INTRODUCTION

African horse sickness (AHS) is endemic in sub-Saharan Africa and is still recognized as one of the major life-threatening diseases of equids in Africa, the Middle East, the Eastern Mediterranean and some parts of Europe (10) because of its high mortality rate (up to 90% in epidemics), particularly in naïve populations (13). AHS is an acute or subacute insect-borne infectious disease of Equidae (horses, mules, donkeys and zebras) caused by African horse sickness virus (AHSV), a double-stranded RNA virus in the genus orbivirus, belonging to the Reoviridae family (2, 4). AHSV exists as nine immunologically distinct serotypes, all of which have been identified and are considered to be enzootic in sub-Saharan Africa (7). Since the first documented outbreak and subsequent isolation of the virus from a dead horse in Nigeria in 1970 (9), sporadic outbreaks of AHS have been reported in Nigeria in the past. Sera collected from 284 horses in seven stables in Kaduna Metropolis, Nigeria, were tested for antibodies against AHS virus (AHSV) using a blocking enzyme-linked immunosorbent assay (ELISA). A high percentage of the sera (86.6%) were positive, indicating continual exposure of Nigerian horses to AHSV. Annual vaccination of horses and vector control to minimize incidence in the region is advocated.

Detection of African horse sickness virus antibodies by ELISA in sera collected from unvaccinated horses in Kaduna Metropolis, Nigeria

D.O. Ehizibolo1* E.C. Nwokike2 Y. Wungak1 C.A. Meseko1

Keywords

Summary
African horse sickness (AHS) is endemic in sub-Saharan Africa and is recognized as one of the major life-threatening diseases of equids in some parts of the world. Several sporadic outbreaks of AHS have been reported in Nigeria in the past. Sera collected from 284 horses in seven stables in Kaduna Metropolis, Nigeria, were tested for antibodies against AHS virus (AHSV) using a blocking enzyme-linked immunosorbent assay (ELISA). A high percentage of the sera (86.6%) were positive, indicating continual exposure of Nigerian horses to AHSV. Annual vaccination of horses and vector control to minimize incidence in the region is advocated.

1. Virology Division, National Veterinary Research Institute, Vom, Nigeria.
2. Equitation Wing, Nigerian Defence Academy, Kaduna, Nigeria.
* Corresponding author
Cell: +234 80 35 92 48 59
E-mail: kingdavid_e@yahoo.com

The population of horses in Nigeria has been estimated at over 1.2 million (18) consisting of both a local breed (Arewa), which is popular with traditional institutions, and exotic breeds (particularly Argentine and Sudanese) mostly kept under intensive management by a few elites for racing and polo games. This study shows the presence of antibodies against AHSV in horses in Kaduna, Nigeria.
**Materials and Methods**

**Study area and stable management**

Kaduna State is located in the Northwestern region of Nigeria, in the Northern Guinea savannah zone. Horses in seven different stables, designated A through G (Figure 1), belonging to government security institutions (n = 2), private organizations (n = 4) and a traditional institution (n = 1) located in Kaduna Metropolis, Nigeria, were used for the study. These stables were kept under intensive to semi-intensive management and each accommodated 30 to 200 horses. The horses were primarily kept for special ceremonial activities, training, crowd control, race competition and polo games. Routine veterinary care was provided for all the stables except stable G. No preventive vaccination is routinely applied against AHSV in Nigeria.

**Sample collection**

Using the principles of convenient sampling, about 50% of the total number of horses in each stable was selected for blood sampling. A total of 284 horses of all ages, different breeds and both sexes were sampled under proper restraint. Approximately ten milliliters of blood was collected from each horse into properly labeled Vacutainer tubes. The blood samples were allowed to clot and were then centrifuged in the laboratory at 1500 g for 10 min. Sera were separated into cryovials and stored at -20°C until tested.

**Test procedure**

An African horse sickness virus blocking enzyme-linked immunosorbent assay (ELISA) kit (INGENASA, Madrid, Spain) was used for the detection of group-specific antibodies to AHSV in equine (i.e., the test detects all nine serotypes [6]) according to the manufacturer’s instruction. Briefly, the antigen was fixed in a solid support (polystyrene plate). After incubation with serum samples, an AHSV specific monoclonal antibody (Mab peroxidase conjugate) was added. If the sample contains specific antibodies to the virus, they will not allow the binding of labeled Mab to the antigen, whereas if the sample does not contain specific antibodies, Mab will bind to the antigen coating the plate. After washing the plate with a multichannel pipetting device suitable for dispensing 300 ml on each well to eliminate all non-fixed materials, presence or absence of labeled Mab can be detected by adding the substrate which, in the presence of peroxidase, will develop a colorimetric reaction. The optical density reading was performed with a spectrophotometer at 405 nm. Samples showing blocking percentages higher than 50% were considered positive for antibodies to AHSV.

**RESULTS**

The stables sampled, breed, sex and age range of the horses are presented in Table I. Results show that overall there was a high prevalence of antibodies in the sera from all the stables included in the study. In total, 246 (86.6%) of the 284 tested horses were positive for antibody to AHSV by ELISA. Stable A had the highest prevalence (100%), whereas stable E had the lowest (73.3%). There was a high prevalence of antibodies in the sera collected from the local (Arewa breed) horses (87.7%; 121/138), as well as in those collected from exotic (Argentine and Sudanese breeds) horses (85.6%; 125/146). The difference between AHSV antibodies detected in local and exotic horses was not statistically significant ($\chi^2 = 0.113; p > 0.05$).

**DISCUSSION**

The prevalence of AHSV antibodies detected by ELISA in the horse stables in Kaduna Metropolis, Northwest Nigeria, was estimated at 86.6%. This high prevalence is consistent with previous serological studies in Nigeria (2, 14, 16), and may be an indication of continual exposure of the horses regardless of age, sex and breed. Our study area (Kaduna) extends from the tropical savannah of Guinea to the savannah of Sudan, with thick vegetation and hot and dry climatic conditions which favor vector propagation and virus transmission. In Nigeria, no preventive vaccination is routinely applied against AHSV, particularly in indigenous and local crossbred horses. There appears to be a form of innate resistance to infection by AHSV as corroborated by Best et al. (3), and Nawathe et al. (14). It could explain the probable absence of reported outbreak of the disease in the region, despite the high prevalence of antibodies. In contrast, newly imported horses, particularly from AHS-free areas, are susceptible to infection and are therefore usually vaccinated before importation. But once established in the country (the animals of exotic breeds examined during the study had been introduced more than ten years ago or were born in the

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**Table I**

Detection of African horse sickness virus antibodies by ELISA in horse sera from Kaduna Metropolis, Nigeria

<table>
<thead>
<tr>
<th>Stable (years)</th>
<th>Num. samples</th>
<th>Breed</th>
<th>Male</th>
<th>Female</th>
<th>Age range</th>
<th>ELISA positive (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>17</td>
<td>Mixed*</td>
<td>10</td>
<td>7</td>
<td>4–10</td>
<td>17 (100)</td>
</tr>
<tr>
<td>B</td>
<td>28</td>
<td>Exotic</td>
<td>12</td>
<td>16</td>
<td>2–9</td>
<td>24 (85.7)</td>
</tr>
<tr>
<td>C</td>
<td>22</td>
<td>Local</td>
<td>15</td>
<td>7</td>
<td>6–22</td>
<td>21 (95.4)</td>
</tr>
<tr>
<td>D</td>
<td>93</td>
<td>Exotic</td>
<td>22</td>
<td>71</td>
<td>3–13</td>
<td>80 (86.0)</td>
</tr>
<tr>
<td>E</td>
<td>15</td>
<td>Exotic</td>
<td>3</td>
<td>12</td>
<td>3–11</td>
<td>11 (73.3)</td>
</tr>
<tr>
<td>F</td>
<td>17</td>
<td>Local</td>
<td>16</td>
<td>1</td>
<td>4–15</td>
<td>14 (82.4)</td>
</tr>
<tr>
<td>G</td>
<td>92</td>
<td>Local</td>
<td>22</td>
<td>70</td>
<td>2–17</td>
<td>79 (85.9)</td>
</tr>
<tr>
<td>Total</td>
<td>284</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>246 (86.6)</td>
</tr>
</tbody>
</table>

* Ten exotic and seven local breeds
Détection du virus de la peste équine africaine au Nigeria

Résumé


La peste équine africaine (PEA), endémique dans les pays subsahariens, est considérée dans certaines parties du monde comme l’une des maladies des équidés les plus mortelles. Plusieurs foyers sporadiques de PEA ont été signalés dans le passé en Afrique. Des anticorps dirigés contre le virus de la PEA ont été recherchés par un test Elisa d’inhibition dans les sérums de 284 chevaux collectés dans sept écuries de la métropole de Kaduna au Nigeria. Un grand pourcentage de sérums (86,6 p. 100) était positif, témoignant d’une exposition permanente des chevaux nigérians au virus de la PEA. La vaccination annuelle des chevaux et la lutte contre les vecteurs du virus sont recommandées pour limiter l’incidence de la maladie dans la région.


Resumen

Ehizibolo D.O., Nwokike E.C., Wungak Y., Meseko C.A. Detección de anticuerpos contra el virus de la peste equina africana mediante ELISA en sueros colectados de caballos no vacunados en Kaduna Metropolis, Nigeria.

La peste equina africana (PEA) es endémica en África subsahariana y es reconocida como una de las enfermedades que amenazan la vida de los equinos en algunas partes del mundo. En el pasado, se han reportado varios brotes esporádicos de PEA en Nigeria. Sueros colectados de 284 caballos en siete establos en Kaduna metrópolis, Nigeria, fueron examina-
dos para anticuerpos contra el virus de PEA (PEAV), utilizando el ensayo por inmunobromación ligado a enzimas (ELISA). Un alto porcentaje de los sueros (86,6%) fueron positivos, indi-
cando una exposición continua de los caballos nigerianos a PEA. Se recomienda una vacunación anual de los caballos y control de vectores para minimizar la incidencia en la región.

Palabras clave: Virus de la peste equina africana – Anticuerpos – ELISA – Nigeria.