Occurrence of African horse sickness in a domestic dog without apparent ingestion of horse meat

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This is the first case of African horse sickness (AHS) in a dog where there was no apparent ingestion of horse meat. Significantly, the dog was part of a colony that resides in a Good Clinical Practice and Good Laboratory Practice accredited facility where complete history, weather and feeding records are maintained. The dog died after a week-long illness despite therapy. The principal post-mortem findings were severe hydrothorax and pulmonary consolidation (red hepatisation of the lungs). Histopathology revealed severe oedema and congestion of the lungs, hyaline degeneration of the myocardium and congestion of the liver sinusoids. Immunohistochemistry detected AHS-positive staining granules in the myocardium, whilst a real-time reverse transcription quantitative Polymerase chain reaction assay of tissue samples was strongly positive for African horse sickness virus nucleic acid. Other dogs on the property showed a 43% seroconversion rate to AHS.

Introduction

African horse sickness (AHS) is caused by African horse sickness virus (AHSV), a virus in the family Reoviridae, genus *Orbivirus*, of which there are nine known serotypes (Coetzer & Guthrie 2004). As the name suggests, it is a disease primarily of Equidae; the horse is most severely affected, whilst donkeys and zebras (*Equus quagga*) are more resistant. The disease occurs regularly in sub-Saharan Africa, although it also appears occasionally in North Africa, from where it can extend into the Middle East and southern Europe. The disease is spread between equids by biting midges (*Culicoides imicola*) and to a lesser extent by *Culicoides bolitinos* in South Africa (Meiswinkel & Paweska 2003). Therefore, in areas where heavy frost occurs, outbreaks are highly seasonal. It has been proposed that AHS is endemic to the eastern parts of the Mpumalanga and Limpopo provinces, where the virus is maintained in zebra reservoirs and from where its spreads southwards during the summer months (Bosman, Brückner & Faul 1995). The importance of the disease in susceptible horses is due to the high mortality rate (70% – 95%), as well as restrictions on the movement of horses to disease-free areas in South Africa and the rest of the world.

Because of the risk of spread and establishment of the disease in previously uninfected countries, the possible epidemiological role of other species in the disease has been investigated by various authors. Antibodies and/or virus have been detected in camelids, bovids, African elephants (*Loxodonta africana*), spotted hyenas (*Crocuta crocuta*), lions (*Panthera leo*), cheetahs (*Acinonyx jubata*), African wild dogs (*Lycaon pictus*), jackals (*Canis* spp.) and genets (*Genetta* spp.) (Alexander *et al*. 1995; Binepal *et al*. 1992; Lubroth 1992). However, the domestic dog is the only other species known to contract the severe form of the disease.

Following the first demonstration of the susceptibility of dogs to the disease by Theiler in 1906, various outbreaks in dogs have been reported (Haig & McIntosh 1956; Piercy 1951; Van Rensburg *et al*. 1981). In all reported field outbreaks in dogs there was a well-documented history of ingestion of horse meat. Transmission of AHSV to dogs by *Culicoides* spp. is not currently considered to be important in the epidemiology of AHS (Maclachlan & Guthrie 2010) as it has been inferred and shown that midges do not readily feed on dogs (Braverman & Chizov-Ginzburg 1996; McIntosh 1955). Nevertheless, there are conflicting opinions regarding the ability of dogs to act as hosts for *Culicoides* spp. For example, it has been suggested that *Culicoides* spp. could indeed infect dogs with bluetongue virus – up to 21% of the population (Oura & Harrak 2011), whilst Braverman and Chizov-Ginzburg (1996) found that all 400 blood meals analysed from *Culicoides* spp. in Israel and Zimbabwe were negative for canine blood. In Siberia, severe midge attacks on dogs (*Culicoides chiopterus, Culicoides pulicaris* & *Culicoides fascipennis*) are reportedly common (Mezenev 1990). Notwithstanding the controversy, it is clear that *Culicoides* spp. do not feed on dogs to the extent that they feed on horses and livestock, although it is possible that dogs could be an incidental host for the midges. Other potential vectors such as mosquitos and ticks (e.g. *Rhipicephalus sanguineus*) have also been suggested and in some cases shown to be capable of transmitting AHS, although the epidemiological importance of this is not known (Alexander *et al*. 1995; Mellor 1993).

Various AHSV serotypes have been detected in the domestic dog: serotypes one and four in dogs in Kenya, serotypes four and seven in dogs in Botswana (Alexander *et al*. 1995) and serotype nine in dogs in Egypt (Salama *et al*. 1981). In South Africa, serotypes three and six have been demonstrated in dogs (McIntosh 1955; Van Rensburg *et al*. 1981). Seroprevalences of 4%, 8%, 9% and 1% have been reported in dogs in Kenya, Botswana, Nigeria and South Africa respectively (Alexander *et al*. 1995; Baba *et al*. 1992; McIntosh 1955). In upper Egypt, live virus was isolated from the blood of 5% of dogs sampled (Salama *et al.* 1981). As the virus was isolated through intracerebral inoculation in mice, this result is difficult to interpret as it is only for a short time during the course of the disease in dogs that the virus can be demonstrated through this technique (Haig & McIntosh 1956). Generally, domestic dogs have a lower seroprevalence of AHSV antibodies than wild carnivores and this is attributed to the greater frequency with which wild carnivores eat or scavenge zebra meat (Alexander *et al.* 1995). As mortality in dogs infected with AHSV ranges from 20% – 78% (McIntosh 1955; Theiler 1906; Van Rensburg *et al*. 1981), it should be kept in mind that the percentage of dogs in which antibodies could be detected might be lower than the percentage of dogs originally infected.

The clinical course of AHS in dogs after ingestion of infected horse meat is 1–2 weeks, although peracute and chronic forms (> 3 weeks) have also been reported. Not all dogs infected with AHSV become clinically ill; some dogs only display a transient fever reaction (Van Rensburg *et al*. 1981). Clinical signs reported in dogs include: pyrexia (although normothermia is also reported in many cases); hyperpnoea; moist rales on auscultation; white foam around nostrils; pharyngitis; coughing; diarrhoea and convulsions (Haig & McIntosh 1956; Theiler 1906; Van Rensburg *et al*. 1981). About 33% of the dogs that Theiler (1906) inoculated intravenously with AHSV died, whereas Van Rensburg *et al.* (1981) reported a mortality rate of 76% of dogs exposed to infected horse meat.

In dogs, macropathological lesions tend to correspond to the 'dunkop' form of the disease in horses, including: severe oedema and hepatisation of the lungs; hydrothorax; petechiation on the pleura; inflammation and congestion of the gastric and intestinal mucosa; blood-stained faeces and enlarged spleen and liver. In chronic cases, dogs only display emaciation on post-mortem examination (Haig & McIntosh 1956; Theiler 1906; Van Rensburg *et al*. 1981). Histopathological lesions consist of acute serofibrinous pneumonia with marked protein-rich oedema, whilst the brain and intestines are congested. In certain cases, there are leucocytic infiltrates into the alveoli and myocardium, whilst lymphoid follicles are small in the spleen and lymph nodes (Van Rensburg *et al*. 1981).

Case presentation

Location

The Malelane Research Unit (MRU) is a Good Clinical Practice and Good Laboratory Practice accredited research facility. The MRU is located in the lowveld of the eastern Mpumalanga Province and is situated at an altitude of 260 m above sea level (25°36'25.35''S; 31°39'48.36''E). This is a subtropical summer rainfall area, receiving an average annual rainfall of 780 mm, which peaks in the months of December through to January. In relation to data for the previous 32 years, the 2011–2012 summer was not particularly wet. However, January 2012, especially the second half, was the second wettest period measured over this time period with 363 mm (Figure 1).

The unit is within 20 km of the southern border of the Kruger National Park and within 5 km of other zebra populations on privately owned land. At the time when the case occurred there were 57 dogs (20 Labradors and 37 cross breed or Africanis [Gallant 2002] type dogs) at the facility that were individually housed in two semi-open enclosures. A complete record of each animal is kept; this includes vaccination record, date of arrival or birth, microchip number and previous treatments. Other records kept include certified water analysis, feeding records and weather records (temperature, wind speed and precipitation). All dogs receive commercial dry (kibbled) pet food (Dogsense Basic Formula for Adult Dogs, Nutroscience [Pty] Ltd, Malmesbury, South Africa). The product label states that it is from a hazard analysis critical control points (HACCP) certified facility and has ISO 9001 traceability. Ingredients are stated on the label as:

> Yellow corn, ostrich (14%), corn prime gluten, wheat bran, polished rice, poultry fat, canola oil, sunflower oil, poultry digest, sodium chloride, potassium carbonate and approved micro minerals and vitamins.

All dogs arrived as pups or were born at the facility. At the time of the case all the dogs had been resident in the dog unit for a minimum of 4 years. Although animals have an outside playpen where they come into contact with each other, this is a fenced off area with no contact with other domestic animal species resident on the facility (which include cattle, horses, sheep and rabbits).

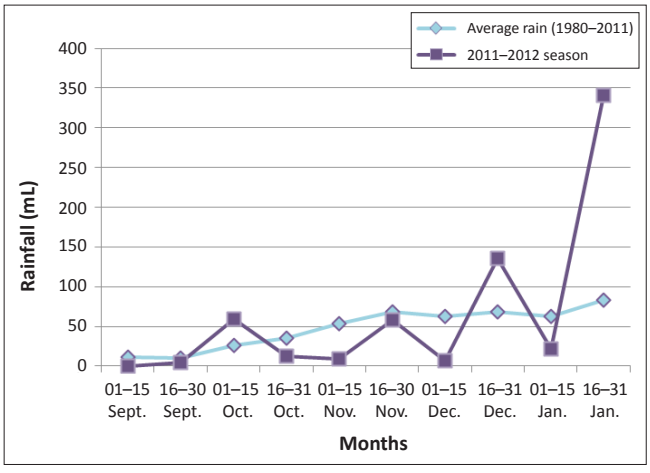


FIGURE 1: Comparison of the average bi-weekly rainfall patterns recorded at the Malelane Research Unit over the period 1980–2011 with that recorded during a period in 2011–2012.

Management and outcome

During the week of 27 February 2012 to 04 March 2012, a 5-year-old male Labrador at the Malelane Research Unit showed progressive appetite loss and displayed a mildly depressed habitus. Apart from the inappetence, no other clinical abnormalities were detected and blood smear evaluation initially did not reveal any abnormalities. The dog was given potentiated sulphonamide (Purbac®, Aspen Pharmacare, South Africa) treatment for the duration of the week. From 03 March 2012 the dog became progressively dehydrated, therefore on 04 March 2012 crystalloid fluid therapy and treatment with tetracycline were instituted. Despite treatment efforts, the dog became progressively depressed during that day. Follow up clinical examination revealed normothermia (39.4 °C), severe hyperpnoea, mild tachycardia, mildly hyperaemic mucous membranes and weakness. Respiratory sounds were increased, but further abnormalities could not be distinguished on auscultation. On blood smear, monocytosis was detected. The dog died early in the evening of 04 March 2012.

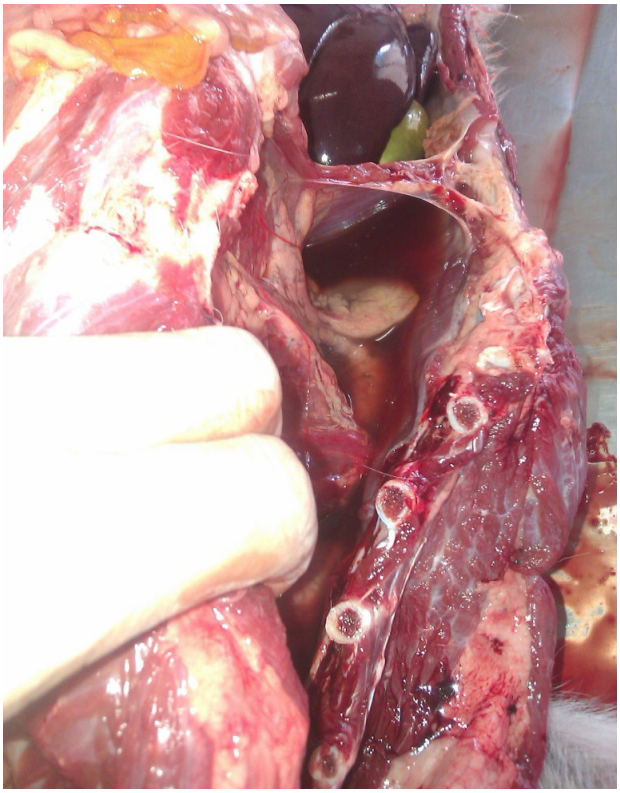
Post-mortem examination

The dog's carcass was refrigerated for approximately 12 h before post-mortem examination commenced. Pink-tinged foam was apparent at the nostrils. Further examination revealed: froth in the trachea; severe hydrothorax (Figure 2); severe oedema of the mediastinum; mild to moderate hydropericardium; severe diffuse pulmonary consolidation (red hepatisation) and oedema (Figure 3); and mild brain and liver congestion.

Samples of the brain, lung, heart, kidney, spleen, liver and various sections of intestine were preserved in 10% buffered formalin (as supplied by the laboratory performing the histopathology) and by freezing in a -40°C freezer. Histopathology revealed severe diffuse congestion and protein-rich oedema of the lungs, accompanied by moderate to severe intra-alveolar haemorrhage, moderate leucocyte infiltration of the lungs, congestion of the centrilobular sinusoids of the liver and moderate brain oedema. Multifocal areas of subendocardial hyaline degeneration and necrosis were present in the heart. The possibility of AHSV was investigated further by immunohistochemical staining of tissue sections (Clift *et al*. 2009). Sparse AHSV-positive staining was identified within the myocardium only, but identification of particles in other organs was made difficult by the presence of large amounts of haematin pigment. Further confirmation was obtained by a strong positive result with a real-time reverse transcription quantitative polymerase chain reaction (RT-PCR) assay for the AHS S7 gene in frozen tissue samples (Quan *et al*. 2010). Recent work has shown the AHSV RT-PCR assay to have high diagnostic sensitivity (97.8%) and specificity (99.9%) (Guthrie *et al*. 2013).

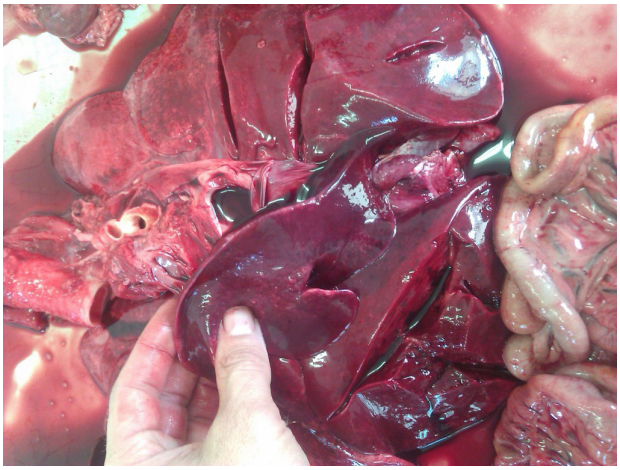
Serology and polymerase chain reaction on resident dogs

On 20 April 2012 whole blood and serum samples were collected from the resident dogs using Ethylenediaminetetraacetic acid and clot-activating tubes respectively. Whole blood



Note: In addition to the hydrothorax there was severe oedema of the mediastinum and moderate hydropericardium.

FIGURE 2: Ventro-cranial view of the opened thorax showing severe hydrothorax.



Note: The The serosanguinous fluid on the post-mortem table originated from the thorax.

FIGURE 3: Severe diffuse congestion, oedema and red hepatisation of the lungs.

samples were assayed for the presence of AHSV nucleic acid with a real-time RT-PCR at the Equine Research Centre of the University of Pretoria, using the method described by Quan *et al*. (2010). Detection of AHSV IgG antibodies was performed using an indirect enzyme-linked immunosorbent assay (iELISA) at the Onderstepoort Veterinary Institute according to the method of Maree and Paweska (2005). A chi-squared test was used to test for significant differences in positive serology between breeds. Relative risk was used to quantify the relationship between breeds and serological statuses. Data were analysed using Excel (Microsoft, USA).

All the whole blood samples tested negative for AHSV by real-time RT-PCR. However, an iELISA for AHS antibodies revealed positive results in 43% of the 56 dogs resident at the time. The positive cases were significantly biased towards Labrador breed dogs ($\chi^2_{(1)} = 11.16$; $p = 0.001$); Labradors were 2.7 times more likely to have a positive serological titre than Africanis or cross-breed dogs.

Discussion

To the authors' knowledge, this report represents the first published case of AHS in a dog without any history of ingestion of horse meat. In horse tissues, immunoperoxidase staining has 100% sensitivity and specificity for AHSV present in heart and lung tissue, with 100%, 98%, 94% and 28% sensitivities for gastrointestinal tract, spleen, liver and kidney tissues respectively (Clift *et al*. 2009). On the other hand, in the brain, lung, heart, kidney, spleen, liver and intestinal tissue of the canine case described here, immunohistochemistry detected viral protein only in the myocardium. This finding may indicate the need for further investigation of the sensitivity of AHS immunohistochemistry in dog tissues.

It is worthy to note that in December 2011 a female Labrador was euthanased at the MRU. This followed a week during which the dog's appetite was reduced and at the end of which, progressive nervous signs developed over a 2 day period. The signs included spastic paresis originating in the hind limbs and progressing towards the front limbs, ataxia, horizontal and vertical nystagmus, hyperpnoea and finally severe recumbent tonic spasticity in the legs and neck (apparently without loss of consciousness). The histopathological changes in the brain, spinal cord, lung, kidney, liver and small intestinal samples of this dog included severe proteinaceous pulmonary oedema, oedema of the nervous tissues, as well as severe congestion of the sinusoidal cords in the liver. No specific diagnosis was made at the time. Subsequent to the confirmed case of AHS a few months later, immunohistochemical staining of tissues from the female Labrador did not identify AHSV. However, myocardial tissue was not available for screening and real-time RT-PCR was not possible because samples were not preserved for this test. There is nevertheless a high suspicion based on similar histopathological lesions, time of outbreak and signalment, that this could have been an early undiagnosed case of AHS in a dog at the MRU.

As there is no known history of ingested equine products within the dog colony at the MRU, other alternative infection routes include unknown or accidental sources of ingestion and vector-borne transmission. Unknown sources of ingestion are unlikely, as this would imply that horse-meat products are added to a commercial dry food ration contrary to what is stated on the label of an ISO-certified manufacturer and that the virus is able to withstand the kibbling process. Furthermore, the serological results do not appear to indicate a common source of exposure, as is seen with toxicological outbreaks, but rather an infectious process that is biased towards the Labrador sub-population.

If vector transmission of AHSV is the route of infection, it is unknown as to which vector is responsible, why there is an over-representation in Labrador dogs compared with the crossbreed or Africanis-type dogs, why this is the first case to be noted since the arrival of the dogs and if the above average bi-weekly rainfall in December and January contributed to the breeding of potential vectors.

The 43% prevalence of dogs with a positive AHS antibody titre is the highest reported in a field outbreak of the disease. Nevertheless, considering that all the dogs had been resident at the MRU for at least 4 years at the time of the outbreak, the mortality rate of a non-food induced AHS infection is low (approximately 5%). Morbiditates are difficult to estimate as mildly depressed habitus, or transient inappetence or pyrexia might not be diagnosed definitively.

AHSV RNA can be detected by real-time RT-PCR in equine whole blood in excess of 130 days after clinical infection (Quan *et al*. 2010). As none of the whole blood samples in the dog colony were positive for AHSV by real-time RT-PCR, this could indicate that the infection in iELISA-positive animals occurred more than four months prior to sample collection, that AHSV is not bound to canine red blood cells to the extent that bluetongue virus is bound to red blood cells (Hassan & Roy 1999; Weyer *et al*. 2013) or that the dogs were sub clinically infected more than 40 days previously, as sub clinically-infected horses only have detectible RNA levels over this period in whole blood (Weyer *et al*. 2013).

Conclusion

It has been found that, contrary to current understanding, AHS could be contracted by natural infection via a non-oral route in dogs. In addition, the highest reported prevalence of naturally occurring antibodies was detected in a population of dogs. Diagnosis of AHS in dogs could, however, be missed if RT-PCR is not requested on tissue samples submitted in conjunction with histopathology and histochemistry. This case has a significant practical implication for the understanding of the epidemiology of AHS. Although vector-borne transmission is likely in this case, further investigations are required before conclusions can be drawn about possible vectors and epidemiology.

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Competing interests

The authors declare that they have no financial or personal relationships that may have inappropriately influenced them in writing this article.

Authors' contributions

S.J.vS. (MSD Malelane Research Unit) initialised and wrote the manuscript, performed the *post-mortem* and assisted in treatment; T.M.D. (West Acres Animal Hospital) co-consulted on the case (suggested African horse sickness as possible infectious process) and made conceptual contributions to the written report; T.S. (MSD Malelane Research Unit) initialised treatments and provided facility support, which aided in diagnosis; J.L.K. (Nkomazi State Veterinarian) facilitated further RT-PCR diagnostics on tissue samples and performed organisation, summary and analysis on weather data; C.W. (University of Pretoria) facilitated further RT-PCR diagnostics on tissue samples and whole blood, facilitated iElisa serology and made conceptual contributions to the manuscript; A.J.G. (University of Pretoria) facilitated diagnostics, made major conceptual contributions to the manuscript and provided valuable guidance to the project.

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