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## SHORT REPORT

# African horse sickness in The Gambia: circulation of a live-attenuated vaccine-derived strain

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### SUMMARY

African horse sickness virus serotype 9 (AHSV-9) has been known for some time to be circulating amongst equids in West Africa without causing any clinical disease in indigenous horse populations. Whether this is due to local breeds of horses being resistant to disease or whether the AHSV-9 strains circulating are avirulent is currently unknown. This study shows that the majority (96%) of horses and donkeys sampled across The Gambia were seropositive for AHS, despite most being unvaccinated and having no previous history of showing clinical signs of AHS. Most young horses (<3 years) were seropositive with neutralizing antibodies specific to AHSV-9. Eight young equids (<3 years) were positive for AHSV-9 by serotype-specific RT-PCR and live AHSV-9 was isolated from two of these horses. Sequence analysis revealed the presence of an AHSV-9 strain showing 100% identity to Seg-2 of the AHSV-9 reference strain, indicating that the virus circulating in The Gambia was highly likely to have been derived from a live-attenuated AHSV-9 vaccine strain.

**Key words:** Animal pathogens, asymptomatic viral infections, immunization (vaccination), veterinary epidemiology, veterinary virology.

African horse sickness virus (AHSV), a double-stranded RNA orbivirus belonging to the family Reoviridae, causes a non-contagious arthropod-borne disease of equidae. The virus is widely distributed across sub-Saharan Africa where it is transmitted between susceptible vertebrate hosts by *Culicoides* biting midges, and also possibly by mosquitoes and ticks [1]. The Sahara desert seems to act as a geographical

barrier to the spread of the virus out of Africa. That said, AHSV serotype 9 (AHSV-9) was responsible for a devastating epizootic in the Middle East and Southwest Asia in 1959 and 1960 which caused the death of around 300 000 horses during the first phase of infection [2]. In the course of this epidemic the ratio of mortality to inapparent infection varied considerably [3] which left many questions unanswered about the factors affecting the pathogenicity of this serotype. In 1965 AHSV-9 again spread beyond its traditional enzootic zones in sub-Saharan Africa into Morocco, Algeria and Tunisia and then into Southern Spain in 1966 [4].

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AHSV-9 has been known for some time to be circulating in the West African equidae population without causing significant clinical disease in the local indigenous horse populations. AHSV-9 was isolated for the first time in Nigeria in 1970 from a horse that died with typical clinical signs of AHS [5]. In a small serological survey carried out in Nigeria, 60% of the horses sampled were found to have neutralizing antibodies to AHSV-9 [6] and no previous vaccination had been carried out in the region. In 1974 about 20 horses that had recently been imported into Nigeria died showing clinical signs of AHS and interestingly at no time during the outbreak were any locally bred horses reported to be ill. An emulsion of spleen tissue from one of the dead horses was injected into a locally bred horse previously shown to have no neutralizing antibodies to AHSV and this animal showed only mild clinical signs followed by complete recovery. This result indicated that local breeds of horses in Nigeria may show a high degree of resistance to infection with AHSV; however, imported 'exotic' breeds appear to be highly susceptible.

Several studies investigating the prevalence of AHS in horses in Senegal and The Gambia have previously been conducted. Mattioli *et al.* [7] tested sera from 112 donkeys and 15 horses in The Gambia for AHS antibodies and found 95.5% of the donkeys and all the horses to be seropositive for AHSV-9. They concluded that these high titres suggested a recent and continuous contact with AHSV-9, although interestingly, with the exception of one suspected but not confirmed case, no clinically sick animals were recorded. Similar high levels of seroprevalence to AHSV-9 were also recorded in horses and donkeys in Senegal [8, 9] which is not surprising as both The Gambia and Senegal are within the same region in which AHS is considered to be endemic. In a separate study conducted in The Gambia by Staeuber *et al.* [10], 81% of the 459 horse serum samples collected tested seropositive for AHSV antibodies. The high percentage of AHSV antibody-positive horses in The Gambia along with a lack of clinical signs suggest that either the local breeds of horses may be resistant to AHS or alternatively the AHSVs circulating may not be virulent. *Culicoides* spp. that are known to transmit or are suspected vectors of AHSV are abundant in The Gambia [11].

We set out to try to address the question of whether the high levels of seroconversion seen in The Gambia were due to an increased resistance of horses living in the region to AHS or whether the AHSV circulating in the region was less virulent and possibly derived

from the live-attenuated vaccine, which had been used in the region for many years. In response to a suspected AHS outbreak in The Gambia and Senegal during September 2007, a vaccination campaign using a monovalent live-attenuated AHSV-9 vaccine from Senegal or a polyvalent live-attenuated vaccine from South Africa (containing serotypes 1, 2, 3, 4, 6, 7, 8) was administered in 2007 to horses in The Gambia. In October 2009 a serological and virological survey for AHS was undertaken. In total 144 equids (horses and donkeys) were sampled from seven villages in the north Nianija district in mid Gambia, where clinical signs consistent with AHS had been recognized in some horses during 2007 (group 1), and 10 villages south of the river Gambia in the district of Niamina, where AHS clinical signs had not previously been reported (group 2). The clinical and vaccination history of each equid was recorded. The median age of the equids (101 horses, 43 donkeys) was 7 years (range 1–20 years). Out of 122 equids from group 1, sampled north of the river Gambia, 21 (17%) were vaccinated as part of the campaign in 2007. None of the 22 equids sampled from south of the river Gambia were vaccinated.

The majority of horses from both groups (96%) were seropositive for AHS antibodies as determined by ELISA (Ingenasa, Spain), despite most being unvaccinated and having no previous history of showing clinical signs of AHS. Five out of 144 equids that tested seronegative were all horses that were aged  $\leq 3$  years. The majority of equids (horses and donkeys) were seropositive for AHS antibodies from an early age ( $> 2$  years). Serum neutralization tests (SNTs) against all nine serotypes of AHSV showed that, in a selection of 15 younger equids that were unvaccinated and antibody-ELISA positive, all had neutralizing antibodies specific to AHSV-9 (Table 1). This indicated that AHSV-9 is likely to be the only serotype that is circulating in the region.

Eight young equids (6 horses, 2 donkeys) that were all aged  $< 3$  years tested positive for AHSV RNA by real-time RT-PCR assay [12] giving cycle threshold ( $C_t$ ) values of between 29 and 41. Live AHSV (dsRNA reference collection GAM2009/01 and GAM2009/02) was isolated on KC (*Culicoides variipennis*) cells from two of these samples with the lowest  $C_t$  values (29.5 and 30.5) indicating that AHSV was circulating freely in the region and was being transmitted by the local midge population (*Culicoides* spp.). Of the eight samples that were PCR positive, seven were also antibody positive in ELISA; however, the remaining

Table 1. Serological (ELISA/serum neutralization test) and virological (real-time RT-PCR) responses in young unvaccinated horses in The Gambia

Horse (ID)	Age (yr)	PCR (C <sub>t</sub> )	ELISA	Serum neutralization titres (log <sub>10</sub> )									
				AHS-1	AHS-2	AHS-3	AHS-4	AHS-5	AHS-6	AHS-7	AHS-8	AHS-9	
9	2	n.d.	+	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	2.68
21	2	n.d.	+	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	2.08
30	2	n.d.	+	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	2.20
37	2	n.d.	+	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	1	1.78
70	2	n.d.	+	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	2.20
83	3	n.d.	+	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	2.68
98	4	n.d.	+	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	1.90
102	4	n.d.	+	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	1.48
111	2	n.d.	+	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	2.08
114	2	n.d.	+	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	3.11
136	2	n.d.	+	Neg	Neg	1.48	Neg	Neg	1.30	1.48	1		> 3.11
46	2	n.d.	+	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	1.78
60	2	n.d.	+	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	2.81
108	3	n.d.	+	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	1	2.68
113	3	n.d.	+	Neg	Neg	Neg	Neg	Neg	Neg	Neg	1	Neg	2.38
6	2	n.d.	-	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
10	2	+(C <sub>t</sub> 37)	-	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg

n.d., No virus detected; C<sub>t</sub>, cycle threshold.

PCR-positive sample, which was taken from a 2-year-old horse, was antibody negative (horse ID no. 10, Table 1), indicating that this animal was likely to be in the early phase of infection before antibodies had developed.

Serotype-specific RT-PCR assays [13] targeting genome segment 2 (Seg-2 – the VP2 gene) identified AHSV serotype 9 in the two samples from which AHSV was isolated. Sequence analysis of Seg-2 confirmed the presence of AHSV-9, and the Seg-2 consensus sequence from both isolates was 100% identical over a 1653-bp region (615–2268 bp) to Seg-2 from the South African AHSV-9 reference strain (PAKrrah/09). As the AHSV-9 live-attenuated vaccine strain used in previous vaccination campaigns in the region is highly likely to have been derived from the AHSV-9 reference strain, we can therefore conclude that the AHSV-9 live-attenuated vaccine strain is likely to be circulating and infecting equids from an early age in The Gambia. Interestingly, the oral susceptibility of livestock-associated *Culicoides* spp. to strains of AHSV has been demonstrated to be higher for vaccine as opposed to field strains of AHSV [14, 15]. Sequence analysis (Seg-2) was also carried out on an AHSV-9 strain isolated from an imported unvaccinated horse from Senegal in 1992 that succumbed to fatal clinical signs of AHS immediately after entry into the country [16]. This revealed a

97.2% identity over 1653 bp to the AHSV-9 reference strain (PAKrrah/09) and the identical strain circulating in The Gambia, indicating that this Senegal isolate was not derived from the AHSV-9 reference/vaccine strain and was likely to be a field strain.

There are three principal explanations as to why horses within the region, despite being infected with AHSV-9, are not showing any overt clinical signs of disease. The first is that a state of ‘endemic stability’ to AHSV-9 exists within the region with protective colostral antibodies to AHSV-9 being passed from dams to their offspring. The young equids are then infected with AHSV-9 from an early age, when they are still partially or completely protected by colostral antibodies, and therefore gain lifelong immunity without showing any clinical disease. The second explanation is that indigenous horses within the region are naturally resistant to AHS having ‘lived’ with the virus for many years. Previous evidence from Nigeria [6] indicates that this may be the case. The third explanation is that the AHSV-9 strain(s) circulating in the region are not virulent and may be derived from tissue-culture-attenuated live vaccine strains used in the region. Sequence analysis presented in this paper indicates that the third explanation is likely to be occurring in The Gambia and that the AHSV-9 strain circulating is likely to have been derived from the live-attenuated AHSV-9 vaccine strain used extensively in

the region for many decades. This does not, however, preclude the possibility that indigenous horses in the region may also show some degree of resistance to AHS. In order to further address these questions it would be necessary to perform detailed experimental infection studies with local and exotic breeds of horses infected with different AHSV serotypes/strains.

We can conclude that AHSV-9 is endemic in equidae in The Gambia with horses and donkeys in the region being infected with the virus from a young age. It is likely that AHSV-9 is being naturally transmitted by *Culicoides* midges in the region and there is no evidence that any of the other nine AHSV serotypes are circulating. We can conclude that one reason why many horses in The Gambia are not showing clinical signs of AHS, despite being infected, is that an attenuated vaccine strain is freely circulating within the region. This circulating AHSV-9 vaccine strain is likely to be 'immunizing' equids from an early age and protecting them from infection with more virulent field strains of the same serotype. Although this may have some beneficial effects, provided that the circulating vaccine-derived strain does not revert to virulence, there is clearly potential for genome segment reassortment with field strains that could cause reversion.

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#### DECLARATION OF INTEREST

None.

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