

Clinical presentation and management of African Horse Sickness in two dogs.

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Abstract:	<p>A ten-month-old, female, spayed Beagle (case 1) and an unrelated two-year-old, female, intact Labrador Retriever (case 2), both living in Pretoria, South Africa, presented individually on separate occasions with acute onset dyspnoea and severe hypoxia. Thoracic radiographs demonstrated severe, diffuse interstitial to alveolar lung patterns with mild pleural and mediastinal effusion. Mixed airway inflammation was seen on trans-tracheal aspirate cytology in case 1.</p> <p>Both cases received supportive therapy but only one dog survived (case 2).</p> <p>African Horse Sickness (AHS) was diagnosed at necropsy based on histopathology and immunohistochemistry in case 1 and ante-mortally in case 2, using reverse-transcriptase polymerase chain reaction (RT-PCR) on whole blood.</p> <p>To the authors' knowledge, this is the first report to detail the haematological-, biochemical-, thoracic radiological-, arterial blood gas- and trans-tracheal aspirate cytology findings of AHS in dogs. This report also describes the treatment of a dog surviving clinical AHS infection.</p>

TITLE OF CASE

Clinical presentation and management of African Horse Sickness in two dogs.

SUMMARY

A ten-month-old, female, spayed Beagle (case 1) and an unrelated two-year-old, female, intact Labrador Retriever (case 2), both living in Pretoria, South Africa, presented individually on separate occasions with acute onset dyspnoea and severe hypoxia. Thoracic radiographs demonstrated severe, diffuse interstitial to alveolar lung patterns with mild pleural and mediastinal effusion. Mixed airway inflammation was seen on trans-tracheal aspirate cytology in case 1.

Both cases received supportive therapy but only one dog survived (case 2).

African Horse Sickness (AHS) was diagnosed at necropsy based on histopathology and immunohistochemistry in case 1 and ante-mortally in case 2, using reverse-transcriptase polymerase chain reaction (RT-PCR) on whole blood.

To the authors' knowledge, this is the first report to detail the haematological-, biochemical-, thoracic radiological-, arterial blood gas- and trans-tracheal aspirate cytology findings of AHS in dogs. This report also describes the treatment of a dog surviving clinical AHS infection.

BACKGROUND

What is AHS

African Horse Sickness (AHS) is a World Organisation for Animal Health (OIE)- listed, non-contagious disease of equids, caused by an arthropod-borne orbivirus of the family *Reoviridae*.(1-3) The African horse sickness virus (AHSV) is endemic in Sub-Saharan Africa with intermittent outbreaks reported in the Near- and Middle East, northern Africa and Europe.(2, 4) In naïve horses, the disease is usually peracute to acute with a greater than 90% mortality rate.

Dogs are the only known non-equid species that contract a highly fatal form of AHS.(2)

African horse sickness virus has been shown to affect dogs after experimental intravenous inoculation with infected horse blood.(5) Serological surveys have reported evidence of widespread natural AHS infection among various African carnivore species with seroconversion in dogs as high as 34-43% reported in endemic areas. (4, 6-9) Infection in these carnivores is proposed to result from ingestion of meat and organs from AHS-infected prey species.(4) Sporadic outbreaks of AHS in domestic dogs have occurred in association with ingestion of virus-contaminated horse meat.(10-12) In 2013, the first case of AHS in a dog without apparent ingestion of horse meat was reported(6) and from 2006-2017, 33 spontaneous cases of canine AHS have been diagnosed post-mortally at the Section of Pathology, Department of Paraclinical Sciences, Faculty of Veterinary Science, University of Pretoria.(13)

The previously reported signs of AHS in dogs have ranged from asymptomatic to transient pyrexia(12), "classical horse sickness symptoms", including hyperpnoea, moist rales on auscultation, white foam around the nostrils, coughing and death.(10, 12) Descriptions beyond basic clinical signs and pathological findings are scant and many reports were published more than 50 years ago. The complete clinical, laboratory and radiological findings of AHS in dogs has not been reported to date.

The authors report the clinical presentation and treatment of two cases of canine AHS, as well as the necropsy findings of one dog and emphasise the consideration of this disease as a differential diagnosis in dogs with acute respiratory signs in areas where equine AHS occurs.

CASE PRESENTATION

Two unrelated dogs presented individually to the Onderstepoort Veterinary Academic Hospital (OVAH), Pretoria, South Africa on separate occasions with acute respiratory distress.

History and Clinical Assessment

Case 1

A ten-month-old spayed female Beagle presented with a one-day history of lethargy, vomiting and acute collapse in May of 2016. The dog was part of the Onderstepoort Teaching Animal Unit (OTAU) breeding colony and was housed on the campus of the University of Pretoria, Faculty of Veterinary Science, in a roofed enclosure with grass pen access, in close proximity to horse paddocks. The faculty is located in an enzootic AHS area. (8)

All horses that reside on the campus are vaccinated annually with a live attenuated African Horse Sickness vaccine 1 (containing AHS serotype 1, 3 and 4) in October and three weeks later with the OBP African Horse Sickness vaccine 2 (containing AHS serotypes 2, 6, 7, and 8) according to manufacturer's instructions (African Horse Sickness Vaccine for horses, mules and donkeys, Onderstepoort Biological Products). Foals are vaccinated at weaning and again six months later.

Nose-to-nose contact between dogs and horses, and sharing of water points is plausible when the dogs are taken on regular walks, but the dogs do not enter the OVAH, Equine Department buildings, where clinically ill or injured horses are stabled. No other dogs in the colony were reported to show clinical abnormalities. Vaccinations of case 1 were fully up to date and a veterinary-formulated, commercial diet was fed solely.

On physical examination, the dog was collapsed with depressed mentation and a body condition score (BCS) of 4/9. Tachypnoea (60 breaths per minute) with severe inspiratory-expiratory dyspnoea, costo-abdominal breathing and bilaterally referred lung sounds and crackles were evident. The remainder of the clinical examination was unremarkable.

Case 2

A client-owned, two-year-old intact female Labrador Retriever presented with a three-day history of partial anorexia and one day of non-productive retching and lethargy in February of 2017. Vaccination history was unclear. The dog was a sole-pet from a low-density suburban, residential area, approximately 15km from the Veterinary Faculty. The dog had no contact with horses and no horses were stabled in the vicinity. The dog had no known access

to horse meat and was fed a commercial diet.

On physical examination, the dog was depressed with BCS 5/9. Marked inspiratory-expiratory dyspnoea with referred lung sounds and crackles were evident. Mild hypothermia (37°C), tachycardia (168 beats per minute) and tachypnoea (100 breaths per minute) was seen. The remainder of the clinical examination was unremarkable.

INVESTIGATIONS

Case 1

Complete blood count revealed haemoconcentration (haematocrit 0.65 L/L, ref: 0.37 – 0.55), lymphopenia ($0.6 \times 10^9/L$, ref: 1 – 4.8), eosinopenia ($0.00 \times 10^9/L$, ref: 0.1 – 1.25) and thrombocytopenia ($118 \times 10^9/L$, ref: 200 – 500). Biochemistry revealed hypoproteinaemia (41.9 g/L, ref: 56 – 73), hypoalbuminaemia (27.2 g/L, ref: 28 – 41), hypoglobulinaemia (14.7 g/L, ref: 20 – 41), increased alkaline phosphatase (496 U/L, ref: 20 – 165), low creatinine (33 $\mu\text{mol/L}$, ref: 59 – 109), hyponatraemia (133 mmol/L, ref: 142 – 151) and hypochloraemia (102 mmol/L, ref: 107 – 117).

Distemper antigen ELISA lateral flow test (Antigen rapid CDV Ag Test Kit, BioNote Inc.) tested negative. This test was chosen for its speed and availability. The dog arrived at the clinic prior to a weekend and serological testing was not available until the following Monday. The rapid death of this dog precluded further ante-mortem testing for distemper.

Unsedated thoracic radiographs (Fig. 1 A & B) revealed a severe perihilar to hilar alveolar lung pattern, extending into the dorso-caudal lungs, with fissure lines and reverse fissure lines indicating mild pleural and mediastinal effusion, respectively. The alveolar pattern had a distinct peribronchial “track-like” appearance.

Arterial blood gas analysis revealed severe hypoxaemia (PaO_2 53.4 mmHg, ref 81 – 103), acute lung injury ($\text{PaO}_2/\text{FiO}_2$ 273) and an increased arterial oxygen gradient (A-a) of 49.2. The A-a gradient did not improve after oxygen administration.

A modified trans-tracheal wash (TTW) was performed under local anaesthesia (lidocaine injection 2%, Bayer Animal Health). Cytological evaluation of the obtained sample revealed a moderately mucoid background with a high number of nucleated cells. The nucleated cells consisted of 30% non-degenerate, mature neutrophils, 5% small lymphocytes and 62.5% large macrophages that were markedly vacuolated and phagocytosing cellular debris. A high number of ciliated and non-ciliated epithelial cells and plaques of basophilic basal epithelial cells (indicating trauma) was visible (Fig. 2 A & B). No microorganisms were seen.

On presentation of this case in 2016, AHS in dogs was not widely known and clinical findings such as radiographic findings had not been described, thus AHS was not considered as a differential diagnosis, especially with case 1 being from the controlled colony of dogs that are not fed horse meat. Prior AHS diagnoses in dogs at the pathology section were all made in dogs referred from other veterinary clinics, or brought in by owners for necropsy. The post-mortem diagnosis was made after TTW fluid and EDTA blood samples were already discarded, hence no RT-PCR was performed in case 1. Retrospective RT-PCR was attempted on wax-embedded histological samples after RNA extraction, but this proved fruitless.

Case 2

Haematology revealed mature neutrophilia ($27.2 \times 10^9/L$, ref: 6-15), monocytosis ($2.72 \times 10^9/L$, ref: 0.15 – 1.35), eosinopenia ($0.00 \times 10^9/L$, ref: 0.1 – 1.25) and basophilia ($0.27 \times 10^9/L$, ref: $0.1 \times 10^9/L$). The biochemistry and urinalysis findings were within normal limits.

Unsedated thoracic radiographs (Fig. 3 A & B) were taken within one hour of presentation and a moderate diffuse interstitial to alveolar pattern affecting all the lungs lobes was present, with an asymmetrical more severely affected right lung appearance. Mild fissure and reverse fissure lines were present. Radiographs were repeated 38 hours later (Fig. 3 C & D) and demonstrated a marked improvement of the lung pattern, and improvement of the fissure and reverse fissure lines.

Peripheral oxygen saturation (SpO_2) (Nonin Model 9847V, Nonin Medical Inc.) on presentation was 85% and remained below 90% despite oxygen supplementation.

Based on an increased awareness of AHS in dogs and a recent diagnosis of AHS in Case 1 with similar presentation, AHSV specific duplex real-time reverse transcriptase quantitative PCR (RT-PCR) was performed ante-mortally in case 2, which tested positive for AHS. The test methodology was performed as described by *Guthrie et al.* (14) This RT-PCR test is OIE accredited (15), and was run at the Veterinary Genetics Laboratory (Equine Research Centre), Faculty of Veterinary Science, University of Pretoria – Onderstepoort.

DIFFERENTIAL DIAGNOSIS

Based on the radiographic and arterial blood gas findings, differential diagnoses included non-cardiogenic pulmonary oedema, most likely as a result of acute respiratory distress syndrome (ARDS). Pneumonia was considered unlikely given the haematology and the radiological findings. Pulmonary haemorrhage with haemothorax and mediastinal haemorrhage was also unlikely as there was no history of trauma or toxin ingestion in either dog, as well as a lack of cytological evidence based on the TTW cytology in dog 1.

TREATMENT AND OUTCOME

Case 1

The dog received 0.3mg/kg promethazine hydrochloride (Phenergan, Sanofi-aventis) IM, 0.125 mg/kg dexamethasone (Kortico injection, Bayer Pty Ltd) IV once, and 20mg/kg ampicillin (Ampicillin-Fresenius, Fresenius Kabi SA Pty Ltd) IV q8h. The dog was maintained in an oxygen tank with oxygen supplementation at 3L/min and a constant rate infusion (22mL/h) of lactated Ringer's (Ringer-Lactate solution, Fresenius Kabi SA Pty Ltd.) was administered.

Case 2

Oxygen was supplemented at 3L/min via intranasal cannula and 0.2mg/kg butorphanol (Dolorex®, Zoetis) IV was given once. The dog then received 0.1 mg/kg dexamethasone (Kortico injection®, Bayer Pty Ltd) IV once, 2mg/kg furosemide (Salix®, MSD Intervet) IV repeated after one hour and continued q8h for three days and thereafter 2mg/kg furosemide (Mylan Furosemide®, Mylan) was given PO q8h for one day and q12h PO for another three days. Additionally Amikacin sulphate at 15mg/kg (Amikacin Fresenius, Fresenius Kabi) IV q24h for five days and 8.75 mg/kg amoxicillin-clavulanic acid (Synulox RTU, Zoetis) SC once followed by 20mg/kg amoxicillin-clavulanic acid (Clavet, Cipla) PO q12h for three days. A constant rate infusion of lactated Ringer's (Ringer-Lactate solution, Fresenius Kabi SA Pty Ltd) was given at 72mL/h.

OUTCOME AND FOLLOW-UP

Case 1

Despite supportive management, the dog's vital signs declined and cardiopulmonary arrest and death ensued 17 hours after initial presentation.

Gross post mortal examination of case 1 revealed generalised congestion, severe pulmonary congestion and oedema associated with diffuse interstitial pneumonia and severe hydrothorax. Histologically, the lungs were characterised by diffuse congestion, protein-rich oedema and small amounts of fibrin filling the alveolar spaces associated with numerous alveolar macrophages. The alveolar walls were distended by mononuclear cells and activated endothelial cells. Small scattered areas of haemorrhage were present throughout the lung parenchyma (Fig. 4 A). African Horse Sickness-specific NS4 immunohistochemical labelling(16) following the standard immunohistochemical protocol(17) revealed numerous positive-labelling intra-alveolar macrophages, confirming an unexpected diagnosis of AHS (Fig. 4 B). The details of this test with both positive and negative controls are described at length by *Zwart et al.* (16)

No serum was stored in Case 1 to perform retrospective serology. Due to the low awareness of the disease, AHS was not considered as a differential for the symptoms at the time the dog presented.

Case 2

Gradual improvement and resolution of hypoxia was seen and the dog was discharged after 72 hours of hospitalisation. At the time of manuscript submission, the dog was reported to

be doing well and no respiratory abnormalities were observed by the owners. A definitive diagnosis of AHS by a positive, AHSV specific duplex real-time reverse transcriptase quantitative PCR was confirmed only after discharging the animal from the hospital.

Case 2 was brought in for a re-evaluation eight months post-recovery, was clinically healthy, and tested positive for AHS titres using VP7-based AHSV iELISA run at the Onderstepoort Veterinary Institute according to the protocol described in *Maree et al.*(18) This test is not validated for canines, but there are no currently available serological tests that are.

DISCUSSION

This case report is, as far as the authors are aware, the first report of the complete haematological, biochemical, thoracic radiographic, arterial blood gas and trans-tracheal wash cytology findings in dogs with AHS.

The clinical signs seen in these two cases are in agreement with previously reported signs of AHS in dogs, including progressive inappetence, depressed habitus, pyrexia, hyperpnoea, tachypnoea, coughing, moist rales on auscultation and white foam around the nostrils.(6, 10, 12, 13) Additional signs of diarrhoea and seizures have been reported by Haig, but some dogs in that report were concurrently affected by Distemper viral infection.(10) More recently, excessive salivation in addition to the abovementioned signs has been described.(13) Not all dogs that contract AHS develop severe clinical signs and some dogs only display mild pyrexia.(12)

The clinical signs were comparable to what is seen in horses affected by the pulmonary form of AHS, with pyrexia, severe respiratory distress and frothy white to blood-tinged fluid at the nostrils.(19) Horses also develop a more subacute or cardiac form of the disease that is associated with pyrexia, subcutaneous oedema, conjunctival congestion, petechiation and signs of colic.(19) Whether these clinical forms are also seen in dogs require verification. The abovementioned signs occur as a result of increased vascular permeability caused by direct and indirect injury to endothelial cells, particularly in organs such as the lungs.(1) It can only be speculated that a similar underlying pathophysiological mechanism occurs in dogs.

The haematological and biochemical findings of the two affected dogs were non-specific. The haemoconcentration in case 1 can be ascribed to dehydration. The lymphopenia, eosinopenia in case 1, and the stress leukogram in case 2, are non-specific findings and are expected in ill dogs. The panhypoproteinaemia and low creatinine in case 1 can be attributed to the dog's young age and thin body condition. Prolonged prothrombin time, activated partial thromboplastin time and increased fibrin degradation products have been described in horses with AHS(20), but coagulation testing was not performed in the reported cases.

Although an alveolar pattern is not a specific finding on radiographs, the severity and diffuse distribution may aid the diagnosis, and follow-up radiographs would be useful to monitor progression of the disease. Mild pleural and mediastinal effusion are compatible with vasculitis, but in this case, no other body cavity effusions could be confirmed on radiographs, which may be expected given the mechanism of disease.

The alveolar arterial oxygen gradient (A-a) of 49.2 was indicative of a severe pulmonary ventilation perfusion mismatch or diffusion impairment. Failure of A-a gradient improvement after oxygen administration was indicative of either alveolar collapse, flooding of alveoli with fluid (pulmonary oedema/acute respiratory distress syndrome ARDS) or alveolar consolidation.(21)

Airway cytological findings have not been reported previously in dogs with AHS. The TTW cytology results in case 1 were consistent with severe inflammation but the presence of macrophages was suggestive of a more sub-acute to chronic condition, although the clinical signs were acute. A mixed inflammatory response is frequently seen in non-infectious pulmonary disease such as inhalation pneumonia, lung lobe torsion or necrosis secondary to a neoplastic lesion.(22) A bronco-alveolar lavage might have enabled collection of a more representative sample, but the dog was not deemed stable enough to perform the procedure.

An ante-mortem diagnosis of AHS can be confirmed by semi-quantitative RT-PCR on EDTA

whole blood.(14) At necropsy, fresh lung tissue can be submitted for RT-PCR and in 10% formalin for routine histopathology and IMP testing.(13, 16, 23) It was attempted to perform IMP retrospectively on the cytological preparations of the TTW sample of case 1 but no positive labelling was observed.

In previous reports, mortality in dogs has ranged from 20-95%.(8, 12, 13, 24) Not all dogs that were fed infective meat developed clinical signs but once symptoms of respiratory embarrassment had set in, the prognosis was poor and mortality high.(12) Case 2 in the current report survived despite severe respiratory disease and hypoxia.

No specific therapy for equine AHS infection is available and treatment is supportive and symptomatic.(19) Based on the tropism and multiplication of the virus in myocardial tissue, regardless of the clinical form of AHS(25), current treatment recommendations by the OVAH equine clinic include the addition of furosemide, angiotensin converting enzyme inhibitor and pimobendan in AHS-affected horses.(26) There are no recommendations regarding treatment in dogs with AHS. The addition of diuretic therapy could have influenced the recovery in case 2 but no conclusions can be made in this regard.

The route of transmission of AHS to dogs that have not ingested horse meat, such as the two dogs involved here, is unknown. Previous studies found that the major vector of AHS, *Culicoides imicola*, is probably not attracted, or only very slightly attracted to dogs, thus dogs are unlikely to act as vectors for AHS virus from infected dogs to horses.(27) Mosquitoes may play a role in transmission of AHS amongst equine populations and do feed on dogs in the absence of other preferable hosts.(27) The apparent increase in the incidence of AHS in dogs, in an endemic AHS area warrants further investigation into the prevalence of the disease in the canine population, the route of transmission to dogs and the current role of canines in the maintenance cycle of AHS.

The apparent increase in AHS occurrence in dogs in South Africa raises the question whether this represents an emerging canine disease. Additionally, this may have implications on international transport and trade of canines in the future.

Control methods in horses in endemic AHS areas are limited to vaccination(28) with the live attenuated AHS vaccination (African Horse Sickness Vaccine for horses, mules and donkeys, Onderstepoort Biological Products) and midge avoidance strategies including stabling during high midge activity, vector proofing stables and insect repellents.(29) On a national and international level, the movement of horses from AHS area is strictly controlled with testing of horses prior to movement.(3)

Due to the lack of knowledge of the current method of transmission, clear control or prevention strategies are premature. Currently, further research is underway to determine the sero-prevalence of AHS in dogs in the Pretoria area as well as potential epidemiological factors that would help shape further studies into prevention strategies. Avoiding the feeding of horse meat to dogs, keeping dogs indoors during times of high midge activity and avoiding direct contact with sick horses (and recently vaccinated horse) or their water bowls would be the presumable preventative measures that owners could adopt.

African horse sickness should be considered as a differential diagnosis in dogs with acute respiratory signs, hypoxia and thoracic radiographic evidence of alveolar lung pattern and pleural/mediastinal effusion in areas where equine AHS occurs.

LEARNING POINTS/TAKE HOME MESSAGES

- Dogs with AHS virus infection may present with acute respiratory distress, tachypnoea, referred lung sound and crackles.
- Hypoxia and severe pulmonary ventilation perfusion mismatch was seen on arterial blood gas analysis.
- Thoracic radiographic findings include a marked diffuse hilar to perihilar alveolar lung pattern with mild pleural and mediastinal effusion.
- The prognosis of dogs that present in acute respiratory distress is poor but treatment with diuretic therapy, antibiotics and oxygen supplementation might be beneficial.
- Increased incidence of AHS in dogs can be anticipated due to the increased

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FIGURE/VIDEO CAPTIONS

FIG 1: Right-lateral recumbent (A) and dorsoventral (B) thoracic radiographs of Dog 1. Note the severe perihilar to hilar alveolar lung pattern with extension into the dorso-caudal lungs. The alveolar pattern has a distinct peribronchial “track-like” appearance There are mild fissure lines and reverse fissure lines present.

FIG 2: Transtracheal aspirate sediment cytology of Dog 1. The sample demonstrated a mucoid background and high cellularity with non-degenerate, hypersegmented neutrophils and markedly vacuolated macrophages (A). A high number of ciliated and non-ciliated respiratory epithelial cells were seen (B).

FIG 3: Right-lateral recumbent (A and C) and dorsoventral (B and D) thoracic radiographs of Dog 2. Radiographs A and B were taken at presentation with the follow up images (C and D) acquired 38 hours later. A moderate mainly perihilar interstitial to alveolar pattern was present. The right lungs were more severely affected. Mild fissure and reverse fissure lines were present. Note the marked improvement of the lung pattern on the follow up images. There is mild focal persistence of the alveolar pattern in the ventral left cranial lung, and subtle improvement of the fissure and reverse fissure lines.

FIG 4: Photomicrograph of the lungs of Dog 1 demonstrating diffuse congestions and protein-rich oedema. Many of the alveolar spaces were filled with fibrin and numerous alveolar macrophages. The alveolar walls were thickened by mononuclear cells and activated endothelial cells (A). AHS NS4 immunoperoxidase labelling of the lung of Dog 1 with numerous intra-alveolar macrophages and endothelial cells displaying positive solid nuclear labelling (brown). For detailed interpretation please see *Zwart et al. (16)* (B).

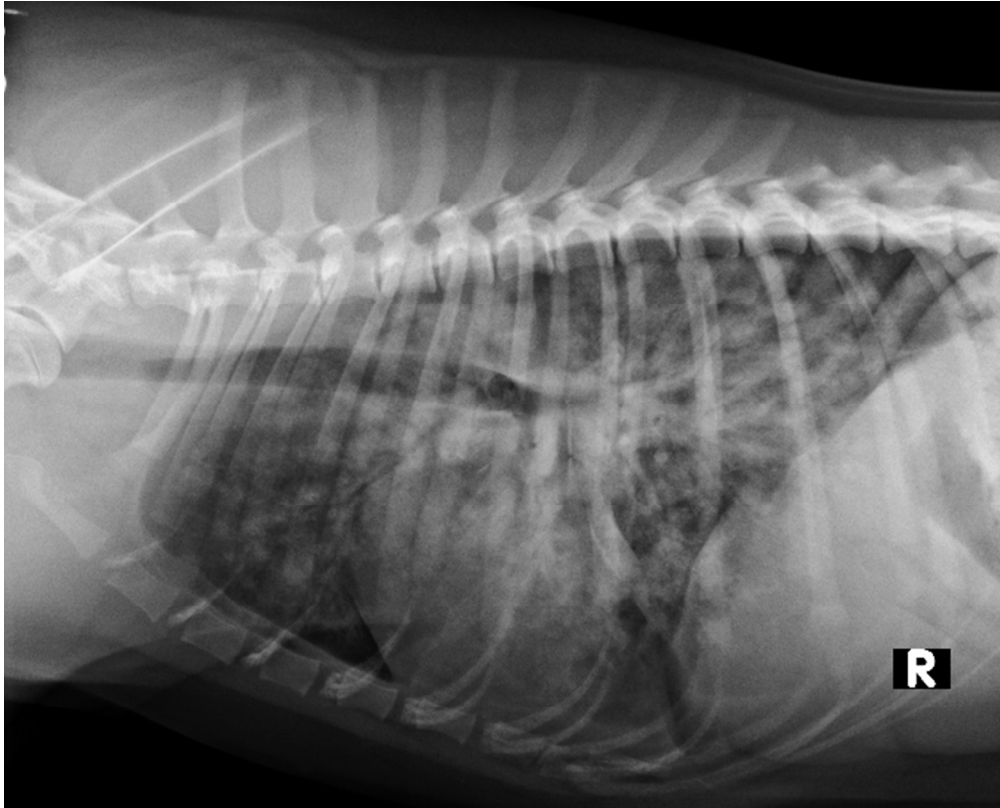


FIG 1: Right-lateral recumbent (a) and dorsoventral (b) thoracic radiographs of Dog 1. Note the severe perihilar to hilar alveolar lung pattern with extension into the dorso-caudal lungs. The alveolar pattern has a distinct peribronchial "track-like" appearance. There are mild fissure lines and reverse fissure lines present.

78x63mm (220 x 220 DPI)



FIG 1: Right-lateral recumbent (a) and dorsoventral (b) thoracic radiographs of Dog 1. Note the severe perihilar to hilar alveolar lung pattern with extension into the dorso-caudal lungs. The alveolar pattern has a distinct peribronchial "track-like" appearance. There are mild fissure lines and reverse fissure lines present.

53x84mm (220 x 220 DPI)

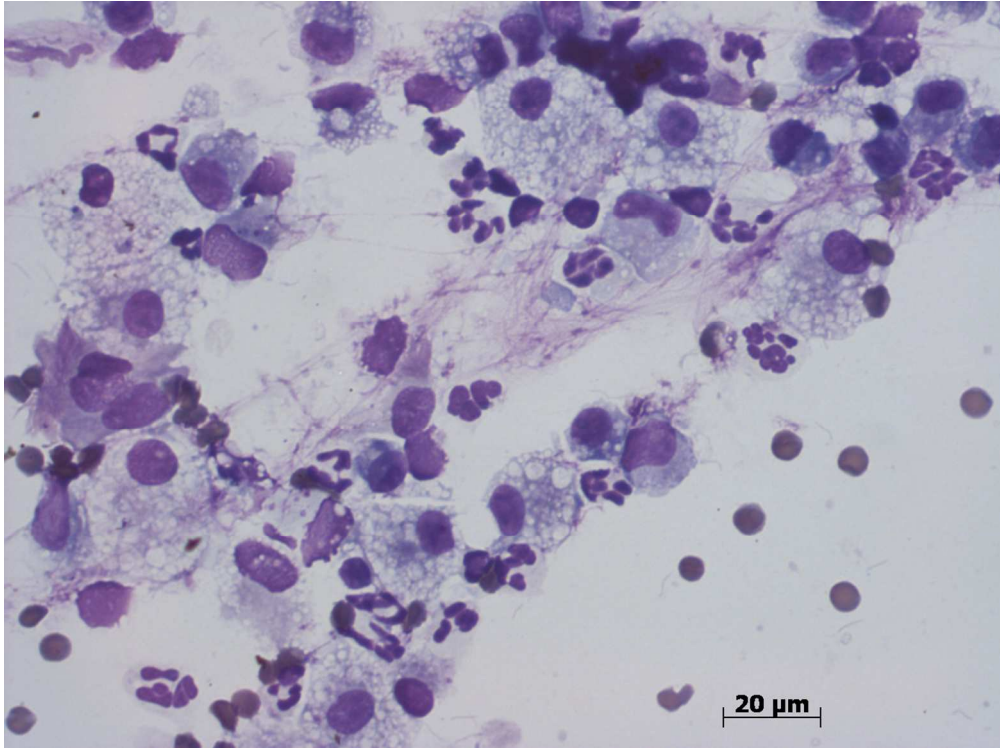


FIG 2: Transtracheal aspirate sediment cytology of Dog 1. The sample demonstrated a mucoid background and high cellularity with non-degenerate, hypersegmented neutrophils and markedly vacuolated macrophages (a). A high number of ciliated and non-ciliated respiratory epithelial cells were seen (b).

368x274mm (96 x 96 DPI)

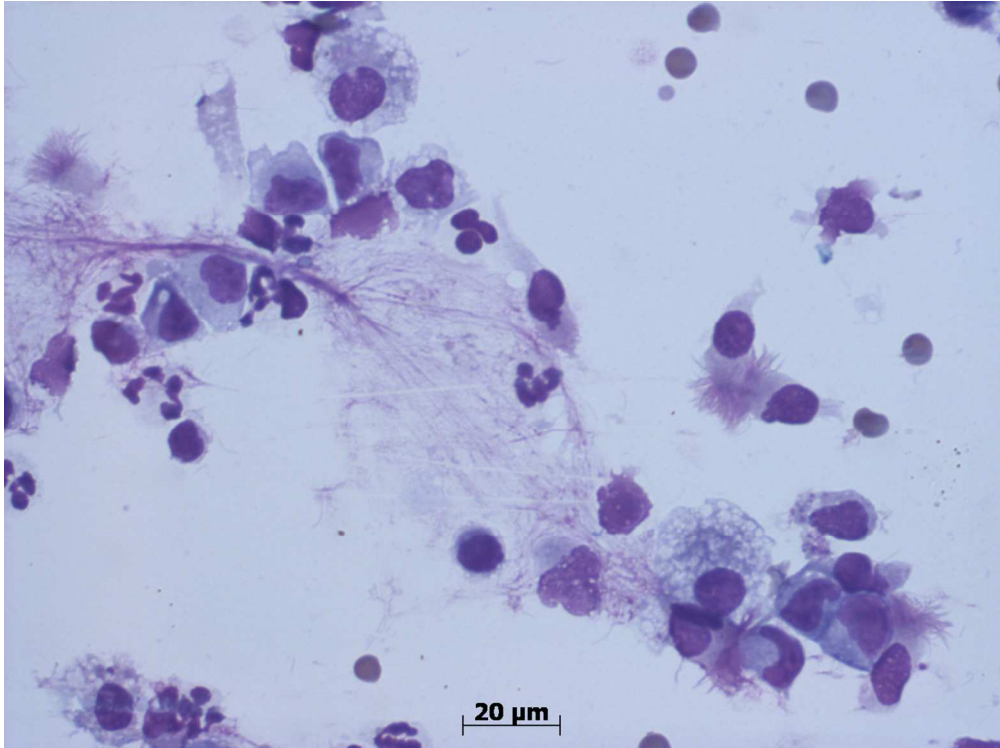


FIG 2: Transtracheal aspirate sediment cytology of Dog 1. The sample demonstrated a mucoid background and high cellularity with non-degenerate, hypersegmented neutrophils and markedly vacuolated macrophages (a). A high number of ciliated and non-ciliated respiratory epithelial cells were seen (b).

368x274mm (96 x 96 DPI)



FIG 3: Right-lateral recumbent (A and C) and dorsoventral (B and D) thoracic radiographs of Dog 2. Radiographs A and B were taken at presentation with the follow up images (C and D) acquired 38 hours later. A moderate mainly perihilar interstitial to alveolar pattern was present. The right lungs were more severely affected. Mild fissure and reverse fissure lines were present. Note the marked improvement of the lung pattern on the follow up images. There is mild focal persistence of the alveolar pattern in the ventral left cranial lung, and subtle improvement of the fissure and reverse fissure lines.

64x61mm (220 x 220 DPI)

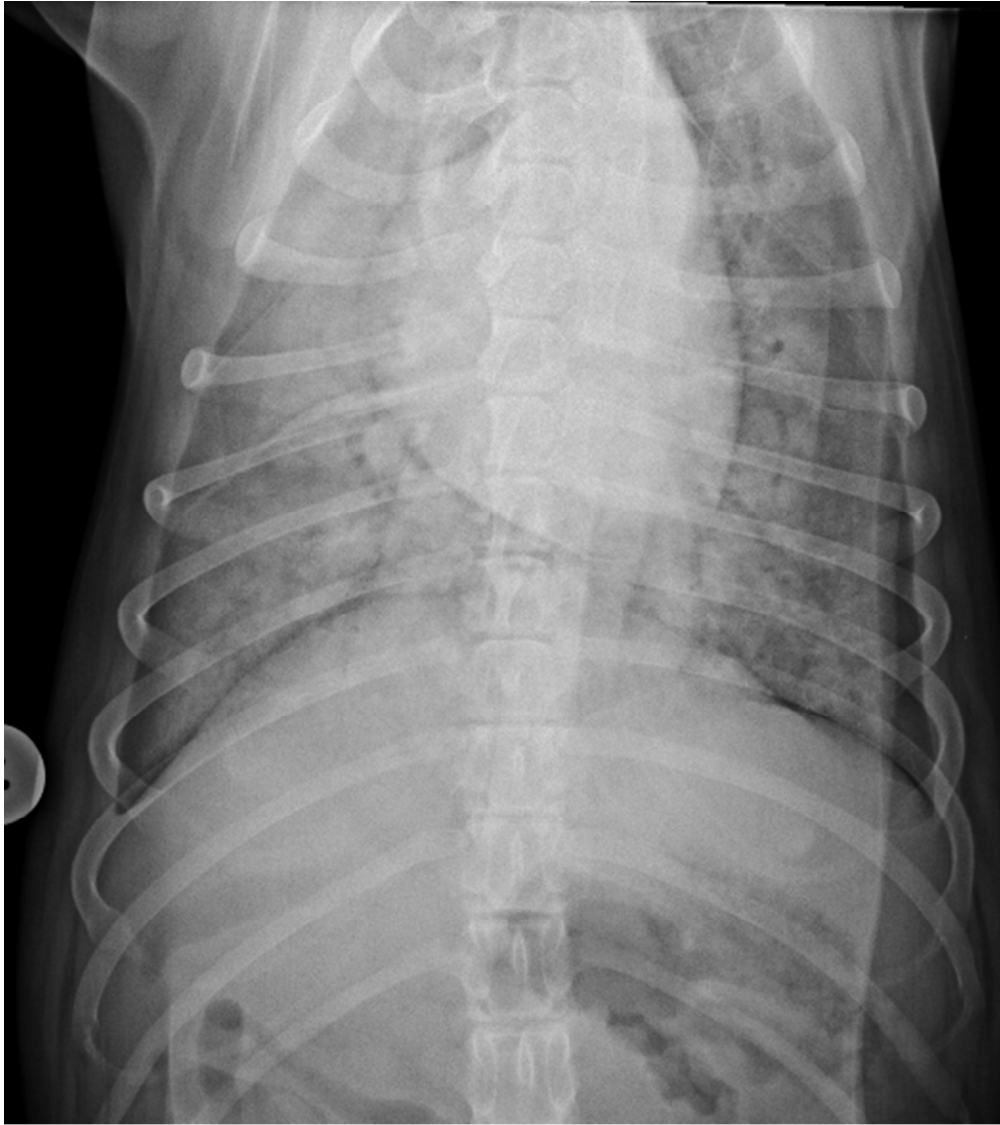


FIG 3: Right-lateral recumbent (A and C) and dorsoventral (B and D) thoracic radiographs of Dog 2. Radiographs A and B were taken at presentation with the follow up images (C and D) acquired 38 hours later. A moderate mainly perihilar interstitial to alveolar pattern was present. The right lungs were more severely affected. Mild fissure and reverse fissure lines were present. Note the marked improvement of the lung pattern on the follow up images. There is mild focal persistence of the alveolar pattern in the ventral left cranial lung, and subtle improvement of the fissure and reverse fissure lines.

60x67mm (220 x 220 DPI)

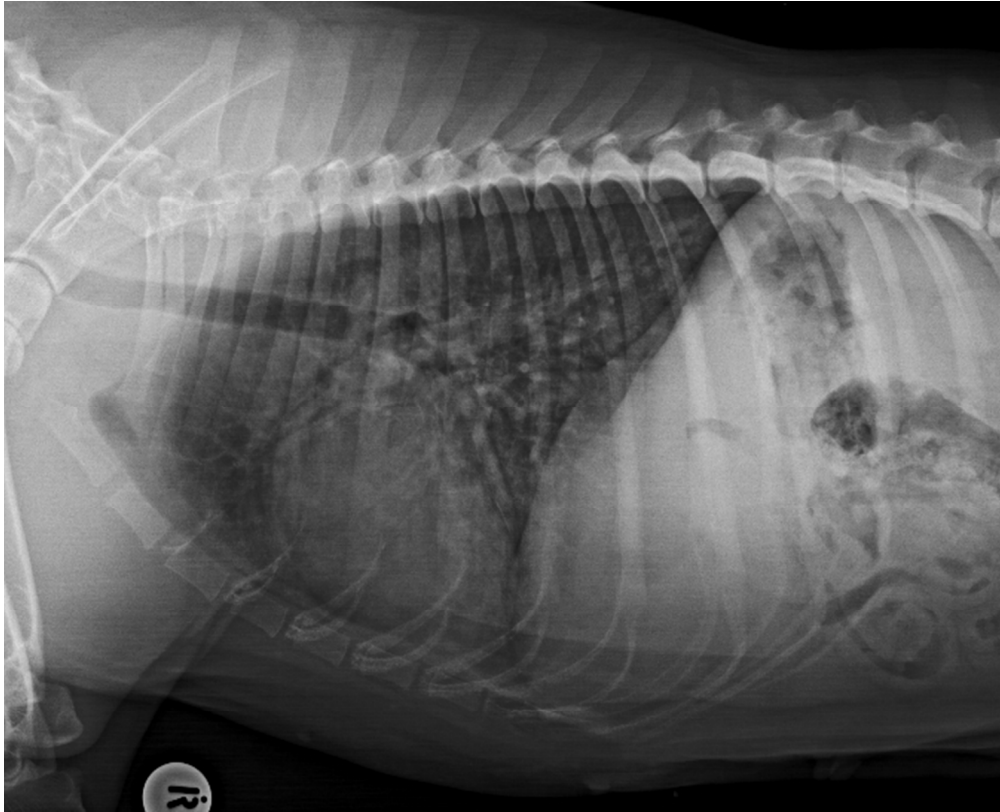


FIG 3: Right-lateral recumbent (A and C) and dorsoventral (B and D) thoracic radiographs of Dog 2. Radiographs A and B were taken at presentation with the follow up images (C and D) acquired 38 hours later. A moderate mainly perihilar interstitial to alveolar pattern was present. The right lungs were more severely affected. Mild fissure and reverse fissure lines were present. Note the marked improvement of the lung pattern on the follow up images. There is mild focal persistence of the alveolar pattern in the ventral left cranial lung, and subtle improvement of the fissure and reverse fissure lines.

77x63mm (220 x 220 DPI)



FIG 3: Right-lateral recumbent (A and C) and dorsoventral (B and D) thoracic radiographs of Dog 2. Radiographs A and B were taken at presentation with the follow up images (C and D) acquired 38 hours later. A moderate mainly perihilar interstitial to alveolar pattern was present. The right lungs were more severely affected. Mild fissure and reverse fissure lines were present. Note the marked improvement of the lung pattern on the follow up images. There is mild focal persistence of the alveolar pattern in the ventral left cranial lung, and subtle improvement of the fissure and reverse fissure lines.

62x82mm (220 x 220 DPI)

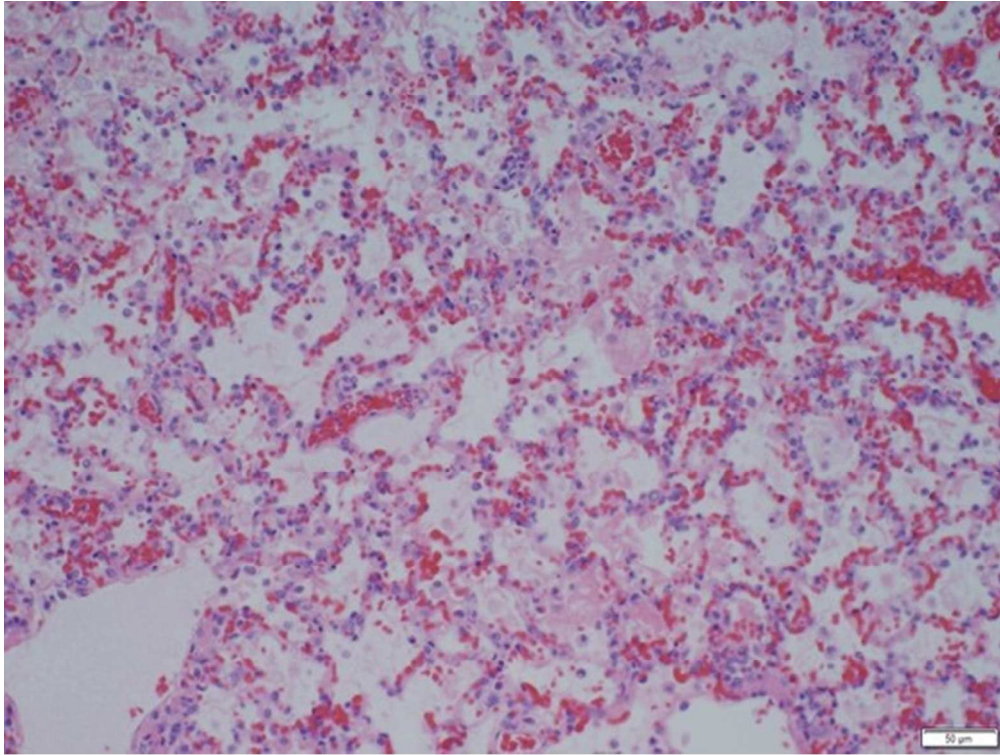


FIG 4: Photomicrograph of the lungs of Dog 1 demonstrating diffuse congestions and protein-rich oedema. Many of the alveolar spaces were filled with fibrin and numerous alveolar macrophages. The alveolar walls were thickened by mononuclear cells and activated endothelial cells (A). AHS NS4 immunoperoxidase labelling of the lung of Dog 1 with numerous intra-alveolar macrophages and endothelial cells displaying positive solid nuclear labelling (brown). For detailed interpretation please see Zwart et al. (16) (B).

64x48mm (220 x 220 DPI)

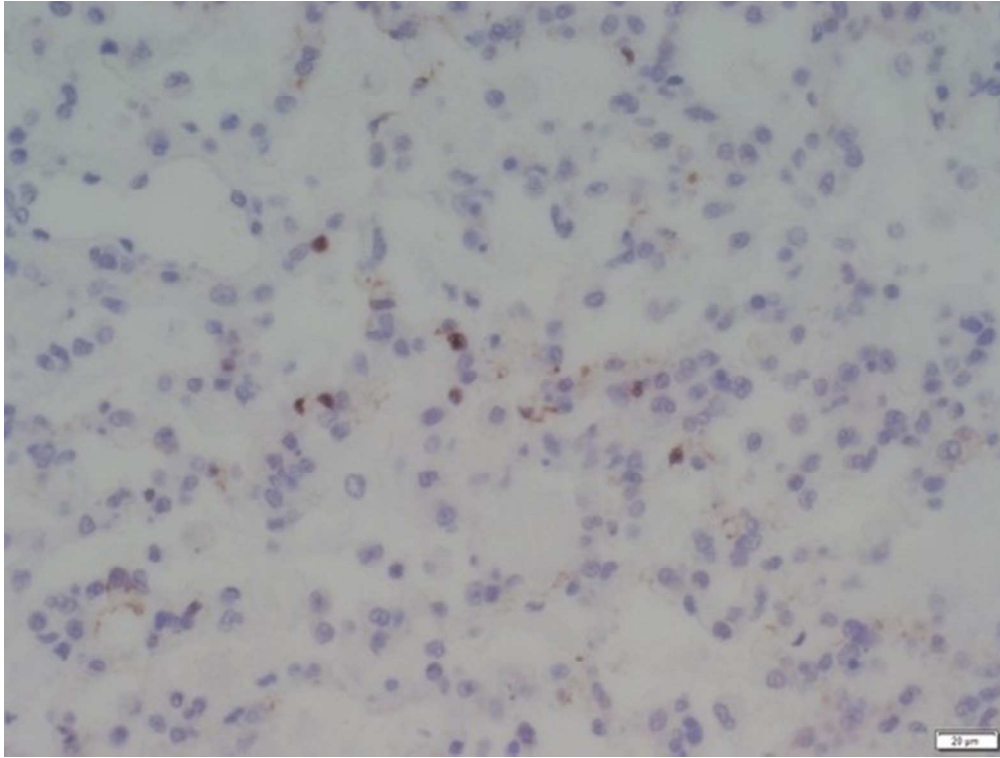


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64x48mm (220 x 220 DPI)