

# Isolation and Identification of Circulating Serotypes of African Horse Sickness Virus in Ethiopia

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## ABSTRACT

An outbreak of African Horse Sickness (AHS) in Ethiopia was investigated in order to isolate and identify circulating serotypes of African Horse Sickness Virus in Ethiopian equines between July and September 2003. Six hundred and fifty (650) equines of different species (horse, donkey, mule) were examined. Viral specimens were collected from 12 acutely sick and moribund animals. Clinical and necropsy findings distinguished two forms of the disease: a peracute pulmonary form and a subacute cardiac form. Laboratory investigation of tissue and whole blood specimens collected from sick and moribund animals identified two different serotypes of AHS viruses: serotype 9 and serotype 6. The identification of serotype 6 represents the first report of this serotype in Ethiopia.

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Incorporation of the newly isolated serotype 6 into the AHS vaccine is recommended.

## INTRODUCTION

African Horse Sickness (AHS) is an acute or subacute vector-borne viral disease of horses and related equine species characterized by severe pyrexia, widespread hemorrhage, and edematous exudations. It is primarily an infection of horses, with mortality as high as 95%. Susceptibility and mortality decreases for mules (80%) and donkeys in that order.<sup>1,2</sup> AHS is caused by an arbovirus for which nine distinct serotypes have thus far been identified. The serotypes of African Horse Sickness Virus (AHSV) are distinguished by differences in virulence, immunogenicity, and production of different disease patterns.<sup>2</sup> Of the nine serotypes identified, type 9 is predominantly found throughout the African continent, and it is the only serotype previously identified in Ethiopia.

The most amenable means of control of AHS for countries such as Ethiopia is

immunization of susceptible hosts with suitable vaccines. The absence of cross protection among different serotypes,<sup>3</sup> however, necessitates incorporation of available serotypes into the vaccines utilized.

In 2002–2003 Ethiopia faced serious and repeated outbreaks of AHS in different regions, including southern, western, central, and northern Ethiopia. The outbreak affected horses vaccinated with monovalent vaccines containing type 9 AHSV (AHS Vaccine, National Veterinary Institute, Debre Zeit, Ethiopia). It is well documented that in spite of its wide distribution, serotype 9 of AHSV has a lower virulence than other serotypes, killing few horses in enzootic areas.<sup>2,3</sup> The outbreak encountered in 2002–2003, however, resulted in high mortality. Donkeys, which were thought to be resistant to AHS,<sup>3,4</sup> were observed to encounter clinical AHS in the Tgrai region of northern Ethiopia.

Considering the diversity of AHSV serotypes and the absence of cross-protection, it has been strongly recommended that polyvalent vaccines be used. However, the absence of previous research on serotype identification in Ethiopia has made it difficult for vaccine manufacturers to shift to polyvalent vaccines. The objective of this study was, therefore, to isolate and identify the circulating AHSV serotypes in Ethiopia.

## **MATERIALS AND METHODS**

### **Study Area and Animals**

The study was conducted in selected areas of Ethiopia where the 2002–2003 outbreaks of AHS were reported, including Southern Ethiopia (Awassa, Hossana, Wondogenet, and Hagereselam); Western Ethiopia (Jimma, Bedelle, Nekemte, Horroguduru, and Chaliya) and Central Ethiopia (Debre Zeit, Meki, Zeway, Filtimo, and Bekejo). Within each of these outbreak regions, areas (villages) with high incidence of epidemics and risk areas were selected for study. Six hundred and fifty (650) equine species (horses, mules, and donkeys) reared in the

selected areas were closely examined and sampled irrespective of age and sex.

### **SAMPLES COLLECTED**

Samples of spleen, lung tissue, and lymph nodes were collected from 12 sick and dead animals to carry out viral isolation and identification and typing of whole blood. Blood was collected (10 mL) in heparinized vacuum tubes from acutely sick animals in the early febrile stages of the disease. Tissue samples were collected from spleen, lung, and lymph nodes obtained from freshly dead and moribund animals. Between 2 g and 5 g of the aforementioned organs and blood collected were kept at +4°C until processed.

### **VIRUS ISOLATION**

To carry out virus isolation, whole blood was washed three times in phosphate buffered saline (PBS). Isolation was facilitated by the addition of sterile distilled water to the blood to hemolyze the erythrocytes. A 10% tissue suspension was made with PBS-containing antibiotics (penicillin and streptomycin). Inoculation of the hemolyzed blood and tissue suspension was performed on VERO cells (Pan African Veterinary Vaccine Center/FAO origin) at the National Veterinary Institute in Debre Zeit, Ethiopia. The inocula prepared were seeded onto early confluent monolayers of Vero cells in roux flasks. The cultures were incubated at 37°C for 30 minutes. After 30 minutes of adsorption time, the cultures were washed with PBS and refilled with maintenance media and incubated at 37°C for 7 days. The development of a cytopathic effect was followed daily.

### **Identification and Typing of Isolates**

Identification of the isolated viral particles was performed using the standard complement fixation test.<sup>1</sup> Designation of serotypes to the identified viruses was carried out by Virus Neutralization Test (VNT) at the University of Pretoria, Faculty of Veterinary Science, Department of Veterinary Tropical Diseases, Onderspoort, South Africa.

## RESULTS

### Clinical and Necropsy Findings

The leading clinical findings recognized in a majority of the animals during the AHS outbreak were pyrexia (41 °C), dyspnea, bouts of coughing, nasal discharge, and sweating. In some cases prominent facial swelling, particularly in the area of eyelids and lips, was encountered. The preponderant lesions observed include edematous lung, fluid in the pleural cavity, and hemorrhages on the serosal and mucosal surfaces of organs such as spleen, heart, and intestine.

### Virus Isolation and Identification

AHSV<sub>s</sub> were isolated from blood, spleen, and lymph nodes. The VNT indicated that two serotypes of AHSV were involved in the outbreak: serotype 9 and serotype 6. The identification of serotype 6 represents the first report of this serotype in Ethiopia.

## DISCUSSION

The clinical and necropsy findings recorded in this study were in agreement with those of other investigators.<sup>2,4-6</sup> The greater proportion of affected animals displayed pulmonary disorders. Pulmonary involvement is typically characteristic of the peracute pulmonary form of AHS.<sup>5,6</sup> This form of the disease was known to be caused by more virulent strains of the causative virus, or it occurs in completely susceptible populations of equines.<sup>2,7,8</sup> This finding showed that most of the animals were severely infected and revealed the involvement of virulent serotypes or strains of AHSV, implying that it may not be due to serotype 9.

We observed the occurrence of the cardiac form of AHS. This form of the disease represents the subacute form of AHS, which is caused by virus strains of lower virulence or by viruses that occur in immune animals infected with heterologous strains of the virus.<sup>2</sup> This could have thus been caused by heterologous strains of serotype 9 for which the animals have long been vaccinated. Since there is the possibility of occurrence of all forms of AHS in one outbreak, other

forms of the disease could have occurred but escaped detection.

The current study established the presence of two serotypes of AHSV in Ethiopia. Isolation and identification of a serotype of AHSV other than serotype 9 has not previously been shown in Ethiopia. Isolation of serotype 6 in this study therefore represents the first report in Ethiopia. The virus could have been introduced into Ethiopian equines by wind-borne infected midges (*Culicoides*) from endemic regions of Africa. The vectors were known to be wind driven and migrate, carrying the virus over 700 km.<sup>2,4,6</sup>

Only monovalent vaccine containing serotype 9 is being fabricated and employed to control AHS outbreaks in Ethiopia. The absence of cross-protection among serotypes of AHSV has been well documented.<sup>2,3,5</sup> Incorporation of serotype 6 into AHS vaccine is therefore recommended. However, it is advisable to assess the degree of interference between the two serotypes. Ozawa and others<sup>9,10</sup> have described the existence of interference among different serotypes of AHSV. The presence of multiple serotypes of AHSV can inflict considerable economic losses for countries like Ethiopia, which has a large equine population. Further surveillance studies are recommended to search for other AHSV serotypes.

## CONCLUSION

This study has shown the widespread occurrence of AHS across various agroecological zones of Ethiopia. In countries such as Ethiopia, where large populations of equines are raised, the presence of multiple serotypes of such a devastating virus poses a serious hindrance to national development.

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## REFERENCES

1. Organization International des Epizootics. *Manual of Standards for Diagnostic Tests and Vaccines*. Paris, Organization International des Epizootics, 2000:178–188.
2. Hess WR. *The Arbovirus: Epidemiology and Ecology*. Vol. 2. Boca Raton, Fla: CRC Press;1988:2–16.
3. Seifert SH. *Tropical Animal Health*. Wageningen, Germany: Kluwer Academic; 1996:235–240.
4. Radostitis OM, Gay CC, Blood DC, Hinchcliff KW. *Veterinary Medicine: The Text Book of Diseases of Cattle, Sheep, Pigs, Goats and Horses*. 9<sup>th</sup> ed. Philadelphia: Saunders; 2000:1038–1041.
5. Wintzer HJ. *Equine Diseases: A Textbook for Students and Practitioners*. Berlin, German: Verlag Paul Parey;1986:388–389.
6. Quinn PJ, Markey BK, Carter ME, Donnelly WJ, Leonard FC. *Veterinary Microbiology and Microbial Disease*. Oxford, England: Blackwell Scientific; 2002:370.
7. Murphy FA, Gibbs PJ, Horzinek MC, Studdert MJ. *Veterinary Virology*. 3<sup>rd</sup> ed. New York: Academic Press;1999:400–402.
8. Mohanty SB, Dutta SK. *Veterinary Virology*. London: Balliere-Tindall;1981:172–174.
9. Ozawa, Y. Interference between African Horse Sickness Viruses in tissue culture. *Am J Vet Res*. 1966;27:106–109.
10. Knipe DM, Howley PM. *Virology*. vol. 2, 4<sup>th</sup> ed. Philadelphia; Lippincott, Williams & Wilkins; 2001:1835–1870.