

Full Length Research Paper

Serological evidence of African horse sickness virus infection of donkeys in Karamoja sub-region, North-eastern Uganda

Jesca Nakayima^{1*}, Mary L. Nanfuka², Daniel Aleper¹ and Duke Okidi¹

¹National Livestock Resources Research Institute (NaLIRRI), P. O. Box 96 Tororo, Uganda.

²National Animal Disease Diagnostics and Epidemiology Centre (NADDEC), P. O. Box 513, Entebbe, Uganda.

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African horse sickness virus (AHSV) causes a non-contagious, infectious insect-borne disease of equids and it is endemic in many areas of sub-Saharan Africa but extends beyond its endemic zones to the Arabian Peninsula, Asia and Europe. The usual mode of transmission is by biting midge, a biological vector and *Culicoides imicola* appears to be the principal vector. Serum samples were screened from camels and donkeys for AHSV antibodies using competitive enzyme-linked immunosorbent assay (cELISA). Results revealed that 16/22 (73%) donkeys had been exposed to AHSV. All 85 camels screened in the study tested negative to AHSV. This was the first study of AHSV in Uganda and it was geared at creating awareness for the veterinary service needs of these animal species which is non-existent so far.

Key words: African horse sickness virus (AHSV), *Culicoides* spp., camels, donkeys, Uganda.

INTRODUCTION

African horse sickness (AHS) is caused by a double stranded RNA virus of the family *Reoviridae* of the genus *Orbivirus*. There are nine antigenically distinct serotypes of AHS virus (AHSV) identified by virus neutralization (Howell, 1962; McIntosh, 1958). The hosts for AHSV are equids: horses, mules, donkeys and zebra. Zebra is believed to be the reservoir host (Barnard, 1998). Antibody is found in camels, African elephants, and black and white rhinoceroses, but their role in epidemiology is unlikely to be significant (OIE, 2009). Dogs acquire peracute fatal infection after eating infected horse meat

(Bevan, 1911; Piercy, 1951), but are not a preferred host by *Culicoides* spp., therefore, are unlikely to play a role in transmission (McIntosh, 1955). Clinical manifestation of AHS in horses involve damage to the circulatory and respiratory systems resulting in serous effusion and haemorrhage in various organs and tissues (Awad et al., 1981; Coetzer and Erasmus, 1994; Lubroth, 1988). African horse sickness (AHS) is peracute, acute, subacute or mild but the disease is more severe in horses. Clinical manifestations of AHSV involve four forms: horse sickness fever, in the majority of cases (which usually

*Corresponding author. E-mail: jescanl2001@yahoo.co.uk .

affects only mules, donkeys and partially immune horses); the subclinical cardiac form is suddenly followed by marked dyspnea and other signs typical of the pulmonary form. This could manifest as the cardio-pulmonary or mixed form or the peracute or pulmonary form (Maurer and McCully, 1963; Newsholme et al., 1983; Theiler, 1921). A nervous form may occur, though it is rare. All forms of disease can occur in any one outbreak but in susceptible populations of horses the mixed and pulmonary forms tend to predominate so mortality rates in these animals will be very high. Mortality rate ranges from 50 to 95% in horses to rare in African donkeys and zebra. Following recovery to AHSV, animals develop good immunity to the infecting serotype and partial immunity to other serotypes. There is no treatment for AHSV; the disease is managed by supportive treatment. Disease prevention is by vaccination with a polyvalent vaccine since all AHSV serotypes are present in South Africa and in most parts of sub-Saharan Africa. Several methods are employed for the diagnosis of AHSV, including virus inoculation of cell cultures, mice inoculation (Howell, 1962), postmortem, serology and molecular assays (Costa et al., 2016; Fowler et al., 2016; de Waal et al., 2016; Sánchez-Matamoros et al., 2016; Weyer et al., 2015). AHS is not contagious, but is known to be spread by insect vectors. The biological vector of the virus is the *Culicoides* (midges) species (Theal, 1900; Wetzel et al., 1970). *Culicoides* midges, in general, breed in damp soil rich in organic matter, however *C. bolitinos* breeds in bovine dung, and it therefore not as dependent on annual rainfall and soil-type. Adult midges become infected by taking blood meals from viraemic animals. However, this disease can also be transmitted by species of mosquitoes including *Culex*, *Anopheles*, and *Aedes*, and species of ticks such as *Hyalomma* and *Rhipicephalus*. Biting flies may also be able to transfer the virus. In Uganda, camels and donkeys are distributed in North-eastern Uganda in Karamoja and Sebei sub-regions. Zebras are found in the various conservation areas throughout the country while horses are sparsely distributed in Uganda. The horse medicine aspect of veterinary service in Uganda is not developed possibly because horses are not common in Uganda and their economic importance is limited. For this reason few people keep horses for prestige and deaths in these horses are common because during an emergency, the Ugandan veterinarians lack the expertise in horse medicine. This is the first report of AHSV in Uganda and it is geared at creating awareness for the need for equine veterinary intervention in these animals.

MATERIALS AND METHODS

Serum samples were collected from Karamoja sub-region in two districts namely: Moroto: N 2° 31' 41.604", E 34° 39' 28.794" and Amudat: N 1° 47' 29.841", E 34° 54' 23.583" districts, Uganda. The camels and donkeys were classified as: infant, juvenile, sub-adult and adult. Both sexes were sampled. Serum was collected from

donkeys and camels from Karamoja sub-region in March, 2016.

Serological analysis

The animals were bled by the jugular vein following restraint. 2.5 ml blood was collected into plain vacutainer tubes without anti-coagulant. Serum was separated from the blood cells by centrifugation at 2500 rpm for 15 min and stored at -20°C until use in a competitive enzyme-linked immunosorbent assay (cELISA) (Inmunologia Y Genetica Aplicad, S. A. Madrid, Spain). In total, 110 serum samples were collected. These included 25 donkeys and 85 camels. Purposive sampling was employed due to the availability of the animals.

RESULTS AND DISCUSSION

16/22 donkeys tested positive to AHSV antibodies. All the 85 camels screened alongside the donkeys tested negative to the viral antibodies. Corrected optical densities (ODs) were calculated from sample ODs and blank ODs. Sample Id represents animal species, age, sex and sample number.

Results revealed that 16/22 (73%) of serum samples from donkeys tested positive to AHSV antibodies (Table 1). All the 85 camels tested negative to AHSV. No previous research has been done on AHSV in Uganda. Literature on AHSV research in Africa and other parts of the world is scanty although reports in South Africa exist (Liebenberg et al., 2016). Not much research interest on biting midges (*Culicoides* spp.) in Uganda (Mayo et al., 2016; Liebenberg et al., 2016; Probst et al., 2015) and not much interest in equine and cameline species in Uganda and their economic importance hence population structure is limited. Nakayima et al. (2017a, b) reported endo-parasites and equine piroplasmiasis in these animals in Karamoja sub-region in the absence of veterinary care and these diseases are also prevalent around the globe (Singh et al., 2012; Sumbria et al., 2016; Singla and Sumbria, 2017).

The distribution of AHSV is determined by several factors including the efficiency of control measures, availability of vertebrate hosts or reservoirs, vector abundance, seasonality and climate. AHSV apparent infection rate rapidly fall to zero at temperatures below 15°C since virus replication does not seem to occur below this temperature (Wellby et al., 1996). However, overwintered midges could harbor "latent" virus in some of these surviving midges that will commence replication and transmission should temperatures rise to permissive levels for example during spring. The major vector of AHSV, *Culicoides imicola* adults are active at temperatures as much as 3°C lower than the minimum required for AHSV replication (Sellers and Mellor, 1993). The seasonality of AHSV is explained by vector activity; after the rainy season in the tropics, in the summer and autumn in temperate regions. Bluetongue virus shares the same vector species (*Culicoides*) (Boorman et al., 1975; Mellor, 2000; Mellor et al., 1975; Venter et al., 2000;

Table 1. Sero-prevalence of AHSV in donkeys from Karamoja sub-region, North-eastern Uganda.

S/N	Animal species	Sample ID	OD reading	Corrected OD	AHSV result
1	Donkey	D/A/F/02	0.152	101.7	Positive
2	Donkey	D/SA/F/03	0.459	83.2	Positive
3	Donkey	D/A/F/04	0.137	102.6	Positive
4	Donkey	D/A/F/05	0.11	104.3	Positive
5	Donkey	D/A/F/06	0.103	104.7	Positive
6	Donkey	D/A/F/07	1.827	0.4	Negative
7	Donkey	D/A/F/08	1.974	-8.5	Negative
8	Donkey	D/A/F/09	1.841	-0.5	Negative
9	Donkey	D/A/M/10	0.126	103.3	Positive
10	Donkey	D/A/M/11	1.972	-8.4	Negative
11	Donkey	D/A/F/12	0.098	105.0	Positive
12	Donkey	D/A/F/13	0.098	105.0	Positive
13	Donkey	D/A/F/14	0.121	103.6	Positive
14	Donkey	D/A/M/15	0.114	104.0	Positive
15	Donkey	D/A/M/16	0.132	102.9	Positive
16	Donkey	D/A/F/17	0.689	69.2	Positive
17	Donkey	D/A/F/18	0.097	105.1	Positive
18	Donkey	D/A/M/19	0.096	105.1	Positive
19	Donkey	D/SA/F/20	1.755	4.7	Negative
20	Donkey	D/A/F/55	0.605	74.3	Positive
21	Donkey	D/SA/F/56	0.335	90.7	Positive
22	Donkey	D/C/M/57	0.135	102.8	Positive

Du Toit, 1944). With the advent of climate change the midge vector has now significantly extended its range northwards into Europe. Since 1998, bluetongue virus has caused disease outbreaks and has become endemic in Europe. AHSV is widely distributed across sub-Saharan Africa (Mellor and Boorman, 1995; Howell, 1963), from Senegal and Gambia in the west to Ethiopia and Somalia in the east, and extending as far south as northern South Africa, and may extend at times to Egypt in the north (Howell, 1963). The Sahara desert serves as an effective geographical barrier preventing the infection from the South spreading north-wards. Probably AHSV has its first historical reference traced to an epizootic in Yemen which occurred in 1327 (Moule, 1896; Sailleau et al., 2000). However, the virus is believed to have originated from Africa following the introduction of susceptible equine breeds during exploration of central and eastern Africa (M'Fadyean, 1900). The earliest account of the disease in Africa traces back to 1569 (Theal, 1900). The first detection of AHSV in South Africa was in 1719, a major outbreak that killed 1,700 animals in the Cape region. However, before this, the wildlife reservoirs could have been circulating the disease (Mornet and Gilbert, 1968). The disease is endemic in these areas with subsequent outbreaks and massive horse deaths (Mellor and Hamblin, 2004). During outbreaks of AHS in endemic areas, different virus serotypes may be active simultaneously within an area,

but one serotype usually dominates during a particular season, followed in the following year by the dominance of another serotype. AHSV is a major challenge to horses in endemic areas in sub-Saharan Africa, but it repeatedly caused large epizootics in the Mediterranean region (North Africa and southern Europe in particular) as a result of trade in infected equids.

Conclusion

AHSV could be endemic in the equine population in Uganda but goes undiagnosed. Zebras in wildlife conservation areas and donkeys could be acting as reservoirs to the infection. No information about the disease is available in Uganda hence no control measures in place. This is a threat to the horse population in Uganda and neighboring countries. There is need to improve knowledge of equine and cameline medicine and welfare in Uganda.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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REFERENCES

- Awad FI, Amin MM, Salama SA, Aly MM (1981). The incidence of African horse sickness in animals of various species in Egypt. *Bull. Anim. Health Prod. Afr.* 29:285-287.
- Barnard JH (1998). Epidemiology of African horse sickness and the role of the zebra in South Africa. *Arch. Virol. Suppl.* 14:13-19.
- Bevan LEW (1911). The transmission of African horse sickness to the dog by feeding. *Vet. J.* 67:402-408.
- Boorman J, Mellor PS, Penn M, Jennings M (1975). The growth of African horse sickness virus in embryonated hen eggs and the transmission of virus by *Culicoides variipennis* Coquillett (Diptera: Ceratopogonidae). *Arch. Virol.* 47:343-349.
- Coetzer JAW, Erasmus BJ (1994). African horse sickness, in: Coetzer JAW, Thomson GR, Tustin RC. (Eds.), *Infectious diseases of livestock with special reference to southern Africa*, Oxford University Press, Cape Town. 1:460-475.
- Costa S, Sastre P, Pérez T, Tapia I, Barrandeguy M, Sánchez-Vizcaino JM, Sánchez-Matamoros A, Wigdorovitz A, Sanz A, Rueda P (2016). Development and evaluation of a new lateral flow assay for simultaneous detection of antibodies against african horse sickness and equine infectious anemia viruses. *J. Virol. Methods* 237:127-131.
- de Waal T, Liebenberg D, Venter GJ, Mienie CM, van Hamburg H (2016). Detection of African horse sickness virus in *Culicoides imicola* pools using RT-qPCR. *J. Vector Ecol.* 41(1):179-185.
- Du Toit RM (1944). The transmission of bluetongue and horse sickness by *Culicoides*. *Onderstepoort J. Vet. Sci. Anim. Ind.* 19:7-16.
- Fowler VL, Howson EL, Flannery J, Romito M, Lubisi A, Agüero M, Mertens P, Batten CA, Warren HR, Castillo-Olivares J (2016). Development of a novel reverse transcription loop-mediated isothermal amplification assay for the rapid detection of african horse sickness virus. *Transbound. Emerg. Dis.* Available at: <http://onlinelibrary.wiley.com/doi/10.1111/tbed.12549/full>
- Howell PG (1962). The isolation and identification of further antigenic types of African horse sickness virus. *Onderstepoort J. Vet. Res.* 29:139-149.
- Howell PG (1963). African horse sickness, in: *Emerging diseases of animals*, FAO, Rome FAO Agricultural Studies. 61:71-108.
- Liebenberg D, Piketh S, Labuschagne K, Venter G, Greyling T, Mienie C, de Waal T, van Hamburg H (2016). *Culicoides* species composition and environmental factors influencing African horse sickness distribution at three sites in Namibia. *Acta. Trop.* 163:70-79.
- Lubroth J (1988). African horse sickness and the epizootic in Spain 1987. *Equine Pract.* 10:26-33.
- Maurer FD, McCully RM (1963). African horse sickness with emphasis on pathology. *Am. J. Vet. Res.* 26:235-266.
- Mayo C, Venter E, Steyn J, Coetzee P, van Vuuren M, Crafford J, Schütte C, Venter G (2016). The prevalence of *Culicoides* spp. in 3 geographic areas of South Africa. *Vet. Ital.* 52(3-4):281-289.
- McIntosh BM (1955). Horse sickness antibodies in the sera of dogs in enzootic areas. *J. South Afr. Vet. Med. Assoc.* 26:269-272.
- McIntosh BM (1958). Immunological types of horse sickness virus and their significance in immunization. *Onderstepoort J. Vet. Res.* 27:465-539.
- Mellor PS (2000). Replication of arboviruses in insect vectors. *J. Comp. Pathol.* 124:231-247.
- Mellor PS, Boorman J (1995). The transmission and geographical spread of African horse sickness and bluetongue viruses. *Ann. Trop. Med. Parasitol.* 89:1-15.
- Mellor PS, Boorman J, Jennings M (1975). The multiplication of African horse sickness virus in two species of *Culicoides* (Diptera: Ceratopogonidae). *Arch. Virol.* 47:351-356.
- Mellor PS, Hamblin C (2004). African horse sickness. *Vet Res.* 35:445-466.
- M'Fadyean J (1900). African horse-sickness. *J. Comp Pathol.* 13:1-20.
- Mornet P, Gilbert Y (1968). La peste équine. In *Les maladies animales à virus*. L'Expansion. 476:195.
- Moule L (1896). *Histoire de la Médecine Vétérinaire*, Maulde, Paris. P 38.
- Nakayima J, Kabasa W, Aleper D, Okidi D (2017a). Prevalence of endoparasites in donkeys and camels in Karamoja sub-region, North-eastern Uganda. *J. Vet. Med. Anim. Health* 9(1):11-15.
- Nakayima J, Nanfuka LM, Aleper D, Okidi D (2017b). Serological prevalence of *Babesia caballi* and *Theileria equi* in camels and donkeys from Karamoja sub-region, North-eastern Uganda. *J. Vet. Med. Anim. Health* 9(6):137-142.
- Newsholme O, Bedford GAH, Du Toit RM (1983). A morphological study of the lesions of African horse sickness. *Onderstepoort J. Vet. Res.* 50:7-24.
- OIE: World Organization for Animal Health (2009). Available at: <http://www.oie.int/>
- Piercy SE (1951). Some observations on African horse-sickness including an account of an outbreak among dogs. *East Afr. Agric. J.* 17:62-64.
- Probst C, Gethmann JM, Kampen H, Werner D, Conraths FJ (2015). A comparison of four light traps for collecting *Culicoides* biting midges. *Parasitol. Res.* 114(12):4717-4724.
- Sailleau C, Hamblin C, Paweska JY, Zientara S (2000). Identification and differentiation of the nine African horse sickness virus serotypes by RT-PCR amplification of the sero type specific genome segment 2. *J. Gen. Virol.* 81:831-837.
- Sánchez-Matamoros A, Nieto-Pelegrín E, Beck C, Rivera-Arroyo B, Lecollinet S, Sailleau C, Zientara S, Sánchez-Vizcaino JM (2016). Development of a luminex-based DIVA assay for serological detection of african horse sickness virus in horses. *Transbound. Emerg. Dis.* 63(4):353-359.
- Sellers RF, Mellor PS (1993). Temperature and the persistence of viruses in *Culicoides* spp. during adverse conditions. *Rev. Sci. Tech. Off. Int. Epizoot.* 12:733-755.
- Singh G, Soodan JS, Singla LD, Khajuria JK (2012). Epidemiological studies on gastrointestinal helminths in horses and mules. *Vet. Practitioner* 13(01):23-27.
- Singla LD, Sumbria D (2017). Equine piroplasmiasis: Belles-lettres update with special reference to Indian scenario. In: *An Update on Diagnosis and Control of Parasitic Diseases*, Ananda KJ, Pradeep BS, Rakesh RL and Malatesh DS (Eds), Department of Veterinary Parasitology, Veterinary College, Shimoga, KVAFSU, Bidar, Karnataka, India. pp. 372-400.
- Sumbria D, Singla LD, Kumar S, Sharma A, Dhayia R, Setia RK (2016). Spatial distribution, risk factors and haematobiochemical alterations associated with *Theileria equi* infected equines of Punjab diagnosed by indirect ELISA and nested PCR. *Acta Trop.* 155:104-112.
- Theal GM (1900). Records of South-Eastern Africa collected in various libraries and archive departments in Europe. Government of the Cape Colony. P 6.
- Theiler A (1921). African horse sickness (*Pestisequorum*), Union S. Africa Dept. Agric. Pretoria, Sci. Bull. P 19.
- Venter GJ, Graham SD, Hamblin C (2000). African horse sickness epidemiology: vector competence of South African *Culicoides* species for virus serotypes 3, 5 and 8. *Med. Vet. Entomol.* 14:245-250.
- Wellby MP, Baylis M, Rawlings P, Mellor PS (1996). Effects of temperature on the rate of virogenesis of African horse sickness virus in *Culicoides* (Diptera: Ceratopogonidae) and its significance in relation to the epidemiology of the disease. *Med. Vet. Entomol.* 86:715-720.
- Wetzel H, Nevill EM, Erasmus BJ (1970). Studies on the transmission of African horse sickness. *Onderstepoort J. Vet. Res.* 37:165-168.
- Weyer CT, Joone C, Lourens CW, Monyai MS, Koekemoer O, Grewar JD, van Schalkwyk A, Majiwa PO, MacLachlan NJ, Guthrie AJ (2015). Development of three triplex real-time reverse transcription PCR assays for the qualitative molecular typing of the nine serotypes of African horse sickness virus. *J. Virol. Methods* 223:69-74.