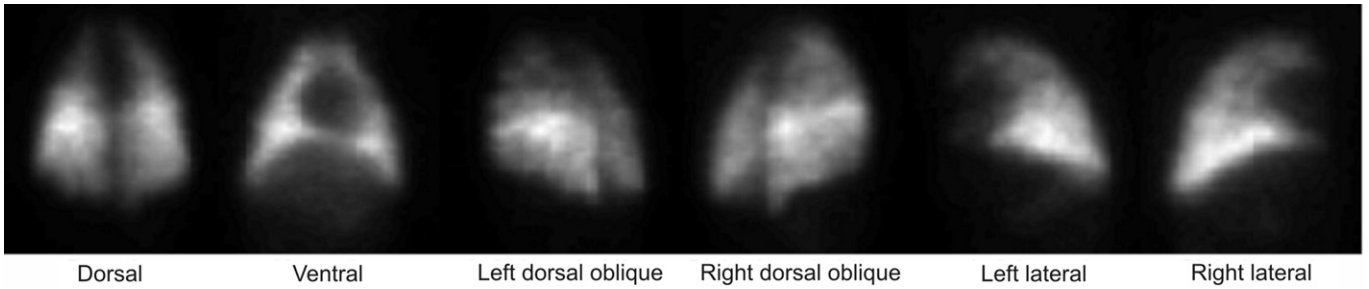

Fig. 3: **Black-and-white pulmonary perfusion scintigrams of dog 20 with an abnormal class 4 pattern. Note the mottled appearance of especially the dorsal oblique images as compared to the images of Fig. 1. For all images, cranial is to the top and caudal to the bottom.**

Table 1: **General results for group 1** (normal dogs) **and group 2** (*Babesia*-infected dogs).

Parameter	Unit	Group 1				Group 2			
		n	Mean	Standard deviation	Range	*n*	Mean	Standard deviation	Range
Age	Months	9	41	2.87	36–43	14	23.36	26.01	6–103
Body weight	Kilograms	9	12.28	1.78	9.9–15	14	15.13	4.92	6.3–25.5
Temperature at admission	°C	9	38.97	0.24	38.6–39.93	14	39.3	1.41	36.4–40.9
Temperature prior to scintigram	°C	9	38.23	0.33	37.5–38.6	14	38.14	0.62	36.7–39.2
Pulse at admission	Beats/minute	9	82	7.94	72–96	14	142.57	29.24	80–200
Pulse prior to scintigram	Beats/minute	9	76	6.71	66–84	14	121.86	21.94	84–154
Respiration at admission	Breaths/minute	9	85.33	29.12	32–100	14	44.14	13.23	20–70
Respiration prior to scintigram	Breaths/minute	9	45.89	31.79	20–100	14	61.93	31.99	28–120

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

Parameter	Unit	Normal values	Group 1				Group 2			
			n	Mean	Standard deviation	Range deviation	*n*	Mean	Standard	Range
Partial pressure of carbon dioxide	mm Hg	30–40	9	34.1	3.27	26.6–37.3	14	30.41	4.75	19.3–38.5
Partial pressure of oxygen	mm Hg	>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	mmo//ℓ	140–155	9	140.67	3.84	131.0–144.0	14	136.79	4.41	127.0–140.0
Potassium	mmo//ℓ	3.6–5.1	9	4.24	0.27	3.95–4.87	14	3.55	0.57	2.69–4.69
Calcium	mmo//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	mmo//ℓ		9	19.16	5.53	4.8–23.8	14	18.07	2.83	13.8–23.8
Standard bicarbonate	mmo//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	mmo//ℓ		9	–6.31	8.26	–28.0–(–0.3)	14	–6.51	2.88	–9.5–0.0
Blood base excess	mmo//ℓ		9	–5.33	8.34	–27.2–0.3	14	–5.87	2.50	–8.4–(–0.2)
Total carbon dioxide content	mmo//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	mmo//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	mm Hg		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

oxygen tension ratio ($P = 0.19$). There was a statistically significant difference for the standard bicarbonate ($P = 0.03$) and estimated oxygen content ($P = 0.0001$).

Thoracic radiographs

Three (33.33 %) group 1 and 7 (50 %) group 2 dogs demonstrated a class 1-type pattern. Three (33.33 %) group 1 and 4 (28.57 %) group 2 dogs demonstrated a class 2-type pattern. Three (33.33 %) group 1 and 3 (21.43 %) group 2 dogs demonstrated a class 3-type pattern. The classification for each dog is given in Table 3. The radiographic classification patterns observed for the 2 groups did not differ significantly ($P = 0.76$, Fischer's exact test).

Pulmonary perfusion scintigrams

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. Five (55.56 %) group 1 dogs were classified as class 1 and 4 (44.44 %) as class 4. Six (42.86 %) group 2 dogs were classified as

Table 3: **Radiographic and scintigraphic classification for the 2 study groups.**

Dog number	Group	Radiographic classification	Adapted 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*-infected	3	2
11	*Babesia*-infected	2	4
12	*Babesia*-infected	1	1
13	*Babesia*-infected	3	4
14	*Babesia*-infected	3	1
15	*Babesia*-infected	1	1
16	*Babesia*-infected	2	4
17	*Babesia*-infected	1	1
18	*Babesia*-infected	2	4
19	*Babesia*-infected	1	4
20	*Babesia*-infected	1	4
21	*Babesia*-infected	1	1
22	*Babesia*-infected	1	1
23	*Babesia*-infected	2	4

class 1 and 7 (50 %) as class 4. Only 1 (7 %) group 2 dog demonstrated a class 2 pattern, due to an enlarged cardiac silhouette. The classification for each dog is given in Table 3. The adapted PISA-PED classification patterns observed for the 2 groups did not differ significantly ($P = 1.00$) and no statistical correlation was found between the radiographic and scintigraphic classification patterns observed for each dog ($P = 1.00$).

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 clinically relevant PTE. Although PTE has never been recorded to be present at *post mortem* examination in CB, thromboemboli were demonstrated in the lungs *post mortem* in dogs with IMHA (for which a similar mechanism of venous stasis, hypercoagulability and endothelial damage as found in CB is proposed)[19]. In the 1 group 2 dog that died, a *post mortem* examination revealed some proteinaceous coagula in numerous small and medium-sized arteries. These were 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, but 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[29], but at the time of the study this diagnostic method was not available for 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[21], but may also be found in complicated CB as a result of the severe anaemia, which causes hypoxaemia and compensatory hyperventilation[34,35]. Finding these clinical signs may thus not always raise 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, but normal values do not exclude it[12,21]. In 1 study, canine patients with experimentally induced PTE revealed no significant differences in blood gas values within 5 minutes or the next half hour after injection[8]. 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 3 dogs[12]. Arterial hypoxaemia and hypocapnoea may also be found[12,21]. The blood pH varies owing to the influences of respiratory alkalosis and metabolic acidosis[12]. The arterial blood gas analysis results demonstrated no statistically significant difference between the 2 groups for partial pressure of carbon dioxide (which indicate that respiratory alkalosis was also not present in group 2) 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 relevant PTE. The standard bicarbonate and estimated oxygen content differed significantly between the 2 groups; the latter could be explained by the presence of anaemia in group 2. Acid–base disturbances were not specifically evaluated in this study. Mixed acid–base disturbances in severe canine babesiosis have been described by Leisewitz *et al.*[22], 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 also to answer the question: 'Does a scintigraphic pattern consistent with clinically relevant PTE occur in dogs hospitalised with naturally occurring infection with *B. canis rossi*?' It was hypothesised that scintigraphic evidence of clinically relevant PTE would indeed occur in these hospitalised patients; this was, however, found not to be the case in this limited study. *Babesia*-infected 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 2 groups if a more quantitative evaluation of the pulmonary perfusion scintigrams was performed, but this was beyond the scope of this study. The presence of small or partially occluding emboli, resulting in a false negative scan, could not be completely excluded. However, if the above were indeed present but undetected, it did not affect the full recovery of group 2 dogs and thus would not be considered clinically relevant.

A generalised mildly mottled appearance was seen especially on the dorsal oblique images of many group 1 and 2 dogs, resulting in many group 1 and 2 dogs being classified as a class 4-pattern according to the adapted PISA-PED classification (Fig. 3). 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[5]. However, since many normal dogs appeared similar, this is not believed to be the 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 3rd, but less likely, possibility is that the appearance is due to uneven distribution because of injection error. The likelihood of this was limited by multiple precautionary measures, *i.e.* all dogs were kept in sternal recumbency during injection and for 5 minutes thereafter, the syringe was gently inverted a few times before injection to ensure good mixing of the particles, the injection was done slowly and evenly and care was taken not to have air in the syringe or to inject air during the process. Owing to agitated patients (especially the normal dogs), it was decided to use a catheter to avoid the possibility of having the dog move and thus possibly cause the radiopharmaceutical to be injected subcutaneously. It is possible that the use of a catheter instead of 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 before injection. Both will result in 'hot clots' rather than photopaenic defects[30].

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 diagnosis[12,21], so that 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[31].

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, group 2 dogs in the same weight range as the group 1 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, which resulted in a small study population. The method of selecting cases did not result in the specific selection of those individuals clinically suspected of PTE, but, 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 1st 5 minutes after the injection of agar, but gradually returned to the initial values[8]. Again, this may mimic dogs with babesiosis. The group 2 dogs in this study, except 1 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 focus on critically ill dogs with respiratory disease and 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.

A further objective of this study was to prospectively evaluate the scintigraphic pulmonary perfusion pattern in hospitalised *Babesia*-infected dogs in an attempt to ascertain whether a scintigraphic pattern consistent with clinically relevant PTE does indeed occur in these patients. Surprisingly, not a single dog in group 2 had wedge-shaped pleural-based perfusion defects that would have resulted in a high probability for clinically relevant PTE. The scintigraphic pulmonary perfusion pattern demonstrated was not significantly different between group 1 and 2 dogs ($P = 1.00$), thus indicating that in this limited study clinically relevant PTE was not a complicating factor in dogs with babesiosis.

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