# Clinical presentation and pathology of suspected vector transmitted African horse sickness in South African domestic dogs from 2006 to 2017

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**Abstract**

African horse sickness (AHS) is a fatal vector transmitted viral disease of horses caused by the African horse sickness virus (AHSV). This disease is characterised by circulatory and respiratory failure, resulting from vascular endothelial injury affecting many organs. The susceptibility of dogs to AHS has been demonstrated in the past following experimental infection through consumption of infected horse meat. Thirty three clinical cases of AHS in dogs (cAHS) have been documented, without a history of ingesting infected horse meat, over a period of 12 years. The clinical cases included in this study presented with a history of acute respiratory distress syndrome or sudden death. The macroscopic and histological changes were mostly characterised by acute interstitial pneumonia, serofibrinous pleuritis and mediastinal oedema. Confirmation of cAHS was obtained by AHS specific NS4 antibody immunohistochemistry and/or AHSV specific duplex real time RT-quantitative PCR. Here, we document the clinical and postmortem diagnostic features of confirmed cAHS cases with no history of ingestion of AHS infected horse meat.

## Introduction

African horse sickness (AHS) is a serious and mostly fatal disease of horses, caused by a double stranded RNA virus transmitted to horses by *Culicoides imicola.* However, other biting insects, such as mosquitoes, may play a minor role.[1] AHS is a non-contagious, arthropod borne disease affecting horses, characterised by failure of the circulatory and respiratory systems, resulting in effusions and haemorrhages in many organs.[2] AHS cases are seen in areas where the haematophagous arthropod vectors naturally occur, especially during the months of increased vector activity (ie, late summer).[3]

The disease is present in most of Sub-Saharan Africa, from Senegal in the west to Somalia in the east, and extends as far south as South Africa.[4, 5] It has been sporadically diagnosed in horses in countries outside Sub-Saharan Africa, including Egypt, Saudi-Arabia, Iran, Afghanistan and Pakistan.[3] In 1966, AHS was first diagnosed in Europe when AHSV-9 was diagnosed in horses in southern Spain; AHSV-9 was the same serotype circulating in North Africa at the time.[6] In 1987, AHS spread to Europe again with horse mortalities reported in Spain. The source of this infection was reported to be from zebra imported to a zoo in Spain from Namibia.[7] It then spread to Portugal, resulting in approximately 2000 horse deaths from AHS.[3]

Four distinct clinical forms of AHS have been described—namely, African sickness fever, subacute or cardiac form, cardiopulmonary form (mixed form) and the peracute or pulmonary form.[3–5, 8] To date, the AHS virus (AHSV) has been classified into nine distinct serotypes,[9–11] and animals who recover from infection of one serotype do not always have adequate cross protection if subsequently infected by a heterologous serotype.[3]

The susceptibility of dogs to AHS was reported over a century ago by Theiler,[12] and since then has been reported from other countries, including Egypt,[13] India,[14] Nigeria[15] and South Africa.[16] In all of these cases the affected dogs were either naturally or experimentally fed on tissues from horses that died from AHS.[17–19] Previous studies by Braverman *et al* [20] indicated that the likelihood of dogs contracting the disease via vector borne transmission is extremely low, due to the fact that dogs are not a preferred host of the vector *Culicoides imicola.*

In this paper, the vector theory is revisited as a possible mode of transmission since at least 33 cases of AHS in domestic dogs (cAHS) were diagnosed from 2006 to 2017 at the Section of Pathology, Department of Paraclinical Sciences, Faculty of Veterinary Science, University of Pretoria. None of these cases had a history of infected horse meat consumption and most of these dogs originated from the area north of Pretoria, South Africa. Another confirmed case was reported by Van Sittert *et al* in 2013 from a research dog colony in the Malelane area.[21]

We document the clinical and postmortem diagnostic features of confirmed cAHS cases at the Section of Pathology, Department of Paraclinical Sciences, Faculty of Veterinary Science, University of Pretoria for the period 2006 to 2017.

**Materials and methods**

Pathology records of dogs diagnosed with cAHS at the Section of Pathology, Department of Paraclinical Sciences, Faculty of Veterinary Science, University of Pretoria, between 2006 and 2017, were reviewed retrospectively.

A diagnosis of cAHS was based on a number of modalities, including gross pathology, histopathology, immunohistochemistry (IHC) and RT-PCR. Epidemiological information regarding the signalment, clinical presentation and diet was collected. The possibility of consumption of AHS infected horse meat was investigated. Complete sets of tissues for diagnostic purposes were not available for all cases. The minimum diagnostic set of samples that defined a case included fresh tissue (lung, spleen, liver or EDTA blood) for RT-PCR and/or formalin fixed lung tissue for histopathology and IHC testing. Fresh tissues and blood were tested with an AHSV specific duplex real time RT-quantitative PCR assay by the Equine Research Centre at the Onderstepoort Genetics Laboratory.[22] The cycle threshold (CT) values and virus typing were determined where possible. Full necropsies were performed on all dogs presented with a suspicion of cAHS. Gross descriptions of lesions were recorded for these cases and fresh organs (liver, spleen and lung) were collected for RT-PCR. A full set of tissues for histopathology and IHC was also collected for microscopical descriptions for these cases. Formalin fixed tissues were prepared for light microscopic examination following standard histopathology processing techniques.[23] Formalin fixed

lung, spleen, heart and liver were labelled with AHSV specific NS4 antibody[24] following the standard immunohistochemical protocols[25] used at the Histopathology Laboratory, Section of Pathology, Department of Paraclinical Sciences, Faculty of Veterinary Science, University of Pretoria. Bluetongue virus infection, a closely related orbivirus, was excluded in all cases by Bluetongue specific immunohistochemistry. For the one suspect case that survived, only EDTA blood was received for RT-PCR testing.

**Results**

Thirty-three dogs were included in this study based on a positive diagnosis of cAHS on either IHC or RT-PCR, or both.

The signalment for these dogs as well as the submitted samples and diagnostic tests performed are listed in Table 1. Twenty-two dogs were male, 9 were female and 2 were unspecified. Twenty-nine dogs were large breed (5 American Pitt-bull Terriers, 6 Boerboel, 5 Rottweiler, 2 Bullmastiff, 2 German Shepard, 1 Labrador, 1 Boxer, 1 Doberman, 1 Great Dane, 1 Ridgeback, 1 Weimaraner, 1 Bull Terrier and 1 Mixed large-breed), 3 were small/medium breed (1 Cocker Spaniel, 1 Beagle and 1 Jack Russel Terrier) and one was unspecified. Of these, 20 were short coated breeds, 10 were long coated breeds and 2 were unspecified.

Most of the dogs in this study presented with a history of anorexia and lethargy of less than 24 hours' duration. On clinical examination, they exhibited signs of severe dyspnoea, excessive salivation, tachypnoea and crackles on lung auscultation. Terminally, copious serous oronasal discharge was occasionally seen. Signs of dyspnoea deteriorated rapidly, terminating in respiratory failure and death. On investigation for evidence of direct exposure to AHSV, either through ingestion of infected horse meat or contact with suspected clinical AHS horse cases, none of the owners of the animals included in this study indicated that these were possible sources.

At necropsy, the most significant macroscopical lesions found affected the lungs and thoracic cavity. The following lesions were consistent throughout all cases, with very little variation between cases. These were characterised by acute interstitial pneumonia, serofibrinous pleuritis and mediastinal oedema (Fig 1).

**TABLE 1: Signalment, samples received and diagnostic tests performed for 33 dogs with African horse sickness**

| Signalment | Samples received | Tests performed |
|---|---|---|
| 5 year male German Shepard | Carcass | PM, histopathology, immunohistochemistry, RT-PCR |
| 3 year female Boxer | Formalin fixed tissue | Histopathology, immunohistochemistry |
| 2 year female Boerboel | Formalin fixed tissue | Histopathology, immunohistochemistry |
| 5 year male Jack Russel Terrier | Formalin fixed tissue | Histopathology, immunohistochemistry |
| 12 year female mixed breed | Formalin fixed tissue | Histopathology, immunohistochemistry |
| 2 year male, breed unspecified | Formalin fixed tissue | Histopathology, immunohistochemistry |
| 4 year male Rottweiler | Formalin fixed tissue | Histopathology, immunohistochemistry |
| 2 year male German Shepard | Formalin fixed tissue | Histopathology, immunohistochemistry |
| 4 year male American Pit-bull Terrier | Carcass | PM, histopathology, immunohistochemistry, RT-PCR (serotype 6) |
| 1.5 year male Rottweiler | Formalin fixed tissue | Histopathology, immunohistochemistry |
| 5 year male Doberman Pincher | Carcass | PM, histopathology, immunohistochemistry, RT-PCR |
| 7 year female Cocker Spaniel | Formalin fixed tissue and fresh lung on ice | Histopathology, immunohistochemistry, RT-PCR (serotype 6) |
| Great Dane, age and sex unspecified | Formalin fixed tissue | Histopathology, immunohistochemistry |
| 2 year male Boerboel | Formalin fixed tissue | Histopathology, immunohistochemistry |
| 3 year male American Pit-bull Terrier | Carcass | PM, histopathology, immunohistochemistry |
| 5 year male Rottweiler | Formalin fixed tissue | Histopathology, immunohistochemistry |
| 5 year female Boerboel | Formalin fixed tissue | Histopathology, immunohistochemistry |
| 3 year male Bull Terrier | Formalin fixed tissue | Histopathology, immunohistochemistry |
| 11 year female Rottweiler | Formalin fixed tissue and fresh lung on ice | Histopathology, immunohistochemistry, RT-PCR |
| 11 year female Beagle | Carcass | PM, histopathology, immunohistochemistry |
| 8 year male Ridgeback | Formalin fixed tissue | Histopathology, immunohistochemistry |
| 4 year male American Pit-bull Terrier | Carcass | PM, histopathology, immunohistochemistry, RT-PCR |
| 4 year male Bullmastiff | Carcass | PM, histopathology, immunohistochemistry |
| 6 year female Labrador-cross | Carcass | PM, histopathology, immunohistochemistry, RT-PCR |
| 4 year male Bullmastiff | Carcass | PM, histopathology, immunohistochemistry, RT-PCR |
| 4 year female Boerboel | Carcass | PM, histopathology, immunohistochemistry, RT-PCR |
| 5 year male American Pit-bull Terrier | Carcass | PM, histopathology, immunohistochemistry, RT-PCR |
| 2 year male Boerboel | Carcass | PM, histopathology, immunohistochemistry, RT-PCR |
| 3 year female Boerboel | Carcass | PM, histopathology, immunohistochemistry, RT-PCR |
| 2 year male Rottweiler | Formalin fixed tissue | Histopathology, immunohistochemistry |
| 3 year male Weimaraner | Carcass | PM, histopathology, immunohistochemistry, RT-PCR |
| 2 year male Boerboel | Carcass | PM, histopathology, immunohistochemistry, RT-PCR |
| 4 year male American Pit-bull Terrier | EDTA blood | RT-PCR (serotype 6) |

PM, postmortem examination.

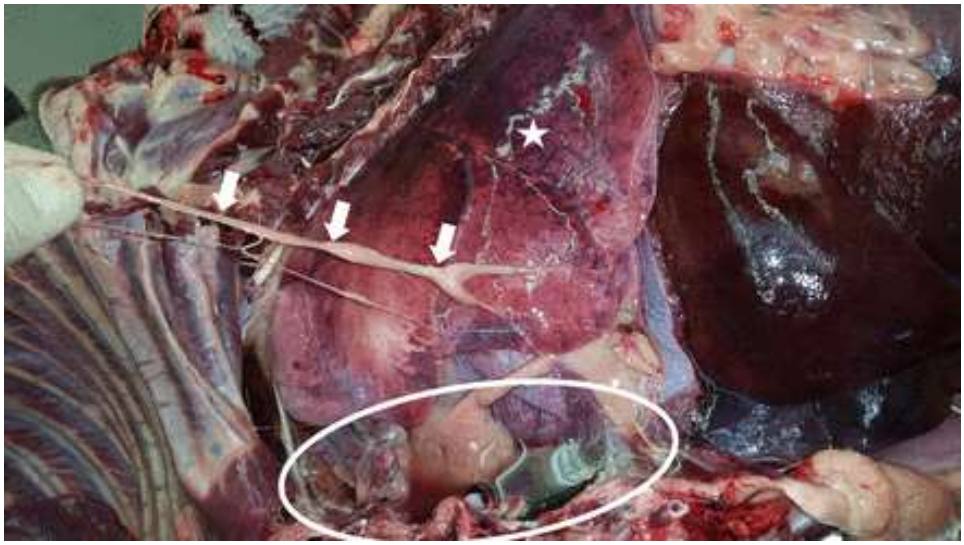

**FIG 1:** Macroscopic appearance of the thoracic viscera. Severe fibrin (arrows) and serous fluid (circle) exudation in the pleural cavity associated with interstitial pneumonia (star).

On histopathology, the lungs of all of the affected animals displayed the most significant lesions. These were characterised by severe protein rich alveolar and septal oedema, severe interstitial pneumonia and scattered areas of haemorrhage. The interstitial pneumonia was characterised by monocytic and lymphocytic leukostasis and exudation, with alveolar macrophage activation, hyperactivation of the alveolar capillary endothelial cells and severe diffuse hyperaemia/congestion (Fig 2). In comparison with the pulmonary changes, histological findings in other tissues were non-specific and mostly insignificant.

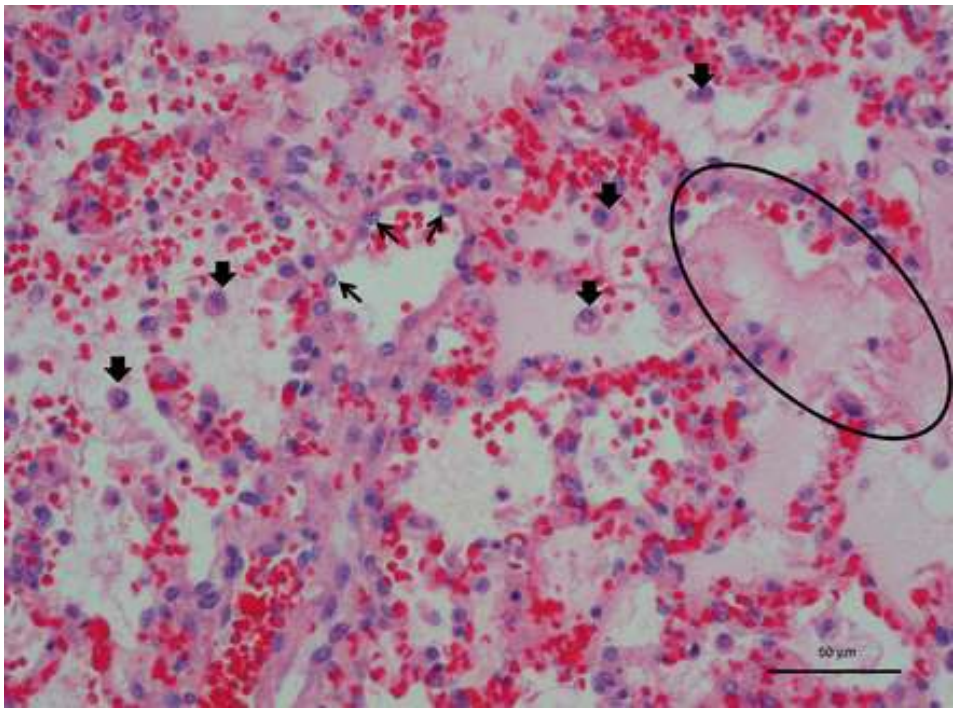


**FIG 2:** Photomicrograph of the lung. Severe serofibrinous interstitial pneumonia characterised by proteinaceous alveolar fluid and fibrin exudation (circle) with alveolar macrophage activation and proliferation (filled arrows). Marked vascular endothelial nuclear hyperactivation (plain arrows); 200× magnification; haematoxylin–eosin staining.

Immunohistochemical labelling for AHSV was most apparent in the lungs. Typical nuclear and cytoplasmic AHSV specific positive labelling of the microvascular endothelial cells as well as scattered monocytes and macrophages was demonstrated (Fig 3). Occasional monocytes and macrophages in the spleen exhibited similar positive labelling.

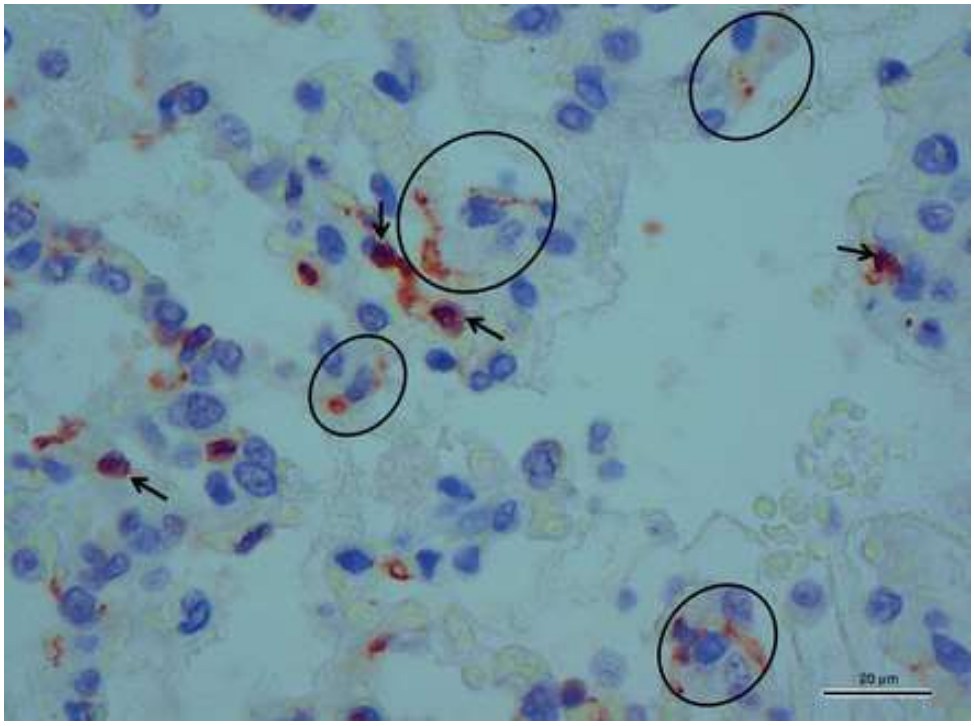


**FIG 3:** Photomicrograph of the lung. Positive solid staining of nuclear antigen (arrows). Granular cytoplasmic staining of endothelial cells (circles); 400× magnification; immunohistochemical staining using African horse sickness virus specific NS4 antibody.

The presence of AHSV was confirmed by RT-PCR in 15 of the 33 dogs for which fresh tissue or blood was available for analysis. The CT values for all the submitted samples (liver, lung, spleen and/or blood) of 11 cases consistently ranged between 20.0 and 31.7, with an average value of 24.3. The CT values for the lung tissues were consistently lower than those of the liver tissues, and the CT values of the spleen tissues were consistently lower than those of the lung tissues in all cases. Serotyping was performed in three of the cases and all were confirmed as serotype 6.

**Discussion**

The results of this retrospective study show that once a dog becomes clinically infected with AHSV, the result is invariably fatal. Most of the dogs presented with a history of acute respiratory distress syndrome or sudden death, which led many owners to suspect malicious poisoning as few of the owners noticed a preceding illness. Most clinical cases presented between January and May, which correlates with increased *Culicoides* midge activity and high levels of vector infection rates. If a radius of 20 km is considered around the Faculty of Veterinary Science, Onderstepoort, RSA, most of the cases originated in the area north to north-east of the faculty. This area is predominated by small holdings with a fairly high concentration of horses and domestic dogs.

The macroscopic changes observed in all cases were indicative of acute pulmonary inflammation of haematogenous origin, resulting in significant and fatal vascular injury. In our experience, these pulmonary findings in combination with the thoracic changes are unique, a consistent feature for cAHS cases and could be regarded as pathognomonic.

Histopathology confirmed the presence of endothelial injury resulting in severe protein rich pulmonary oedema and interstitial pneumonia. The clinical and pathological findings are similar to what has been described for the peracute respiratory form affecting horses. In the cases we examined, none of the dogs showed changes similar to the more subacute cardiac form commonly observed in horses.[4, 5, 8, 26] The reason for this is not clear.

If a case presents with clinical signs and pathology suggestive of cAHS, laboratory confirmation should be obtained by IHC and/or PCR. At necropsy, fresh lung tissue should be submitted for RT-PCR and in 10 per cent buffered formalin for routine histopathology and IHC testing. In live animals, semiquantitative RT-PCR could be performed on EDTA blood.

The types of dogs reported to be affected in this study suggests that large dogs with no breed predilection are at increased risk of contracting cAHS. Larger dog breeds are more likely to be used as guard dogs that are generally kept outdoors and would thus be at increased risk for midge and tick exposure in comparison with dogs that are kept indoors during the night. This finding supports the vector transmission theory of cAHS. Exposure is most likely to occur at night when the *Culicoides* vector is most active. Although *Culicoides* mediated transmission is considered to be the most likely, other vectors, especially ticks, also need further investigation. Male, short haired dogs were overrepresented. This finding is suspected to be associated with the specific preferred breeds and increased aggression in male dogs being more sought after as guard dogs. Dogs with short coats may also prove to be a preferred canine host by vectors if investigated further. Most cases originated from dogs living on small holdings. Other animals, including domestic livestock (cattle, sheep and goats) and horses are regularly kept on these properties. Ruminants and horses are the preferred hosts for *Culicoides* spp, and hence will provide feeding opportunities for the *Culicoides* spp that will increase their numbers, but also increase *Culicoides* spp infection rates with pathogenic viruses such as orbiviruses (especially AHSV and Bluetongue virus). This may be a very important risk factor for dogs to contract cAHS, as they will potentially suffer much higher exposure rates than urbanised dogs.

Symptomatic treatment of dogs that presented to veterinarians showing signs of acute respiratory distress syndrome, typical of cAHS, were unsuccessful in >95 per cent of cases. It would thus appear that once the infection dose of the AHSV exceeds the threshold for clinical disease to manifest, the prognosis deteriorates significantly.

In conclusion, cAHS is an important differential diagnosis that should be considered in dogs (especially large breed, male dogs from small holdings) presenting with clinical signs of acute respiratory distress syndrome. Based on findings in this study, it is believed that cAHSV may be vector transmitted in dogs, and not only by consumption of infected horse meat. Further investigation is necessary to determine the epidemiology of cAHS.

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