Serological evidence of African horse sickness virus infection of donkeys in Karamoja sub-region, North-eastern 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
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 AHHSV, animals develop good immunity to the infecting serotype and partial immunity to other serotypes. There is no treatment for AHHSV; the disease is managed by supportive treatment. Disease prevention is by vaccination with a polyvalent vaccine since all AHHSV serotypes are present in South Africa and in most parts of sub-Saharan Africa. Several methods are employed for the diagnosis of AHHSV, 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). AHHSV 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 AHHSV 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 anticoagulant. 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 AHHSV 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 AHHSV antibodies (Table 1). All the 85 camels tested negative to AHHSV. No previous research has been done on AHHSV in Uganda. Literature on AHHSV 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 piroplasmosis 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 AHHSV is determined by several factors including the efficiency of control measures, availability of vertebrate hosts or reservoirs, vector abundance, seasonality and climate. AHHSV 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 AHHSV, Culicoides imicola adults are active at temperatures as much as 3°C lower than the minimum required for AHHSV replication (Sellers and Mellor, 1993). The seasonality of AHHSV 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.

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

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 northwards. 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 (MFAdyean, 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 camelid medicine and welfare in Uganda.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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REFERENCES


