Isolation and identification of African horse sickness virus during an outbreak in Lagos, Nigeria


Summary: An outbreak of African horse sickness involving two horse stables in Lagos, Nigeria, was investigated. Inoculation of blood from infected horses into suckling albino mice resulted in isolation of a virus which was identified as African horse sickness virus by the complement fixation test. The clinical, pathological and epizootiological findings (reported elsewhere) were consistent with African horse sickness. Potential threats of the epidemic to international horse trade are briefly highlighted.


INTRODUCTION

African horse sickness (AHS) is still one of the major infectious diseases threatening the horse industry in Africa, the Middle East, the Eastern Mediterranean and some parts of Europe (8). The disease is enzootic in Africa south of the Sahara (8).

In the recent past, severe epizootics of AHS have occurred in parts of Africa and Asia outside the recognised areas of distribution, indicating potential spread of the virus to hitherto free areas (2, 6). In Nigeria, sporadic outbreaks of AHS have been reported, mostly from the northern parts of the country (3), apparently because of the higher horse population in the north than in the south. Recently, the authors investigated an outbreak of AHS involving two horse stables in Lagos, Nigeria. This paper describes the virus isolation and identification procedures used.

MATERIALS AND METHODS

Blood sample collection and inoculation of mice

Thirty-nine blood samples were collected from horses in the two stables involved in the outbreak. Approximately 10 ml of blood were collected from clinically-diseased and contact animals by jugular venepuncture using sterile needles and vacuum tubes as described by Kemp and colleagues (7). Approximately 1 ml of whole blood was
preserved in ethylene diamine tetra-acetic acid (EDTA) for virus isolation, while the remainder was used for serology. Sera were separated from blood clots by centrifugation at 1,800 rpm and stored at -20°C until tested. Whole blood from each animal was frozen and thawed once and inoculated intracerebrally into a litter of six suckling mice (two to three days old). Each mouse received 0.02 ml of the suspension. The inoculated mice were observed daily for 14 days for signs of illness or death. Infected mice were removed daily when they became sick or moribund and stored at -70°C until tested for virus.

**Complement fixation test**

Serotype 9 of AHS virus, isolated by Best and colleagues (3), was used in the complement fixation test (CFT). The virus had undergone four passages in suckling mouse brain. Virus antigen was prepared by sonication and sucrose acetone extraction in infected suckling mouse brains as described by Clarke and Casals (4).

Detection of antibodies to AHS virus in the horse sera and identification of virus isolates were performed on plastic plates using the modified microtitre technique described by Sever (9). Sera were inactivated at 56°C for 30 min and tested in two-fold serial dilutions with veronal buffer against optimum dilutions (obtained by checkerboard titration) of the antigen.

A 10% suspension of the infected mouse brain was made in sterile veronal buffer and tested against optimum dilution of the immune mouse ascitic fluid previously prepared against AHS virus serotype 9 as described by Tikasingh and colleagues (11).

**RESULTS**

Of the 39 horse sera tested, 37 (95%) contained antibody to AHS virus. High complement fixation antibody titres (>1:16) were frequently obtained in the sera tested (Table I).

<table>
<thead>
<tr>
<th>Reciprocal of antibody titre</th>
<th>Positive No.</th>
<th>(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;4</td>
<td>2</td>
<td>5.4</td>
</tr>
<tr>
<td>4</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>8</td>
<td>2</td>
<td>5.4</td>
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<td>16</td>
<td>1</td>
<td>2.7</td>
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<td>32</td>
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<td>24.3</td>
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</tr>
<tr>
<td>256</td>
<td>3</td>
<td>8.1</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>37</strong></td>
<td></td>
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</tbody>
</table>

**Table I**

Results of the complement fixation test for antibodies to African horse sickness virus in sera from horses in two stables in Lagos, Nigeria
In addition, 3 (7.7%) of the 39 blood samples from diseased and contact horses inoculated into suckling mice yielded viruses identified as AHS viruses by CFT.

**DISCUSSION**

The specificity of CFT in the identification of virus isolates and the diagnosis of recent arbovirus infections has been well established (10, 12, 13). Recently, House and colleagues (5) demonstrated that CFT compares well with virus neutralisation and enzyme immunoassay in terms of sensitivity and specificity for rapid diagnosis of AHS virus infections.

The results of this investigation indicated presence of AHS virus in the horse population studied. Isolation of the virus from some of the infected horses further confirmed the high level of complement fixation antibodies detected in the animals and the presence of other epidemiological determinants of AHS in the study area. The outbreak occurred during the peak of the rainy season in the area, which results in a high vector population and high virus activity. In addition, Lagos is situated in the swamp forest zone of Nigeria where the climate and vegetation favour high vector density. Furthermore, the animals had not been immunised against AHS for at least seven years before the outbreak. The animals could therefore be regarded as particularly susceptible to the disease.

In comparison with serum, whole blood was a much more suitable source for virus isolation. Preservation of blood in EDTA anticoagulant enabled testing of undiluted blood. This is an advantage over the use of other preservatives (such as potassium oxalate, carbolic acid and glycerine) recommended by the Onderstepoort Laboratory (1); because of toxicity to mice, the latter must be diluted at least five-fold. Direct intracerebral inoculation of suckling mice with whole blood is therefore a reliable method for isolation of AHS virus. The low virus yield (7.7%) obtained in this study may be due to high antibody titres already developed by the animals. However, this low virus yield is due, most probably, to the use of whole blood for virus isolation, while a better yield could have been obtained by using buffy coat. The inoculation of whole blood may have another disadvantage, as whole blood harbouring many non-specific horse antigens will create non-specific horse antibodies which may interfere in the CFT by giving non-specific reactions. Identification of virus isolates and/or examination of sera from infected animals by CFT are probably the simplest methods for establishing diagnosis of AHS in developing countries with less sophisticated laboratories.

This paper focuses on the characteristics of AHS virus serotype 9, of which a well-known strain was examined (3). However, the investigation of existing complement fixation antibodies against other AHS virus serotypes would be interesting. The rapid spread and resurgent outbreaks of AHS from different continents in recent times present a potential threat to the horse industry in countries of the Western world (6). Increases in international horse trade and global horse movements further emphasise the importance of disseminating information on outbreaks in order to effectively control the spread of this disease.
ISOLEMENT ET IDENTIFICATION DU VIRUS DE LA PESTE ÉQUINE LORS
D’UNE ÉPIDÉMIE À LAGOS, NIGERIA. – L.A. Oladosu, O.D. Olayeye, S.S. Baba et
S.A. Omilabu.

Résumé : L’épidémie de peste équine, survenue dans deux écuries de Lagos,
Nigeria, a fait l’objet d’une étude. L’inoculation de sang provenant de chevaux
infectés à des souriceaux albinos nouveau-nés a permis d’isoler un virus,
reconnu comme étant celui de la peste équine par le test de fixation du
complément. Les données cliniques, pathologiques et épizootiologiques
(publiées par ailleurs) ont confirmé le diagnostic de la peste équine. Les
menaces éventuelles de l’épidémie pour le commerce international des équidés
sont brièvement évoquées.


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AISLAMIENTO E IDENTIFICACIÓN DEL VIRUS DE LA PESTE EQUINA
DURANTE UN BROTE EN LAGOS, NIGERIA. – L.A. Oladosu, O.D. Olayeye, S.S. Baba
y S.A. Omilabu.

Resumen: Los autores estudiaron un brote de peste equina que afectó dos
establos de Lagos, Nigeria. La inoculación de sangre de caballos infectados a
ratoncillos albinos recién nacidos permitió aislar un virus e identificarlo
mediante la prueba de fijación del complemento como el virus de la peste
equina. Los datos clínicos, patológicos y epizootiológicos (publicados en otro
informe) concordaban con el diagnóstico de la peste equina. Los autores se
refieren por último, brevemente, a las amenazas que la epidemia podría
representar para el comercio internacional de équidos.


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REFERENCES


4. CLARKE D.H. & CASALS J. (1958). – Technique for haemagglutination and
haemagglutination inhibition tests with arthropod-borne viruses. Am. J. med. Hyg., 7,
561-573.


